Ultra-clean pure shift NMR with optimal water suppression for analysis of aqueous pharmaceutical samples

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Electronic Supporting Information

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Sample information:

1) Bupivacaine HCl: 10.9 mg of bupivacaine hydrochloride from Sigma-Aldrich (lot P500128, catalog # PHR1128-1G) was diluted in 0.55 mL of a mixture of 90% H₂O/10% D₂O (v/v), and this solution was transferred to a 5 mm NMR tube.

2) Aurobasidin A (AbA): Approximately 2 mg of purified AbA from Merck & Co. Inc., Kenilworth, NJ, USA was dissolved in 150 µL of a mixture of CD₃CN:H₂O (vide manuscript) and transferred to a 3 mm NMR tube.

NMR parameters and instrumentation:

All data was acquired in a Avance III 600 MHz Bruker instrument equipped with a 5mm TCI cryoprobe at 308 K. 8.0 µs was used for the duration of hard 90° ¹H pulses for all experiments. The duration of the SAPPHIRE NOESY pre-stauuration, iES and iW5 were approximately 1h 12 min, 44min, and 42 min, respectively, for the AbA sample. The duration of the double Saltire was set to 30ms, with a bandwidth of 10 kHz and flip angle of 15°, for all pure shift experiments. RSNOB pulses of 37 ms duration (50 Hz bandwidth) were used for all ES experiments. SINE.100 was used for gradient shape in all pure shift experiments, except in the double Saltire element, where RECT.1 was used. 8 scans and 4096 complex points were used in the acquisition of all SAPPHIRE experiments, with 4 increments in the SAPPHIRE dimension (TD2) and 50 chunks (TD1) of 10 ms each. The delay to keep T2 weighting constant between SAPPHIRE increments (d2) was set to 6 ms for all SAPPHIRE experiments. 4 drop points (cnst4) was used for all pure shift experiments.

Data acquisition and processing of SAPPHIRE data follow the same steps as described by Moutzouri et al. [1] The interferogram FIDs were reconstructed with TopSpin 3.x macros available at https://www.nmr.chemistry.manchester.ac.uk/. The reconstruction for SAPPHIRE data is a two-step process: (i) the FID chunks are arrayed to form new FID interferograms, one per SAPPHIRE increment; (ii) each SAPPHIRE increment is added for the final averaged pure shift FID. For TopSpin 4.x the macro “sertoint.ptg” should be used before step (i) to convert the FID file into integer.
Fig. ESI 1. $^1$H NMR pure shift spectra of bupivacaine hydrochloride in 90% H2O/10% D2O (v/v) (1) conventional $^1$H, (2) SAPPHIRE-iW5, (3) SAPPHIRE-iES, and (4) PSYCHE-NOESY-pre-saturation.

Fig. ESI 2. Zoom of Fig. ESI 1 for strongly coupled signals. (1) SAPPHIRE-iW5, (2) SAPPHIRE-iES, and (3) PSYCHE-NOESY-pre-saturation.
**Fig. ESI 3.** Full $^1$H NMR pure shift spectra of Aureobasidin A 10% H$_2$O/90% CD$_3$CN for: (1) SAPPHIRE-NOESY-pre-saturation, (2) SAPPHIRE-iES, and (3) SAPPHIRE-iW5.

**Fig ESI 4.** HSQC real-time pure shift with multiplicity editing of Aureobasidin A in 90% H$_2$O/10% D$_2$O (v/v). Vertical projection was replaced by $^{13}$C($^1$H) spectrum and top projection was replaced by SAPPHIRE-iES.
**Fig ESI 5.** Suppression profiles for the PSYCHE-SAPPHIRE-iES experiment
Fig ESI 6. Suppression profiles for the PSYCHE-SAPPHIRE-iW5 experiment
Sideband Averaging with Periodic PHase Incrementation of Residual J Evolution for the acquisition of clean PSYCHE pure shift spectra with excitation sculpting

The pulse sequence involves a 3D acquisition scheme. F3 is the direct dimension. F1 is the incremented dimension for the reconstruction of the pure shift interferogram. F2 is the incremented dimension for the J-evolution.

