Electronic Supporting Information

Recovering sensitivity lost through convection in pure shift NMR

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1 Experimental details

1.1 NMR experimental details

The helium-cooled probe used for these experiments was a Bruker TCI cryoprobe equipped with a z-gradient coil with a maximum nominal gradient strength of 53 G cm–1, fitted to a 500 MHz NEO spectrometer (PRISM core facility, Biogenouest, Univ Rennes, Univ Angers, INRAE, CNRS, FRANCE). The cooling unit for temperature regulation was a Bruker BCU 05. Experiments were performed using the Bruker Topspin (v4.2.0) software package.

The nitrogen-cooled probe used for these experiments was a Bruker Prodigy BBFO 5 mm cryoprobe equipped with a z-gradient coil with a maximum nominal gradient strength of 66 G cm^{-1}, fitted to a Bruker AVIII HD 500 MHz spectrometer (Department of Chemistry, University of Manchester, UK). The cooling unit for temperature regulation was a Bruker BCU II, set on low power. Experiments were performed using the Bruker Topspin (v3.6.2) software package.

The room-temperature probe used for these experiments was a Bruker BBO probe equipped with a z-gradient coil with a maximum nominal gradient strength of 53 G cm^{-1}, fitted to a 500 MHz AV III HD spectrometer (PRISM core facility, Biogenouest, Univ Rennes, Univ Angers, INRAE, CNRS, FRANCE). The cooling unit for temperature regulation was a Bruker BCU II, set on low power. Experiments were performed using the Bruker Topspin (v3.6.1) software package.

1D ^1H pure shift NMR spectra were recorded using the sequences shown in Figure 1 of the main text, with 5 kHz spectral width, 16 t_1 increments, and 4096 complex points. A chunk duration of 20 ms was used in all pure shift experiments. Active spin refocussing (ASR) pulse sequence elements and gradients were different for band-selective, Zangger-Sterk and PSYCHE experiments, and are described in the relevant captions. Coherence transfer pathway (CTP)-enforcing gradient pulses G_1 and G_2 were half-sine shaped, with 1 ms duration followed by a 1 ms recovery delay. All data were processed using the reconstruction macro pshift4f (available from https://www.nmr.chemistry.manchester.ac.uk/) in Topspin for interferogram construction, with zero-filling, Gaussian apodization and Fourier transformation. The resulting 1D pure shift spectra were then opened in MNova software and signal intensities were obtained from the MNova Data Analysis module.

In the following, 'gradient' will be used as shorthand for 'pulsed field gradient'.

1.2 Strychnine sample

The sample was prepared by dissolving 22.5 mg of commercial strychnine in 0.5 mL of $CDCl_3$ to give approximately 150 mM final concentration. The 5 mm tube was sealed to avoid solvent evaporation or sample degradation.

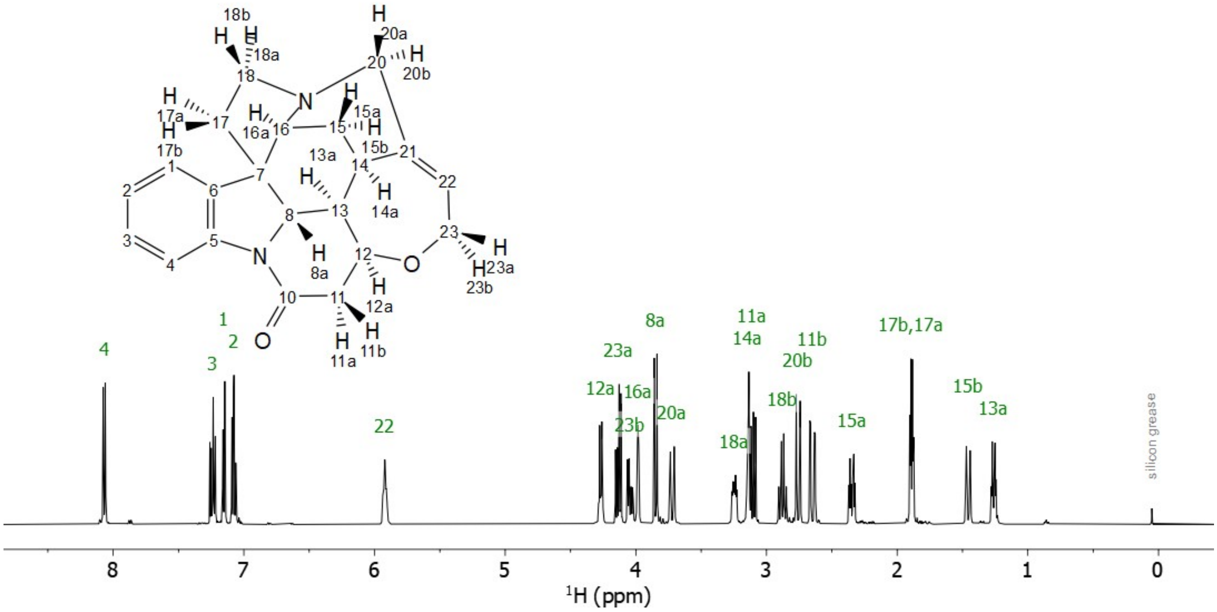


Figure S1: 500 MHz ^{1}H NMR spectrum of strychnine in $CDCl_3$ showing assignments.

