An Alternate Strategy for the Quantification of the *in vivo* Glutamate/Glutamine (Glx) Peak at 2.35 ppm

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Introduction: Quantification of the metabolites especially Glutamate/Glutamine (Glx) at 2.35 ppm from proton magnetic resonance spectroscopy (^{1}H MRS) of human brain is often confounded by overlap with varying compositions of lipids and macromolecules. Level of this contamination varies across the brain and introduces operator bias and thus reduces the reproducibility of the measurements, which poses a significant problem in acute drug studies. In this study, we propose and demonstrate a new method for quantification of the Glx at 2.35 ppm from the spectra obtained at 7T that exhibits enhanced reproducibility and can be generalized to other metabolites of interest.
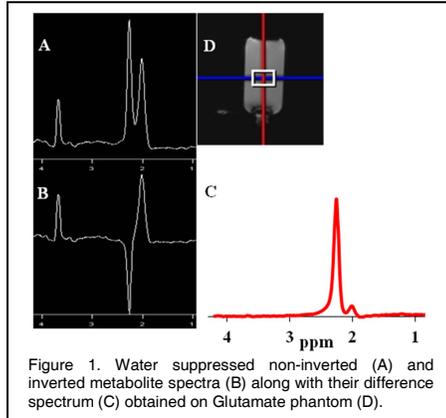

Figure 1. Water suppressed non-inverted (A) and inverted metabolite spectra (B) along with their difference spectrum (C) obtained on Glutamate phantom (D).

Methods: ^{1}H MRS was performed on Glutamate phantom at room temperature (Figure 1) and on normal healthy volunteers (aged 19-33 years) under an approved Institutional Review Board protocol of the University of Pennsylvania using Siemens 7T whole body scanner with a vendor supplied 32-Channel head coil. A total of six different voxel locations were chosen as shown in images in Figure 2 consisting of mid-frontal grey matter, MFGM ($15\times15\times15$ mm^3), left frontal white matter, LFWM ($15\times15\times15$ mm^3), left dorsolateral prefrontal cortex, LDLPFC ($20\times30\times20$ mm^3), left prefrontal cortex, LPFC ($20\times20\times20$ mm^3), occipital cortex, OCC ($20\times30\times20$ mm^3) and posterior cingulate cortex, PCC ($20\times30\times20$ mm^3) areas of brain. Automated shimming of the B_0 field was performed on the voxel in order to obtain localized water line width of ~30 Hz or less using FASTMAP shim method [1, 2] provided by SEIMENS as a works in progress (WIP) package. Single voxel spectra (SVS) for Glx were obtained with a custom sequence that acquires a water reference spectrum, a water suppressed metabolite spectrum and a water suppressed metabolite spectrum with a narrow band frequency inversion in a single acquisition using following parameters: number of points = 2048, averages =

8/32/32, TR = 3000 ms and TE = 21 ms. Total acquisition time to obtain the spectra were 4 min. Frequency selective editing pulses were used to invert Glutamate -CH$_2$ protons attached to β-carbon (at 2.35 ppm). The inversion width used was 20 Hz which was based on the line-width of water after eddy current compensation (usually varies from ~18-21 Hz). Each subject was scanned twice for two different voxels, to examine between day reproducibility in Glx concentrations across time and the voxel was positioned by using automated custom built software (ImScribe) [3] for the second scan, to maintain the consistency in voxel placement. For post processing we have used the raw multi-channel time domain data from the scanner. From the water reference data, channel wise time dependent phase shifts due to eddy current and amplitude scale factors were obtained and saved. All the three spectra were obtained after channel wise eddy current correction and adaptive combination [4]. Subtraction of inverted from non-inverted water suppressed spectra results in twice the amplitude of Glx signal. Amplitude of the Glx peak at 2.35 ppm, thus obtained from the areas of interest in brain were halved before being fitted as two peaks (since there is a slight contamination from γ-CH$_2$ protons of Glutamate at 2.13 ppm) by Lorentzian functions with non-linear least squares fitting (MATLAB "nlinfit" routine) followed by integration and then normalized by water reference signal for absolute quantification of Glx. Metabolite peaks from water suppressed non-inverted spectrum were fitted as Lorentzian functions with non-linear least squares fitting (MATLAB "nlinfit" routine) by taking into account the prior knowledge of the ten macromolecular peaks and 15 metabolite peaks over the frequency range of 0.5 to 4.3 ppm [5] followed by integration and then normalized by water reference signal for absolute quantification of Glx. Base SNR for both the spectra in all cases were greater than 500.
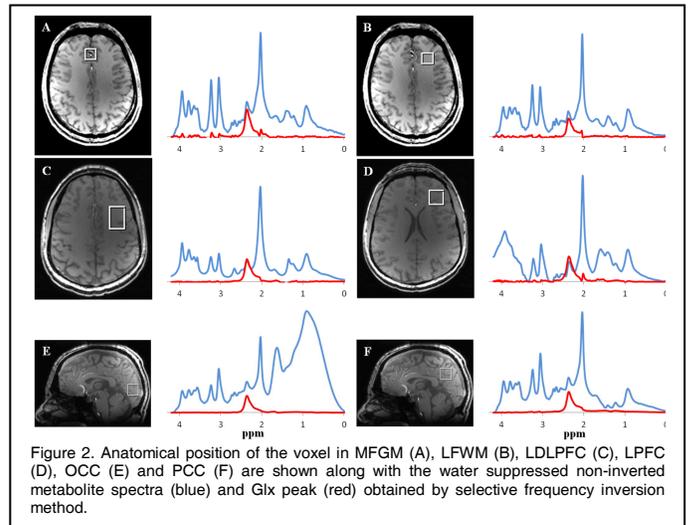