The data can be reconstructed using the two following AU programs (downloaded from: http://nmr.chemistry.manchester.ac.uk)

1) pm_pshift (produces pure shift spectra for each different J-evolution time, adjusting the length of the first chunk appropriately)

2) pm_fidadd (averages the pure shift spectra acquired with different J-evolution times)

;CLASS=HighRes
;DIM=3D
;TYPE=
;SUBTYPE=
;COMMENT=

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

define delay tauA
define delay tauAA
define delay tauB
define delay tauBB
define delay tauBBB
define delay tauBBBB
define delay tauC
define delay tauD
"p2=p1*2"
"l0=0"
"l8=0"
"in0=inf1/2"
"in10=inf2"
"d0=inf1/2"
"d10=inf2"
"d30=in0/2"
"d40=in0/2-in10"
"cnst5=(td2/2)+1"
"tauA=0"
"tauAA=inf1/4"
"tauB=d2-p17-2*d16-20u"
"tauBB=d2-p17-2*d16-20u-inf2"
"tauBBB=d2-p17-2*d16-20u"
"tauBBBB=d2-p17-2*d16-20u+inf2"
"tauC=inf2"
"tauD=(dw*2*cnst4)"

"cnst50=(cnst20/360)*sqrt((2*cnst21)/(p40/2000000))"
"p30=1000000.0/(cnst50*4)"

"cnst31= (p30/p1) * (p30/p1)"
"spw40=plw1/cnst31"

"p10=p40"

;*************** WaveMaker Parameters ***************
"d11=30m+1s/(cnst12)-1s/(cnst12)"
"d11=30m+1s/(cnst13)-1s/(cnst13)"
;sp12:wvm:rsnob:f1 rsnob(cnst12 Hz, cnst13 ppm; PA=0.5, NPOINTS=5000);

"d11=30m"

"acqt0=0"
baseopt_echo
aqseq 312
1 ze
2 50m
d1 pl1:f1
3 50u UNBLKGRAD

if "l8 < cnst5"
{
    "tauA=(l8*in10)"
    "tauB=d2-p17-2*d16-20u-(l8*in10)"
    "d30=(in0/2+(l8*in10))+((l0-1)*in0)"
    "tauBBB=(d2-p17-2*d16-20u)-(l8*in10)"

    p1 ph1

    if "l0==0"
    {
        tauA
    }
    else

{    tauAA
}
p16:gp1
d16
8u
p2 ph2
4u p10:f1
(p12:sp12 ph5):f1
4u p11:f1
p16:gp1
d16

if "l0==0"
{
    tauA
}
else
{
    tauAA
}

if "l0==0"
{
}
else
{
    d30
}

if "l0==0"
{
    tauB
}
else
{
    tauBBB
}

d16
p17:gp2
d16
20u p10:f1
;
( center (p40:sp40 ph3):f1 (p10:gp10) )
;
20u p11:f1
d16
p17:gp2
d16

if "l0==0"
{ 
    tauB
}
else
{
    tauBBB
}

tauD
p18:gp3
d16 p10:f1
4u
(p12:sp12 ph6):f1
4u p11:f1
p2 ph4
8u
p18:gp3
d16 BLKGRAD

if "l0==0"
{

}
else
{
d30
}

lab1, goto lab7
}
else
{
    "tauC=(in10)+((l8-cnst5)*in10)"
    "tauBB=(d2-p17-2*d16-20u-in10)-((l8-cnst5)*in10)"
    "d40=((in0/2-in10)-((l8-cnst5)*in10))+((l0-1)*in0)"
    "tauBBBB=(d2-p17-2*d16-20u+in10)+((l8-cnst5)*in10)"

    p1 ph1
    if "l0==0"
    {
    }
else
    {
        tauAA
    }
}
p16:gp1
d16
8u
p2 ph2
4u p10:f1
if "$l0==0"
{
}
else
{
    tauAA
}

if "$l0==0"
{
}
else
{
    d40
}

if "$l0==0"
{
    tauBB
}
else
{
    tauBBBB
}

d16
p17:gp2
d16
20u pl0:f1
;
( center (p40:sp40 ph3):f1 (p10:gp10) )
;
20u pl1:f1
d16
p17:gp2
d16
if "$l0==0"
{
    tauBB
}
else
{
    tauBBBB
}

tauD

if "$l0==0"
else
{

}

if "l0==0"
{
    tauC
}
else
{

}

if "l0==0"
{

}
else
{
d40
}

lab2, goto lab7
}

lab7, go=2 ph31
50m mc #0 to 2
    F1QF(calc(l0,1))
    F2QF(calc(l18,1))
exit

ph1 =0 0 1 1 0 0 1 1 0 0 1 1 0 0 1 1
ph2 =0 0 0 0 1 1 1 1 0 0 0 0 1 1 1 1
ph3 =0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1
ph4 =0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1
ph5 =2 2 2 2 3 3 3 3 2 2 2 2 3 3 3 3
ph6 =2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
ph31=0 2 3 1 2 0 1 3 2 0 1 3 0 2 3 1
;POWER LEVEL
;p0 : zero power (0W)
p1 : power level for pulse (default)
spw4 : power level of PSYCHE selective pulse