Table S1 . Assignments of ^{1}H signals of strychnine sample, obtained from routine structural elucidation experiments (^{1}H, ^{13}C{^{1}H}, edited-HSQC, COSY, NOESY and HMBC).

^{1}H label	Chemical shift (ppm)	Multiplicity	J_{HH} (Hz)
4	8.07	dt	0.85, 0.85, 8.04
3	7.24	ddd	1.37, 7.35, 8.23
1	7.15	dd	1.38, 7.62
2	7.08	td	1.08, 7.47, 7.48
22	5.92	tt	1.93, 1.93, 5.18, 5.18
12a	4.27	dt	3.28, 3.28, 8.40
23a	4.13	dd	6.90, 13.80
23b	4.05	m	
16a	3.98	dd	2.15, 4.04
8a	3.85	d	10.48
20a	3.72	dq	1.67, 1.67, 1.66, 14.66
18a	3.25	ddd	3.10, 4.88, 10.21
14a	3.14	s	
11a	3.11	dd	8.51, 17.48
18b	2.88	td	8.51, 10.24, 10.10
20b	2.76	d	14.73
11b	2.65	dd	3.33, 17.41
15a	2.35	dt	4.36, 4.36, 14.49
17a,17b	1.89	m	
15b	1.46	dt	2.10, 2.10, 14.59
13a	1.26	dt	3.23, 3.23, 10.53

2 Convection measurements

2.1 Pulse Sequence

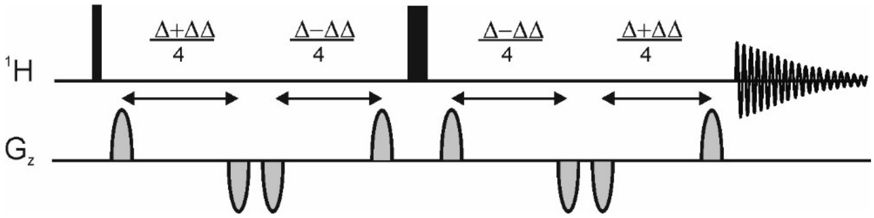


Figure S2: Convection compensated spin-echo sequence adapted, by the inclusion of a delay imbalance ΔΔ, for the measurement of convection. The narrow and wide rectangles represent hard 90° and 180° radiofrequency pulses, respectively. Gradient pulses are half-sine shaped, of 1 ms duration followed by a 0.5 ms recovery delay, and G_1 is 90% of the nominal maximum gradient of 53.5 G cm^{-1}.

2.2 Convection measurement by NMR

In order to measure the rate of convection in a sample it is usual to adapt a convection-compensated pulse sequence designed for diffusion measurements. The sequence of Figure S2 is designed to cancel the effects of constant flow when the diffusion delay imbalance ΔΔ is zero, allowing diffusion-weighted data to be acquired without interference from convection. When ΔΔ is non-zero, the signals from species with magnetogyric ratio γ, moving with constant z velocity v acquire a phase shift $-\gamma\delta G_1 v\Delta\Delta$ radians, so by varying ΔΔ it is possible to map out the Fourier transform of the velocity distribution.

A common approximation used in convection measurement by NMR is to assume a rectangular distribution of signal as a function of flow velocity, with signal constant from velocity $-v_{max}$ to $+v_{max}$ and zero outside this range. This yields a signal amplitude attenuation as a function of delay imbalance, $S(\Delta\Delta)$, that is a *sinc* (sin[x]/x) function:

$$S(\Delta\Delta) = S_0 \sin\left(\gamma\delta G A_G v_{max}\Delta\Delta\right)/\left(\gamma\delta G A_G v_{max}\Delta\Delta\right) \qquad \text{(S1)}$$

A more realistic model for the velocity distribution is to use the theoretical form for the convective flow driven by a horizontal temperature gradient,[1] leading to:

$$S(\Delta\Delta) = 2\int_0^1 rJ_0\left(k\alpha r\left[1-r^2\right]\right)dr\,dk \qquad \text{(S2)}$$