Figure 2. Anatomical position of the voxel in MFGM (A), LFWM (B), LDLPFC (C), LPFC (D), OCC (E) and PCC (F) are shown along with the water suppressed non-inverted metabolite spectra (blue) and Glx peak (red) obtained by selective frequency inversion method.

Results and Discussion: The concentrations of Glu at 2.26 ppm for the 9.3mM Glutamate phantom from water suppressed non-inverted spectrum and from selective frequency inversion method were 9.27 and 9.79mM, respectively (Figure 1). The concentrations of Glx at 2.35 ppm from water suppressed non-inverted spectrum and from selective frequency inversion method for both the scans from the normal healthy volunteers are tabulated in Table 1. The relative changes in absolute Glx concentration from between day scans (Figure 3) for voxel positioned in different anatomical areas are lower for selective frequency inversion method when compared to traditional water suppressed metabolite spectra with contaminations.

Voxel	Traditional method			Inversion method		
	Glx (mM)		Rel Ch (%)	Glx (mM)		Rel Ch (%)
	Scan1	Scan 2		Scan 1	Scan 2	
MFGM	12.97	13.91	7.25	14.51	14.18	2.27
LFWM	10.18	11.21	10.12	13.25	13.09	1.21
LDLPFC	12.05	12.7	5.39	14.21	14.61	2.81
LPFC	9.48	8.22	13.29	15.93	15.3	3.95
OCC	13.66	14.98	9.66	16.13	15.35	4.84
PCC	13.83	13.36	3.40	14.7	14.52	1.22

Table 1. Glx concentrations obtained from composite peak fitting of water suppressed non-inverted spectrum and from selective frequency inversion method are shown above.
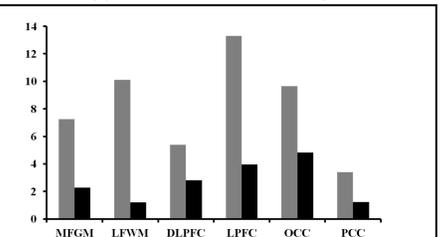

Figure 3. Relative changes in absolute Glx concentrations from between day scans for water suppressed non-inverted spectra (grey) and from selective frequency inversion method (black).

Conclusions: We have demonstrated the successful implementation of a selective frequency inversion method that simplifies the quantification of Glx in *in vivo* brain spectra. While the inversion approach shows within subject variability of less than 5% the conventional water suppressed metabolite spectra shows a larger range (3-13%) for voxels positioned in different regions of the brain. Implementation of the same at 3T and additional work is in progress for comparison of data obtained from frequency selective inversion method with quantification from LC model and JMRUI software of normal spectra.

References. [1]. Gruetter, R., et al. (1993) *Magn. Reson. Med.* 29, 804-811. [2]. Gruetter, R., et al. (2000) *Magn. Reson. Med.* 43, 319-324. [3]. Wolf, D. H. et al. (2011) *Psychopharmacology* 218, 503-512. [4]. Natt, O., et al. (2005) *Magn Reson Med.* 53, 3-8. [5]. Pfeuffer, J., et al. (1999) *J. magn. Res.* 141, 104-120.