;PULSE DURATION
p1 : high power 90 pulse width
p2 : high power 180 pulse width
p10 : duration of weak gradient during PSYCHE pulse element
p12 : duration of water excitation sculpting
p40 : duration of double-chirp PSYCHE pulse element

;PULSE SHAPE
spn12 : file name for the 180 water excitation sculpting
spn40 : file name for PSYCHE pulse element

;GRADIENT DURATION
p16 : CTP gradient pulse width
p17 : CTP gradient pulse width
p18 : CTP gradient pulse width

;GRADIENT SHAPE
gpn1 : SINE.100
gpn2 : SINE.100
gpn3 : SINE.100
gpn10 : RECT.1

;GRADIENT STRENGTH
gpz1 : CTP gradient [77%]
gpz2 : CTP gradient [49%]
gpz3 : CTP gradient [63%]
gpz10 : weak gradient during PSYCHE element (1-4%)
gpz10 : weak gradient during PSYCHE element (1-4%)

;DELAES
d1 : relaxation delay; 1-5 * T1
d16 : gradient stabilisation delay
d2 : delay to keep the T2 weighting constant between the pure shift experiments acquired with different evolution time [greater than 1/4SW1+p16+2*d16]

;CONSTANTS
cnst4 : number of points to drop when collecting FID
cnst5 : (td2/2)+1
cnst12 : bandwidth for selective inversion pulse (in Hz)
cnst13 : chemical shift for selective inversion pulse (in ppm)
cnst20 : desired flip angle for PSYCHE pulse element (degree) (normally 10-25)
cnst21 : bandwidth of each chirp in PSYCHE pulse element (Hz) (normally 10000)
;td1: number of chunks to be acquired
;td2: number of different J evolution times to be averaged (N)
;ns: 8 * n, total number of scans
;ds: 8, number of dummy scans
;sw1: sw3/n (n must be an integer number)
;sw2: 2*N*sw1 where N is the steps in the SAPPHIRE suppression (this pulse sequence works when N is an even number)
;2sw3/sw2 should be integer
;in10: 1/sw2
;l8: loop counter for F2 dimension
;l0: loop counter for F1 dimension
;FnMODE1: QF
;FnMODE2: QF

;sapphire_W5
Sideband Averaging with Periodic PHase Incrementation of Residual J Evolution for the acquisition of clean PSYCHE pure shift spectra

Hard 180° pulses were replaced by W5 water-saturation

The pulse sequence involves a 3D acquisition scheme. F3 is the direct dimension. F1 is the incremented dimension for the reconstruction of the pure shift interferogram. F2 is the incremented dimension for the J-evolution.

The data can be reconstructed using the two following AU programs (downloaded from: http://nmr.chemistry.manchester.ac.uk)

1) pm_pshift (produces pure shift spectra for each different J-evolution time, adjusting the length of the first chunk appropriately)

2) pm_fidadd (averages the pure shift spectra acquired with different J-evolution times)

$CLASS=HighRes$DIM=3D$TYPE=

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

define delay tauA
define delay tauAA
define delay tauB
define delay tauBB
define delay tauBBBB
define delay tauC
define delay tauD

"p2=p1*2"

"l0=0"
"l8=0"

"in0=inf1/2"
"in10=inf2"

"d0=inf1/2"
"d10=inf2"
"d30=in0/2"
d40=in0/2-in10

cnst5=(td2/2)+1

tauA=0
tauAA=inf1/4
tauB=d2-p17-2*d16-20u
tauBB=d2-p17-2*d16-20u-inf2
tauBBBB=d2-p17-2*d16-20u-inf2
tauC=inf2
tauD=(dw*2+cnst4)

cnst50=(cnst20/360)*sqrt((2*cnst21)/(p40/200000))
p30=1000000.0/(cnst50*4)

cnst31= (p30/p1) * (p30/p1)

spw40=plw1/cnst31

p10=p40

d11=d11+cnst7*d11-cnst7*d11
d11=d11+cnst29*d11-cnst29*d11
cnst28=01
cnst30=(cnst29*bf1)-cnst28
cnst27=0