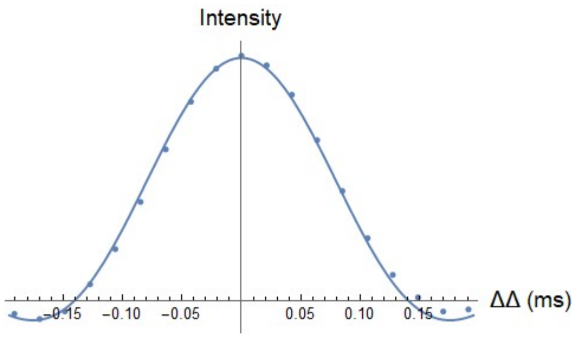
with $k = \gamma\, A_G\, G\, \delta\, \Delta\Delta$ and $\alpha = \frac{3\sqrt{3}}{2} v_{max}$

where J_0 is a Bessel function of the first kind, *r* is the tube radius, γ is the magnetogyric ratio, *G* is the gradient amplitude, A_G is the gradient pulse shape factor (2/π for half-sine pulses), v_{max} is the maximum convection velocity and ΔΔ is the delay imbalance. Fitting experimental data to Equation S2 allows v_{max} to be determined. (The Mathematica notebook used is freely available at https://doi.org/10.48420/23864202).

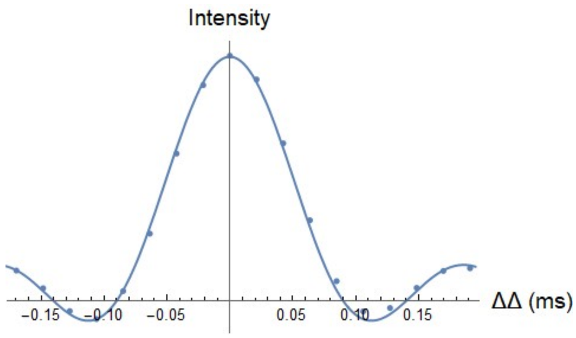
[1] I. Swan, M. Reid, P. W. A. Howe, M. A. Connell, M. Nilsson, M. A. Moore and G. A. Morris, *J. Magn. Reson.*, **2015**, *252*, 120–129.

2.3 Helium-cooled probe

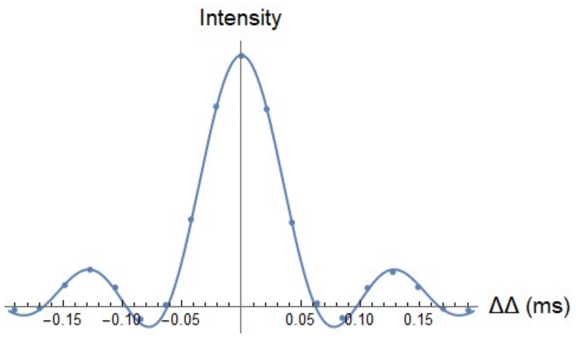
Convection depends on the conditions used for temperature regulation. With regulation at a nominal temperature of 288 K, convection rate was measured using different values of the variable temperature (VT) regulation gas flow rate.



