2.1.

A. $\delta_{water\text{-}NAA}$ = 801 Hz/300 Hz/ppm = 2.67 ppm, such that T = 309.9 K

B. $\delta_{water\text{-}NAA}$ = (11 + 796) Hz/300 Hz/ppm = 2.69 ppm, such that T = 308.0 K

2.2.

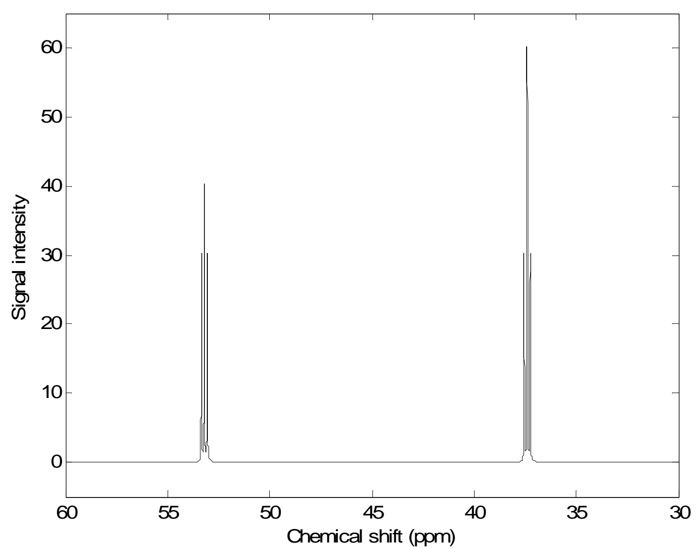
- Comparison to an *in vitro* NMR spectrum from an isolated 'candidate' compound.
- Modulating the 'candidate' compound by physiological intervention (e.g. the concentration of GABA can be increased by anti-epileptic drugs).
- Varying the echo-time (uncoupled spins do not modulate and can be seen at longer echo-times)
- Spectral editing
- Two-dimensional correlation spectroscopy (COSY)
- Two-dimensional spin-echo spectroscopy (JRES)
- High-resolution NMR, MS or biochemical assays on brain tissue extract.

2.3.

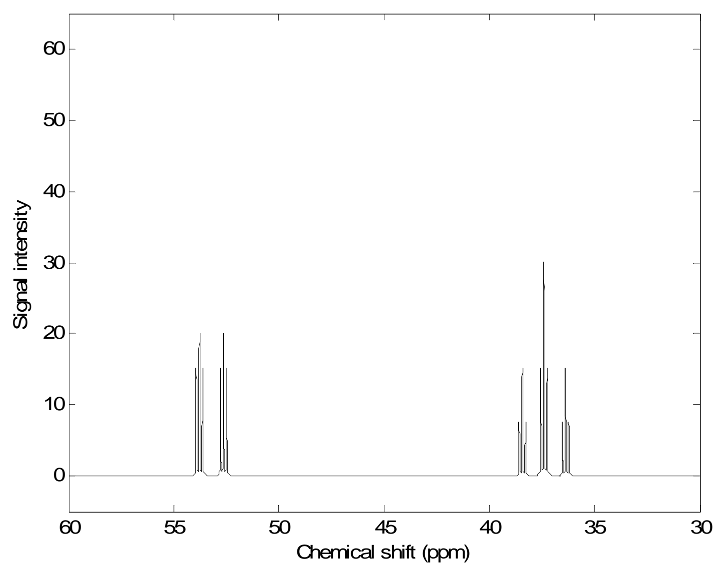
- Phosphorylcholine and glycerophosphoryl can be detected separately with ^{31}P NMR.
- While the large methyl singlet resonances overlap at 3.22 ppm, the smaller methylene groups do not overlap and can be detected separately in high-resolution or two-dimensional NMR spectra.
- Small differences in ^{13}C NMR chemical shifts also allow separation of the three compounds.

2.4. pK_A = 6.77, δ_A = 5.70 ppm δ_{HA} = 3.23 ppm, such that pH = 6.88 ppm.