p27=1000000.0/(cnst6*4)
cnst46= (p27/p1) * (p27/p1)

plw18=plw1/cnst46

cnst7=18*d19+5.95*p27+4*d12

d11=30m
d12=20u
acqt0=0

baseopt_echo

aqseq 312
1 ze
2 50m
d1 pl1:f1
3 50u UNBLKGRAD

if "l8 < cnst5"
{
  "tauA=(l8*in10)"
  "tauB=d2-p17-2*d16-20u-(l8*in10)"
  "d30=(in0/2+(l8*in10))+(l0-1)*in0"
  "tauBBBB=(d2-p17-2*d16-20u)-(l8*in10)"

  p1 ph1
if "l0==0"
{
    tauA
}
else
{
    tauAA
}
p16:gp1
d16 p118:f1
d12 fq=cnst30:f1
d12
p27*0.087 ph2
d19*2
p27*0.206 ph2
d19*2
p27*0.413 ph2
d19*2
p27*0.778 ph2
d19*2
p27*1.491 ph2
d19*2
p27*1.491 ph3
d19*2
p27*0.778 ph3
d19*2
p27*0.413 ph3
d19*2
p27*0.206 ph3
d19*2
p27*0.087 ph3
d12 fq=cnst27:f1
d12 p11:f1
p16:gp1
d16

if "l0==0"
{
    tauA
}
else
{
    tauAA
}

if "l0==0"
{
}
else
{
    d30
}
if "$\text{lo}==0$"
{
    tauB
}
else
{
    tauBBB
}

d16
p17:gp2
d16
20u pl0:f1
;
( center (p40:sp40 ph4):f1 (p10:gp10) )
;
20u pl1:f1
d16
p17:gp2
d16

if "$\text{lo}==0$"
{
    tauB
}
else
{
    tauBBB
}

tauD
p18:gp3
d16 p118:f1
d12 fq=cnst30:f1
d12
p27*0.087 ph5
d19*2
p27*0.206 ph5
d19*2
p27*0.413 ph5
d19*2
p27*0.778 ph5
d19*2
p27*1.491 ph5
d19*2
p27*1.491 ph6
d19*2
p27*0.778 ph6
d19*2
p27*0.413 ph6
d19*2
p27*0.206 ph6
d19*2
if "l0==0"
{
}
else
{
d30
}
lab1, goto lab7
}
else
{
"tauC=(in10)+((l8-cnst5)*in10)"
"tauBB=(d2-p17-2*d16-20u-in10)-((l8-cnst5)*in10)"
"d40=((in0/2-in10)-((l8-cnst5)*in10))+(l0-1)*in0"
"tauBBBB=(d2-p17-2*d16-20u+in10)+((l8-cnst5)*in10)"
p1 ph1
if "l0==0"
{
}
else
{
  tauAA
}
p16:gp1
d16 p118:f1
d12 fq=cnst30:f1
d12
p27*0.087 ph2
d19*2
p27*0.206 ph2
d19*2
p27*0.413 ph2
d19*2
p27*0.778 ph2
d19*2
p27*1.491 ph2
d19*2
p27*1.491 ph3
d19*2
p27*0.778 ph3
d19*2
if "l0==0"
{
}
else
{
    tauAA
}
if "l0==0"
{
}
else
{
    d40
}
if "l0==0"
{
    tauBB
}
else
{
    tauBBBB
}
d16
p17:gp2
d16
20u pl0:f1;
( center (p40:sp40 ph4):f1 (p10:gp10) )
;
20u pl1:f1
d16
p17:gp2
d16
if "l0==0"
{
    tauBB
}
else
{
    tauBBBB
}
\begin{verbatim}

\textcolor{red}{\textbf{tauD}}

\textcolor{blue}{\textbf{if}} "\textcolor{red}{l0==0}" \\
\{ \\
  \textcolor{green}{\textbf{tauC}} \\
\}

\textcolor{blue}{\textbf{else}} \\
\{ \\
\}

\textcolor{blue}{p18:gp3} \\
\textcolor{blue}{d16 p118:f1} \\
\textcolor{blue}{d12 fq=cnst30:f1} \\
\textcolor{blue}{d12} \\
\textcolor{blue}{p27*0.087 ph5} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*0.206 ph5} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*0.413 ph5} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*0.778 ph5} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*1.491 ph5} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*1.491 ph6} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*0.778 ph6} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*0.413 ph6} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*0.206 ph6} \\
\textcolor{blue}{d19*2} \\
\textcolor{blue}{p27*0.087 ph6} \\
\textcolor{blue}{d12 fq=cnst27:f1} \\
\textcolor{blue}{d12 p11:f1} \\
\textcolor{blue}{p18:gp3} \\
\textcolor{blue}{d16 BLKGRAD} \\
\textcolor{blue}{if} "\textcolor{red}{l0==0}" \\
\{ \\
  \textcolor{green}{\textbf{tauC}} \\
\}