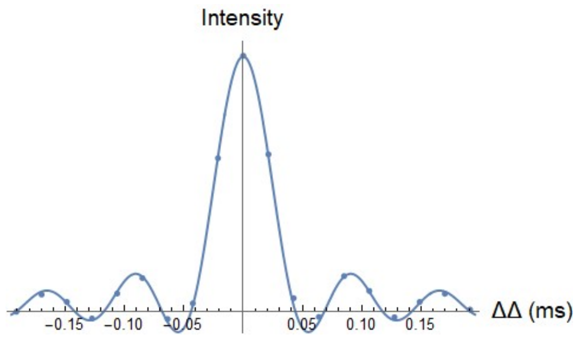
800 lph
Fitted ν_{max} = 0.33 mm s^{-1}



700 lph
Fitted ν_{max} = 0.51 mm s^{-1}



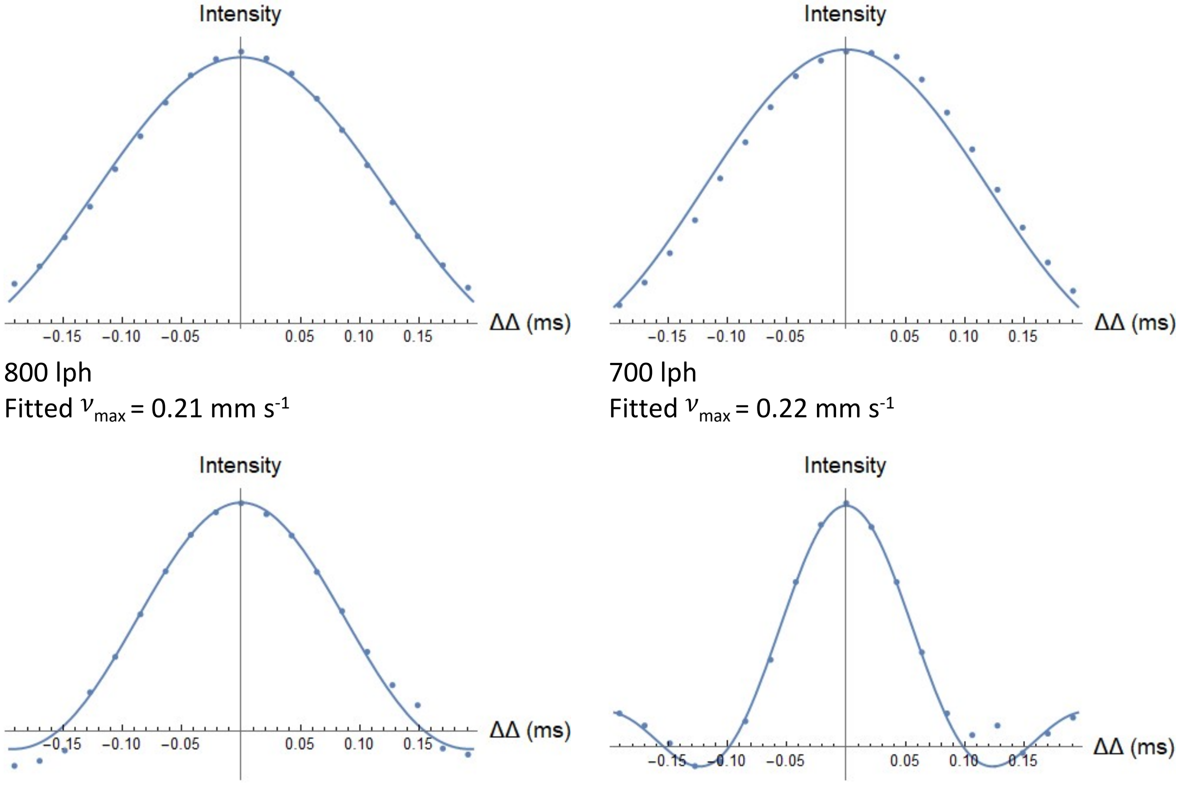
600 lph
Fitted ν_{max} = 0.74 mm s^{-1}



500 lph
Fitted ν_{max} = 1.05 mm s^{-1}

Figure S3: Normalized intensity of the signal at 4.18 ppm (left hand doublet of the 23a ^{1}H dd) in the NMR spectrum of strychnine plotted as a function of the imbalance delay ΔΔ / s. The signals were measured using the convection-compensated spin-echo sequence of Figure S2 for different thermal conditions on a helium-cooled TCI probe (total diffusion delay Δ = 200 ms, 1 ms gradient pulses of 90% nominal strength). All spectra of each set were similarly phased and 2 Hz line-broadening was used. Experimental peak intensities are plotted as blue points and were fitted to Equation S2 in Mathematica.

2.4 Room temperature BBO probe



800 lph
Fitted ν_{max} = 0.21 mm s^{-1}

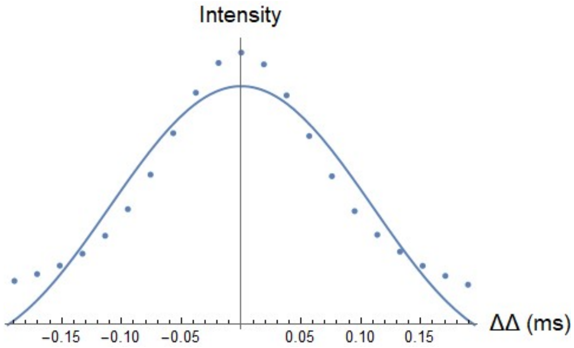
700 lph
Fitted ν_{max} = 0.22 mm s^{-1}

600 lph
Fitted ν_{max} = 0.30 mm s^{-1}

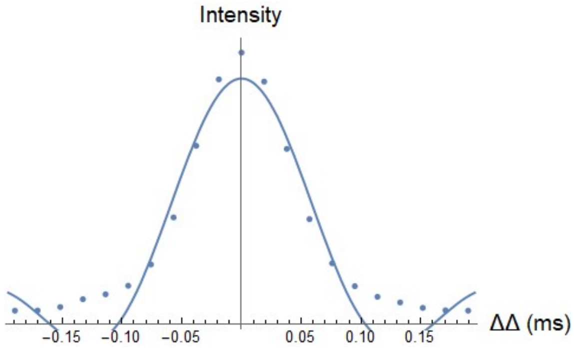
500 lph
Fitted ν_{max} = 0.47 mm s^{-1}

Figure S4: Normalized intensity of the signal at 4.18 ppm (left hand doublet of the 23a ^{1}H dd) in the NMR spectrum of strychnine plotted as a function of the imbalance delay ΔΔ / s. The signals were measured using the convection-compensated spin-echo sequence of Figure S2 for different thermal conditions on room-temperature BBO probe (total diffusion delay Δ = 200 ms, 1 ms gradient pulses of 90% nominal strength). All spectra of each set were similarly phased and 2 Hz line-broadening was used. Experimental peak intensities are plotted as blue points and were fitted to Equation S2 in Mathematica.