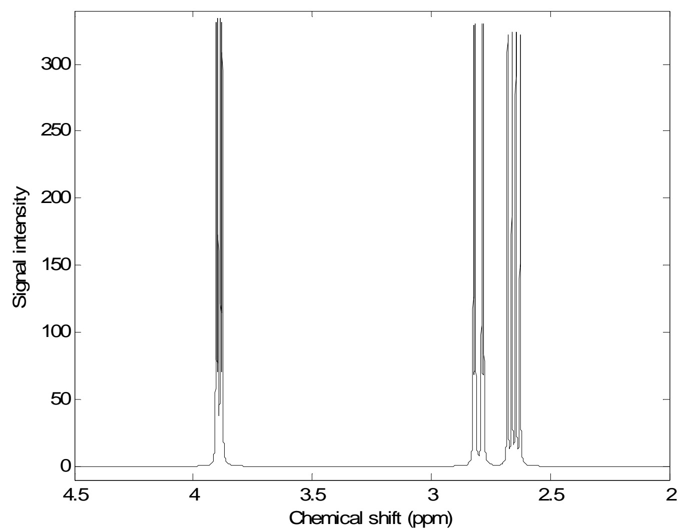
2.5.A. ^{13}C NMR spectrum with ^{1}H decoupling.



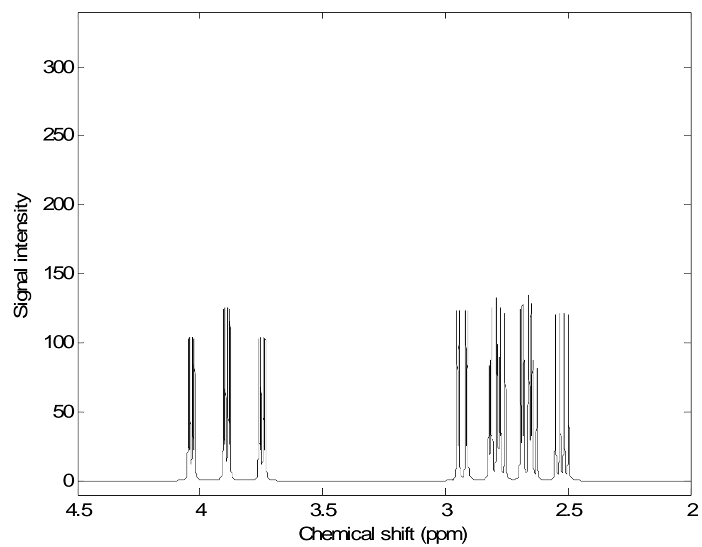
B. ^{13}C NMR spectrum without ^{1}H decoupling.



C. ${}^{1}H$ NMR spectrum with ${}^{13}C$ decoupling.



${}^{1}H$ NMR spectrum without ${}^{13}C$ decoupling.



2.6. The general expression for the longitudinal magnetization following a double inversion recovery is given by:

$$M_z(TR, TI1, TI2) = M_0 - M_0[2-(2-e^{-TR/T_1})e^{-TI1/T_1}]e^{-TI2/T_1}$$

Therefore the signal recovery for metabolites and macromolecules are given by:

$M_z(T_1 = 1250 \text{ ms}) = 0.0086\ M_0$

$M_z(T_1 = 1500 \text{ ms}) = -0.0086\ M_0$

$M_z(T_1 = 1750 \text{ ms}) = -0.0079\ M_0$

$M_z(T_1 = 300 \text{ ms}) = 0.7553\ M_0$

$M_z(T_1 = 400 \text{ ms}) = 0.5882\ M_0$

$M_z(T_1 = 500 \text{ ms}) = 0.4412\ M_0$

Therefore, even in the worst case ($M_z(T_1 = 500 \text{ ms})/M_z(T_1 = 1250 \text{ ms})$) the metabolites are suppressed > 50× relative to the macromolecules resonances. There is however a spread in the macromolecular recovery efficiency that is dependent on the T_1 relaxation time.

2.7.A. Differences in choline and creatine T_2 relaxation can not explain the observed ratio, as the tCho/tCr ratio should increase at longer echo-times. Furthermore, the decay of macromolecular resonances or the appearance of an anti-phase GABA resonance underneath the creatine resonance can also not explain the observed ratio. The decreased ratio is most likely not caused by an increase in the creatine signal, leaving the only other option in that choline must have decreased relative to creatine. The key point to the answer is that the choline resonance intensity is obtained by numerical integration, which means that choline can not be differentiated from other (partially) overlapping resonances, such as taurine. Taurine is a strongly-coupled spin-system that has a significantly reduced multiplet intensity at TE = 75 ms relative to TE = 10 ms. Therefore the ‘overall integrated choline resonance’ has decreased at longer echo-times, offering a possible explanation for the observed ratios.

B. Spectral fitting algorithms such as LC model (see Chapter 9) can separately quantify choline and taurine at any echo-time. Following a correction for T_2-relaxation-induced signal loss the tCho/tCr ratio should be constant for all echo-times.

