# The influence of template metabolite omissions on 1H-MRS quantification

Reggie Taylor[1,2], Peter Williamson[1,3], and Jean Théberge[1,2]

[1]*Medical Biophysics, University of Western Ontario, London, ON, Canada,* [2]*Medical Imaging, Lawson Health Research Institute, London, ON, Canada,* [3]*Psychiatry, University of Western Ontario, London, ON, Canada*

**Introduction:** The human brain $^{1}$H-MRS spectrum consists of signal contributions from the $^{1}$H nuclei of several metabolites. Each metabolite contributes area to the total area under the metabolite spectrum with a characteristic peak pattern (spectral signature). Variations in electron shielding cause each $^{1}$H nuclei to resonate at unique chemical shifts, but there remains a large degree of overlap between spectral signatures due to line broadening effects of transverse relaxation and local field fluctuations. Sample noise also adds complexity to the metabolite spectrum. Some metabolites have relatively uncomplicated spectral signatures that result in prominent spectral peaks allowing them to be easily identified despite relatively small contribution to total spectral area. Other metabolites contribute to a large proportion of the total spectral area, making their inclusion in metabolite quantification templates mandatory. However, other metabolites have modest contributions to total spectral area. These metabolites are often omitted from $^{1}$H-MRS templates, usually owing to poor quantification reliability related to SNR limitations. Inclusion of poorly quantified metabolites in the fit may complicate the quantification of other metabolites, resulting in an over- or under-estimation of their concentrations. Omitting the spectral signature of any metabolite in the basis set results in remaining spectral area only accounted for by mis-estimation of other metabolites. Correct interpretation of MRS data relies heavily on exact knowledge of contributors to a metabolite's concentration estimate. Therefore, the purpose of this study is to systematically examine the influence of metabolite omission on the estimation of metabolite concentrations using Monte Carlo simulations. Metabolite quantification improves at high magnetic field strengths[1]. Therefore, this study will conduct simulations of 7 Tesla spectral signatures to examine the influence of metabolite omissions on the high quality, simpler spectra typically obtained at this field strength before moving to the more complicated spectra observed at lower field strengths.

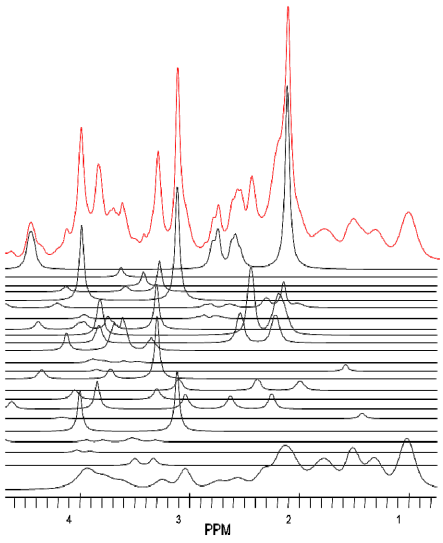


Figure 1. The simulated *in vivo* spectrum (red) along with all metabolite and MM spectral signatures (black). From the top, NAA, Gly, Scy, Cho, Cr, NAAG, Asp, GPC, Gln, Myo, α-Glc, Ala, PC, GABA, PEth, GSH, Lac, PCr, β-Glc, Ser, Tau and MM.

| Metabolite | Total Sum of Squares |
|---|---|
| MM | 48.497 |
| NAA | 42.360 |
| Glu | 18.398 |
| Cr | 5.481 |
| Myo | 5.330 |
| Gln | 4.761 |
| GSH | 4.097 |
| PC | 3.842 |
| PCr | 3.073 |
| GABA | 2.565 |
| NAAG | 1.340 |
| PEth | 1.283 |
| GPC | 1.277 |
| Tau | 0.980 |
| Cho | 0.800 |
| α-Glc | 0.434 |
| Gly | 0.332 |
| ß-Glc | 0.279 |
| Asp | 0.164 |
| Scy | 0.146 |
| Ser | 0.123 |
| Ala | 0.030 |
| Lac | 0.005 |

Table 1. The total sum of squares of the differences between other metabolite's expected and observed concentrations when that metabolite was omitted from the spectrum. Units are in $mmol^2/kg_{ww}^2$.