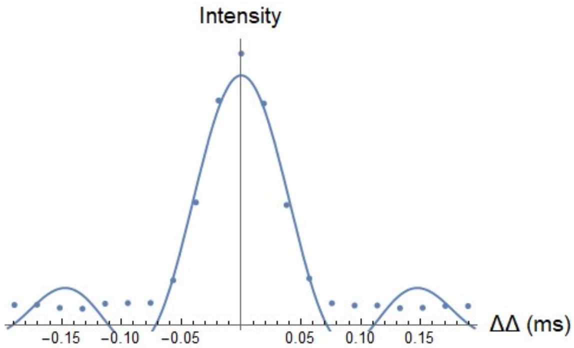
2.5 Nitrogen-cooled probe



500 lph
Fitted ν_{max} = 0.19 mm s^{-1}



400 lph
Fitted ν_{max} = 0.36 mm s^{-1}



300 lph
Fitted ν_{max} = 0.52 mm s^{-1}

Figure S5: Normalized intensity of the signal at 3.89 ppm (8a ^{1}H d) in the NMR spectrum of strychnine plotted as a function of the imbalance delay ΔΔ / s. The signals were measured using the convection compensated spin-echo sequence of Figure S2 for different thermal conditions on a nitrogen-cooled Prodigy probe (total diffusion delay Δ = 177.5 ms, 1 ms gradient pulses of 90% nominal strength). All spectra of each set were similarly phased and 2 Hz line-broadening was used. Experimental peak intensities are plotted as blue points and were fitted to Equation S2 in Mathematica.

Experimental data obtained for helium-cooled and room-temperature probes agree very well with to the Hadley convection model (see Section 2.2), driven by horizontal temperature gradients. For the nitrogen-cooled probe, however, the experimental data suggest the presence of more complicated convective flow, presumably as a consequence of the probe design.

3 Convection measurement using Zangger-Sterk experiments

Since the signal intensity obtained in Zangger-Sterk experiments is strongly affected by convection, data were collected for different temperature regulation conditions using three different probes and the results fitted for v_{max}.

3.1 Helium-cooled probe

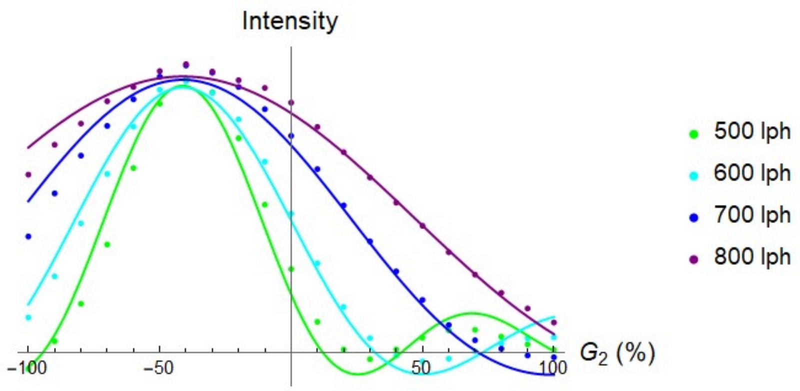


Figure S6: Intensity in arbitrary units of the pure shift H15a signal of strychnine as a function of the percentage gradient strength G_2 (100% is a nominal gradient of 53 $G.cm^{-1}$). Spectra were measured at 288 K with different gas flow rates using the pulse sequence of section 8, with a 74 ms duration rSNOB selective pulse and a spatial encoding gradient G_0 of +1.5 %. Experimental data are plotted as points, and the solid lines were obtained by fitting the experimental data to the attenuation calculated for this pulse sequence for Hadley convection in an infinite cylinder, with v_{max} as the only adjustable parameter.

3.2 Room-temperature probe

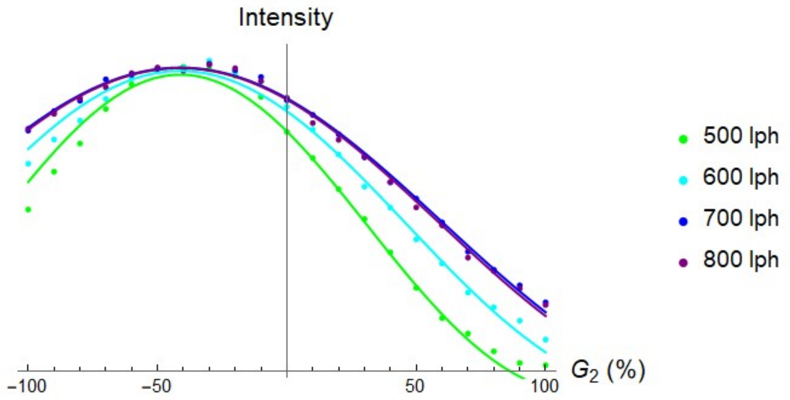


Figure S7: Intensity in arbitrary unit of the pure shift H15a signal of strychnine as a function of the percentage gradient strength G_2 (100% is a nominal gradient of 53 $G.cm^{-1}$). Spectra were measured at 288K with different gas flow rates using the pulse sequence of section 8, with a 74 ms duration rSNOB selective pulse and a spatial encoding gradient G_0 of +1.5 %. Experimental data are plotted as points, and the solid lines were obtained by fitting the experimental data to the attenuation calculated for this pulse sequence for Hadley convection in an infinite cylinder, with v_{max} the only adjustable parameter.