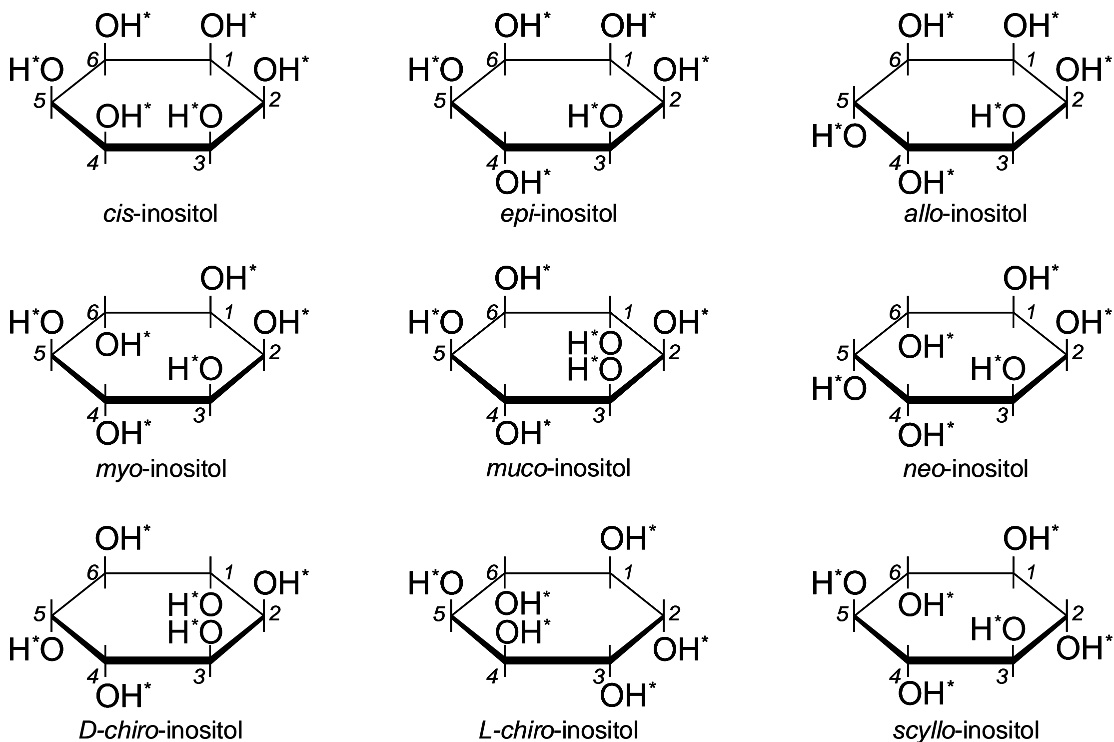
2.8.

In the presence of heteronuclear decoupling, halothane appears as a singlet resonance at 0.0 ppm, isoflurane appears as two singlets at circa –3.4 ppm and –9.7 ppm, while the CH group in enflurane appears as a triplet at circa –80.5 ppm. The CF_2 group next to the CF group appears as a doublet at circa –11.2 ppm, while the other CF_2 group appears as a singlet at circa –9.4 ppm.

In the absence of heteronuclear decoupling, all resonances in halothane and isoflurane appear as doublets. The CF resonance in enflurane appears as a double triplet, while the –11.2 ppm and –9.4 ppm CF_2 resonances appear as double doublet and single (regular) doublet signals, respectively.

Note that *in vivo*, the T_2 relaxation of all three compounds is so short that the resonances appear as apparent singlets, even in the absence of heteronuclear decoupling.

2.9.



2.10A. T_2 = 10 ms, thus FWHM = $1/(\pi T_2)$ = 31.83 Hz. However, in the presence of a 5 Hz residual inhomogeneity, the real FWHM = 31.83 + 5 = 36.83 Hz, making T_2^* = $1/(\pi\text{FWHM})$ = 8.64 ms.

Using the integral given in the solution to Exercise 1.5D, it follows that the integration boundaries are ±116.3 and ±234.1 Hz for 90% and 95% integrals, respectively.

Therefore, in order to quantify a Lorentzian-shaped line of FWHM = 36.83 Hz for > 95% the integration boundaries must be set to > 12 times the line width!

B. The integral over the range [–2FWHM, +2FWHM] equals $0.422M_0$ or 84.4% of the maximum integral.

2.11.

- The presence of nearby electronegative nuclei
- Chemical exchange
- Ring-currents in aromatic rings
- The presence of shift reagents
- pH
- temperature
- The presence of magnesium, calcium or other ions.