**Methods:** Metabolite spectral signatures were simulated individually for 22 metabolites (Figure 1) using software based on the GAVA simulation environment[2] and modified in-house (JT). Chemical shifts, coupling constants, and concentrations were obtained from literature values[3],[4]. An empirically determined macromolecular (MM) baseline was added to the spectra as well. The templates were made to represent true *in vivo* spectra that would be acquired using an ultra-short echo time STEAM sequence (TE=6ms, TM=32ms) with the number of transients (NT) set to 64 using a 7T MRI. Global constraints were set for phase (0th and 1st order), chemical shift, and LW, but all metabolites had independent amplitudes. An additional chemical shift constraint was placed on the MM signals, leading to a total of 27 fitting parameters in the full template. Lorentzian linewidths were initialized to 9.45 Hz based on the literature[5]. SNR was determined relative to the height of a reference peak equivalent to the NAA CH3 singlet at 2.01ppm often used to measure SNR. This peak was placed at 0 ppm sufficiently offset from the metabolites so as to not interfere with the quantification. The literature suggests an SNR of 20-25 is possible at 7T and NT=1 [6]. The average of these values (22.5) was used to calculate the theoretical SNR for NT=64, which was 180. Noise was added by a program developed in house (JT). There were 23 templates created that each lacked one metabolite's (or the MM's) spectral signature in its basis set along with one full template. All templates were used to successively fit 200 noisy realisations of the simulated full spectrum containing all the metabolites using our fitting software (fitman spectral analysis suite)[7]. Average metabolite concentration estimates were compared to their known concentrations. In total, 4800 simulated spectra were quantified.

**Results and Discussion:** Every metabolite influenced at least one (typically more) other metabolite's estimate by 10%, or greater, with the exception of Lac only. Results were placed in a spreadsheet (not included) indicating percent variation from expected concentration estimates upon the omission of a metabolite. A total sum of squares analysis was used to demonstrate the most influential spectral signatures on metabolite concentration estimates (Summarized in Table 1). For each metabolite omission, it was observed how its absence altered total creatine, choline, and glucose estimates, rather than their individual components (Cr and PCr, PC, GPC, and Cho, α- and β-Glc, respectively). For example, with PC omitted it would be observed how it altered total choline and not GPC or Cho. This was done to preserve the integrity of Table 1 and to avoid the obvious result that the individual components would account for each other. Not surprisingly, the MM baseline had the largest influence due to its massive overlap with the entire spectrum. Fitting errors due to metabolite omissions were the largest in Ser, Asp, α- and β-Glc, with average errors of 90%, 34%, 49% and 38%, respectively. The only metabolites influencing the total spectral area by more than 5% were Glu at -7.7% and Gln at -5.2%. NAA's omission only resulted in a -3.3% fluctuation in total area, demonstrating how efficiently the fitting algorithm utilizes the template to minimize residual area. A somewhat surprising result is that no metabolite omission influenced Scy concentration estimates by more than 5%. Spectral overlap with an omitted metabolite typically resulted in incorrect concentration estimations, but even non-overlapping metabolites were also affected in some instances. This is likely due to a chain reaction of metabolites compensating for each other. Our next step is the extension of this work at lower field strengths where spectral signatures show greater overlap, more complicated coupling patterns and often limited basis sets are used in quantification.

**Conclusion:** We were able to demonstrate and quantify the influence of spectral area unaccounted for by the spectral quantification template due omitted metabolites using Monte Carlo simulations. These results underscore the importance of careful interpretation of MRS data quantified using a limited basis set and illustrate that there can be numerous contributions to a metabolite's concentration estimate, including some from metabolites with no direct overlap, even at 7T.

**References** [1] Tkac et al. *Magn Reson Med*46:451-456 (2001). [2] Soher BJ, et al. *J Magn Reson*. 185: 291-299 (2007). [3] Govindaraju et al**.** *NMR Biomed* 13:129-153 (2000). [4] Krawcsyk and Gradowska. *Journal of Pharmaceutical and Biomedical Analysis***:** 31:455-463 (2003). [5] Deelschand et al. *J Magn Reson* 206: 74–80 (2010). [6] Mangia et al. *J Cereb Blood Flow Metab* 27: 1055-1063 (2007). [7] Bartha et al. NMR Biomed 12:205-216(1999).