\textcolor{blue}{\textbf{else}} \\
\{ \\
\}

\textcolor{blue}{if} "\textcolor{red}{l0==0}" \\
\{ \\
\}

\textcolor{blue}{\textbf{else}} \\
\{ \\
\}
\end{verbatim}
lab2, goto lab7
}
lab7, go=2 ph31
50m mc #0 to 2
  F1QF(calc1c(l0,1))
  F2QF(calc1c(l8,1))
exit

ph1 =0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2 0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2 2 2
ph2 =0 0 1 1 0 1 1 0 0 1 1 0 1 1 0 0 1 1 0 0 1 1 0 0 1 1 0 0 1 1 0 0 1 1
ph3 =2 2 3 3 2 2 3 3 2 2 3 3 2 2 3 3 2 2 3 3 2 2 3 3 2 2 3 3 2 2 3 3 2 2
ph4 =0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1
ph5 =0 0 0 0 1 1 1 1 0 0 0 0 1 1 1 1 0 0 0 0 1 1 1 1 0 0 0 0 1 1 1 1 0 0
ph6 =2 2 2 2 3 3 3 3 2 2 2 2 3 3 3 3 2 2 2 2 3 3 3 3 2 2 2 2 3 3 3 3 2 2
ph31=0 2 2 0 2 0 2 2 0 0 2 0 2 2 0 0 2 0 2 2 0 0 2 0 2 2 0 0 2 0 2 2 0

;POWER LEVEL
;pl0 : zero power (0W)
;pl1 : power level for pulse (default)
;pl18 : power level for 3-9-19-pulse (watergate)
;spw40 : power level of PSYCHE selective pulse

;PULSE DURATION
;p1: high power 90 pulse width
;p2: high power 180 pulse width
;p10 : duration of weak gradient during PSYCHE pulse element
;p27 : 90 degree pulse at pl18
;p40 : duration of double-chirp PSYCHE pulse element

;PULSE SHAPE
;spnam40: file name for PSYCHE pulse element

;GRADIENT DURATION
;p16: CTP gradient pulse width
;p17: CTP gradient pulse width
;p18: CTP gradient pulse width

;GRADIENT SHAPE
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam3: SINE.100
;gpnam10: RECT.1

;GRADIENT STRENGTH
;gpz1 : CTP gradient [77%]
;gpz2 : CTP gradient [49%]
;gpz3 : CTP gradient [63%]
;gpz10 : weak gradient during PSYCHE element (1-4%)

;DELAYS
;d1: relaxation delay; 1-5 * T1
;d16: gradient stabilisation delay
;d2: delay to keep the T2 weighting constant between the pure shift experiments acquired with different evolution time [greater than 1/4SW1+p16+2*d16]
;d19: delay for binomial water suppression
; d19 = (1/(2*cnst10)), d = distance of next null (in Hz)

;CONSTANTS
;cnst4: number of points to drop when collecting FID
;cnst5: (td2/2)+1
;cnst6: bandwidth of WATERGATE transfer [10k - 20k Hz]
;cnst10: distance of next null (in Hz) [2500-5000 Hz]
;cnst17: duration of W5 pulse (in sec)
;cnst20: desired flip angle for PSYCHE pulse element (degree) (normally 10-25)
;cnst21: bandwidth of each chirp in PSYCHE pulse element (Hz) (normally 10000)
;cnst29: solvent frequency (in ppm)

;OTHERS
;td1: number of chunks to be acquired
;td2: number of different J evolution times to be averaged (N)
;ns: 8 * n, total number of scans
;ds: 8, number of dummy scans
;sw1: sw3/n (n must be an integer number)
;sw2: 2*N*sw1 where N is the steps in the SAPPHIRE suppression (this pulse sequence works when N is an even number)
;2sw3/sw2 should be integer
;in10: 1/sw2
;l8: loop counter for F2 dimension
;l0: loop counter for F1 dimension
;FnMODE1: QF
;FnMODE2: QF

REFERENCES