3.3 Nitrogen-cooled probe

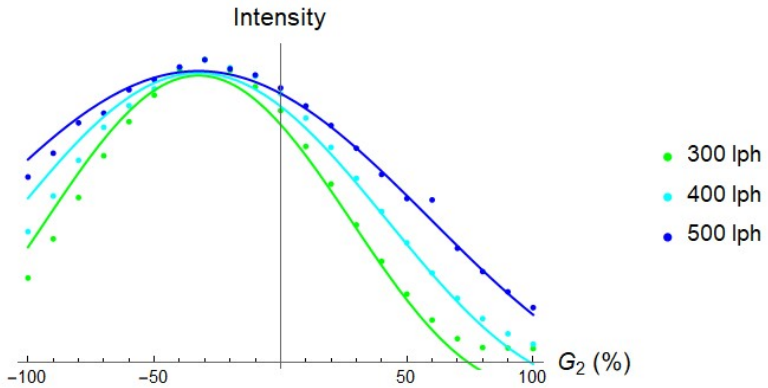


Figure S8: : Intensity in arbitrary unit of the pure shift H15a signal of strychnine as a function of the percentage gradient strength G_2 (100% is a nominal gradient of 66 G.cm^{-1}). Spectra were measured at 288K with different gas flow rates using the pulse sequence of section 8, with a 59.7 ms duration rSNOB selective pulse and a spatial encoding gradient G_0 of +1.5 %. Experimental data are plotted as points, and the solid lines were obtained by fitting the experimental data to the attenuation calculated for this pulse sequence for Hadley convection in an infinite cylinder, with v_{max} the only adjustable parameter.

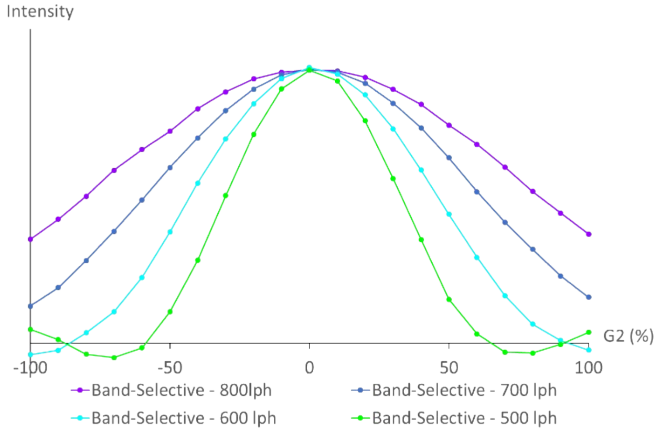
4 Comparison of convection v_{max} values obtained from spin echo and pure shift measurements

Table S2: Maximum velocity (v_{max}) measured in strychnine-$CDCl_3$ sample.

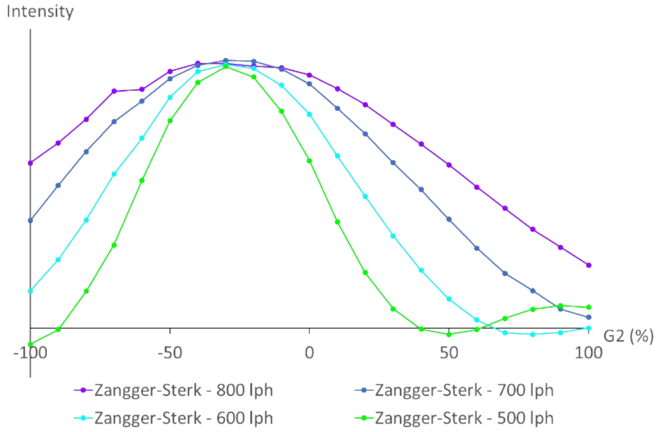
Probe	**Temperature** (K)	**Gasflow** (lph)	v_{max} **(mm s^{-1})** determined using the sequence shown in Figure S2	v_{max} **(mm s^{-1})** obtained from the variation in pure shift signal with G_2 gradient strength
Helium-cooled	288	500	1.05	1.01
		600	0.74	0.74
		700	0.51	0.48
		800	0.33	0.35
Room-temperature	288	500	0.47	0.42
		600	0.30	0.35
		700	0.22	0.30
		800	0.21	0.31
Nitrogen-cooled	288	300	0.52	0.50
		400	0.36	0.41
		500	0.19	0.33

5 Comparison of different pure shift schemes with the same ASR duration, for a helium-cooled probe

a- Band Selective



b- Zangger-Sterk



c- PSYCHE

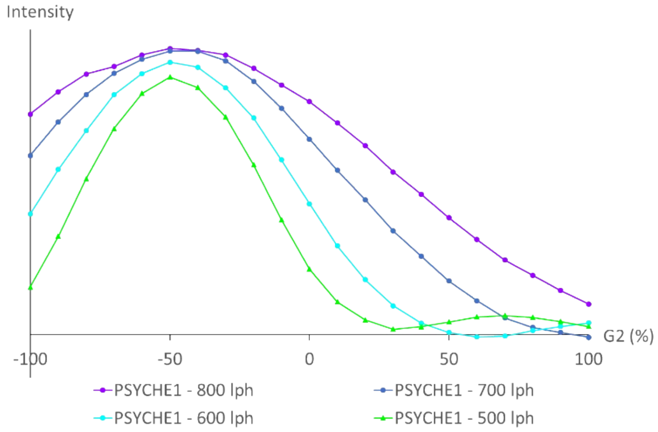
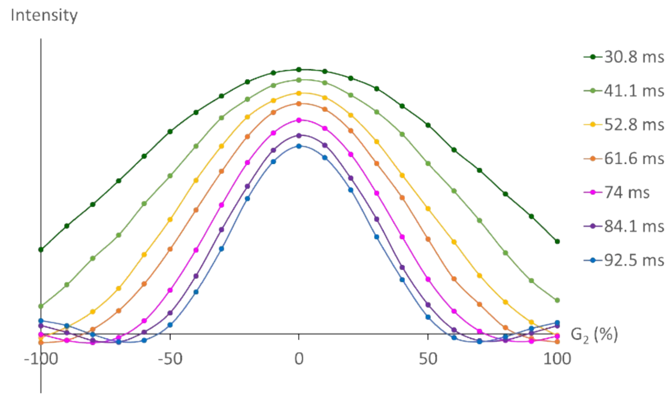


Figure S9: Intensity in arbitrary units of the pure shift H15a signal of strychnine as a function of the percentage gradient strength G_2. Spectra were measured on a helium-cooled TCI probe at 288 K with different gas flow rates using the pulse sequence of section 8 at 288 K with different gas flow rates with a) band-selective (59.7 ms rSNOB selective pulse of 31 Hz selectivity), b) Zangger-Sterk s (59.7 ms rSNOB selective pulse of 31 Hz bandwidth with +1.5% spatial encoding gradient G_0), and c) PSYCHE sequence (two 30 ms CHIRP frequency-swept pulses of 10 kHz sweep width with +3.0% gradient G_0).
NB: absolute intensities are very different from one type of pure shift experiment to the other, band-selective experiments offer nearly 100% sensitivity, whereas Zangger-Sterk and PSYCHE retain about 5% or 15% of the original ^{1}H signal respectively.

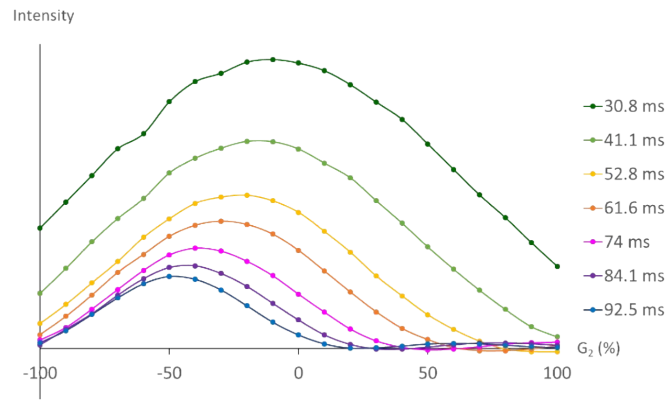
6 Effect of varying ASR duration, for a helium-cooled probe

Flow encoding occurs mainly during the second echo of pure shift experiments, since the ASR elements needs to be long enough to achieve active spin selection. Figure S10 shows the combined effects of relaxation and convection during the ASR for three different ASRs.

d- Band Selective



e- Zangger-Sterk



f- PSYCHE

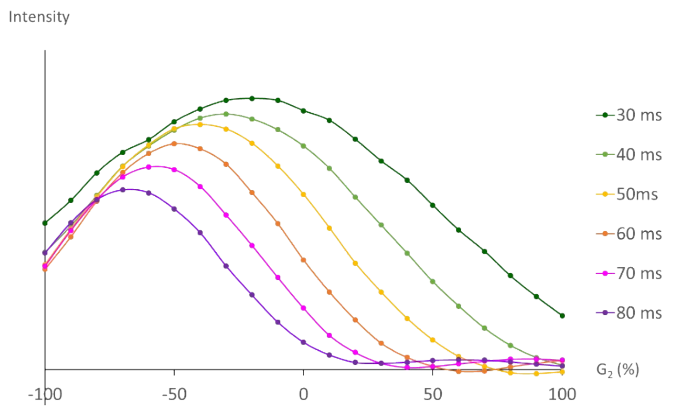


Figure S10 Intensity in arbitrary units of the pure shift H15a signal of strychnine as a function of the percentage gradient strength G_2. Spectra were measured on a helium-cooled TCI probe at 288 K with different gas flow rates using the pulse sequence of section 8 at 288 K with a gas flow rate of 600 lph using a) band-selective (rSNOB selective pulse of varying durations), b) Zangger-Sterk (rSNOB selective pulse of varying durations with +1.5% spatial encoding gradient G_0), and c) PSYCHE sequence (two Chirp frequency swept pulse of 10 kHz bandwidth and varying durations – duration mentioned in the legend is the total duration - with +3.0% spatial encoding gradient G_0).

7 Bruker pulse sequences with automatic calculation of gradient G_2 strength (GPZ2)

7.1 1D pure shift Zangger-Sterk pulse sequence with convection compensation.

```
;broadband pure shift NMR using
;interferogram Zangger-Sterk method
;automatic calculation of GPZ2 gradient strength for convection compensation

; Developed by Manchester NMR Methodology group
; Departement of Chemistry, University of Manchester, United Kingdom
; April 2023 - Elsa Caytan and Howard M. Foster
;
;References:
; K. Zangger and H. Sterk J Magn Reson, 1997, 124, 486-489.
; Juan A. Aguilar, Stephen Faulkner, Mathias Nilsson, and Gareth A. Morris Angew. Chem. Int. Ed. 2010,
49, 3901-3903.

;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

;cnst50: bandwidth of the selective refocusing pulse [Hz]
;userA1: waveform for selective refocusing [rsnob/reburp]
;sp12(p12):wvm:ec_ZS:f1 userA1(cnst50 Hz; NPOINTS=1000)


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


define delay tauA
define delay tauD
define delay denominator


"if (d18 < 0.000001) {d18=0.000035;}"
"if (d19 < 0.000001) {d19=0.000020;}"

"p2=p1*2"
"d11=30m+1s/(1+cnst50)"
"d11=30m"

"d0=0"
"in0=inf1/2"

"if (d16 < 0.00011) {d16=0.0001;}"
```

```
"d17=d16-50u"

"tauA=in0/2-p16-d16"
"if (tauA < 0.00001) {tauA=0.00001;}"

"tauD=de+p1*2/3.1416+(dw*2*cnst4)"

"denominator=2.0*(p16/1e6)*(d16+p12+p16)"
"gpz2=0.01*((-
1.0*gpz0*(p12/2e6)*PI*(p12/2e6))+(2.0*d16*gpz1*p16/1e6)+(2.0*gpz1*(p16/1e6)*(p16/1e6)))/den
ominator"

"acqt0=0"
baseopt_echo


1 ze
2 d11
  50u BLKGRAMP
  50u LOCKH_OFF
  d1 pl1:f1
  50u LOCKH_ON
  50u UNBLKGRAMP

3 p1 ph1
  d0
  tauA
  p16:gp1
  d16
  p2 ph2
  p16:gp1
  d16
  tauA

  tauD

  p16:gp2
  d16

  d18
  d19 gron0
  (p12:sp12 ph3):f1
  d18
        d19 groff

  p16:gp2
  50u
  d17 BLKGRAMP
  d0

  go=2 ph31
  d11 mc #0 to 2 F1QF(id0)
```

```
 50u LOCKH_OFF
exit

; ns =   2    4       8             16                          32                                                   64
 ph1 = 0 2  0 2  0 2 0 2  1 3 1 3 1 3 1 3  0 2 0 2 0 2 0 2 1 3 1 3 1 3 1 3
 ph2 = 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   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
 ph3 = 0 0  1 1  0 0 1 1  0 0 1 1 0 0 1 1  2 2 3 3 2 2 3 3 2 2 3 3 2 2 3 3
ph31 = 0 2  2 0  2 0 0 2  1 3 3 1 3 1 1 3  0 2 2 0 2 0 0 2 1 3 3 1 3 1 1 3


;pl1 : high power (dB)
;p1  : 90 degree high power pulse
;p16 : duration of CTP gradients [0.5ms-1ms]
;p12 : duration of ASR pulse
;d0  : incremented delay
;d1  : relaxation delay
;d16 : recovery delay for gradients [1ms]
;d18 : gradient group delay adjustment [20-200us]
;d19 : gradient group delay adjustment [20-200us]
;gpz1: CTP gradient (47%)
;gpz2: CTP gradient, will be set to the optimal value (31%)
;gpz0: weak gradient during ASR [1-3%]
;userA1: waveform of ASR pulse [rsnob/reburp]
;gpnam1: shape of CTP gradient [SINE.100]
;gpnam2: shape of CTP gradient [SINE.100]
;cnst4: number of drop points [4*i, i=1,2,...]
;cnst50: bandwidth of the ASR pulse in Hz
;in0 : 1/(2 * SW) = DW
;td1 : number of chunks
;FnMODE: QF(no-frequency)
;ns : 2*n
;ds: 4*m
;for processing data used the pshift4f macro
```