

Spectral Editing – Uncovering Hidden Metabolites

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Introduction

Separating the Wheat from the Chaff, a subtitle from one of the first papers to use phase modulation effects for spectral editing, provides a useful metaphor for the NMR editing process. Any difference between buried and overlapping signals can be used for separation and editing. Magnetic resonance provides a wide variety of properties that can be used, including spin-coupling effects such as J-modulation and multiple quantum behavior. Relaxation, diffusion and dynamic effects can also be used. In the case of J-modulation and multiple quantum spin states, the difference is often exploited via a weaker, but resolved spin-coupled signal. In cases where all of the coupled signals of a desired metabolite are buried, 2D-spectroscopy may provide the necessary resolving power. In the case of dynamic spectroscopy using exogenous stable isotopes, simple difference spectroscopy may be sufficient. Spectral editing is especially important in proton spectroscopy, where the signals from up to 20 metabolites are crowded into a spectral region of less than 4 ppm. Adding to the difficulty for proton spectroscopy is the dominating water and lipid signals, which can complicate the spectral baseline and give rise to overlapping artifact signals. In a way, artifact reduction is a special case of spectral editing. A few examples of spectral patterns of metabolites commonly edited for are shown in figure 1.

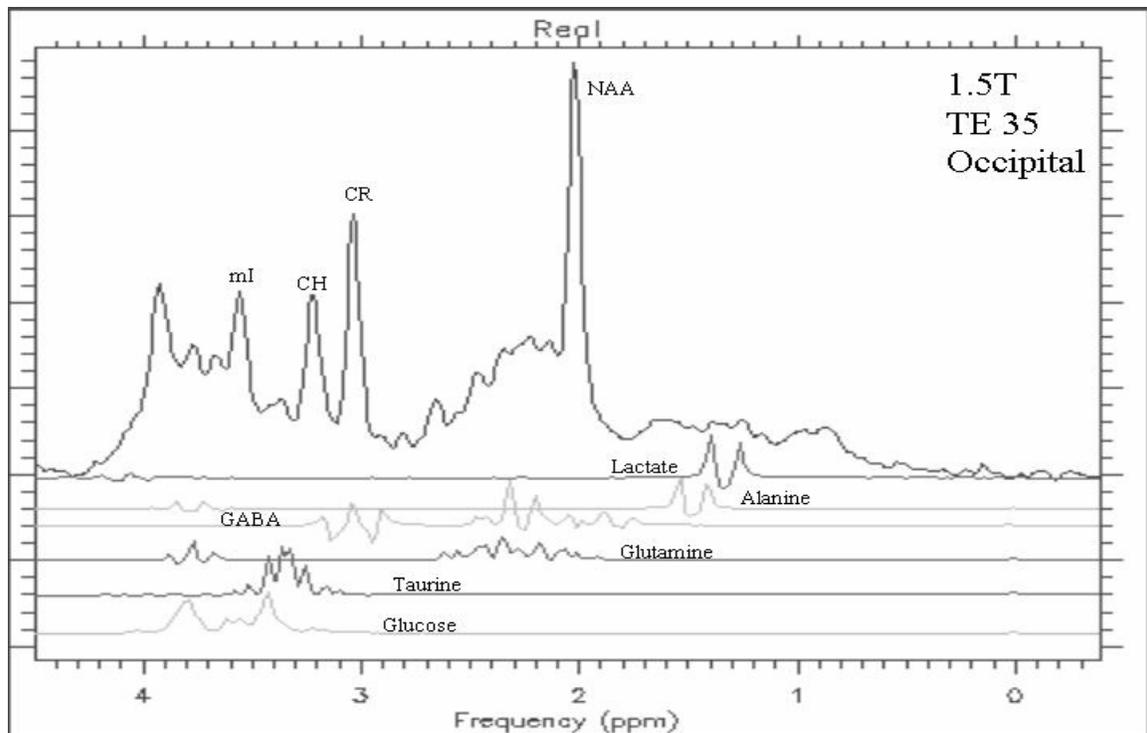


Fig. 1. Subspectra of lactate, alanine, GABA, glutamine, taurine and glucose at TE 35 along with an in-vivo spectrum.

¹³C-H from ¹²C-H: The Original Wheat From Chaff Example.

One popular way to edit is by spin-echo difference spectroscopy. This method takes advantage of the phase modulation that occurs when two or more resonances in a coupled spin system are simultaneously refocused via a spin-echo sequence (90- τ -180- τ -). If the refocusing of one of the coupled spins is avoided or reversed on an alternate scan, then the characteristic phase modulation does not occur, and a simple subtraction of the two scans provides the editing. This is illustrated in the wheat from chaff example with the separation of the carbon-13 satellites (1% at natural abundance) from the protons attached to the carbon-12 parent. In a proton-only spin-echo, carbon-13 spins are not refocused, and hence no heteronuclear J_{CH} phase modulation occurs. A second acquisition, in which the carbon-13 spins are refocused, provides the modulation and the basis for subtractive editing. Figure 2 shows the magnetization vectors for this editing process.

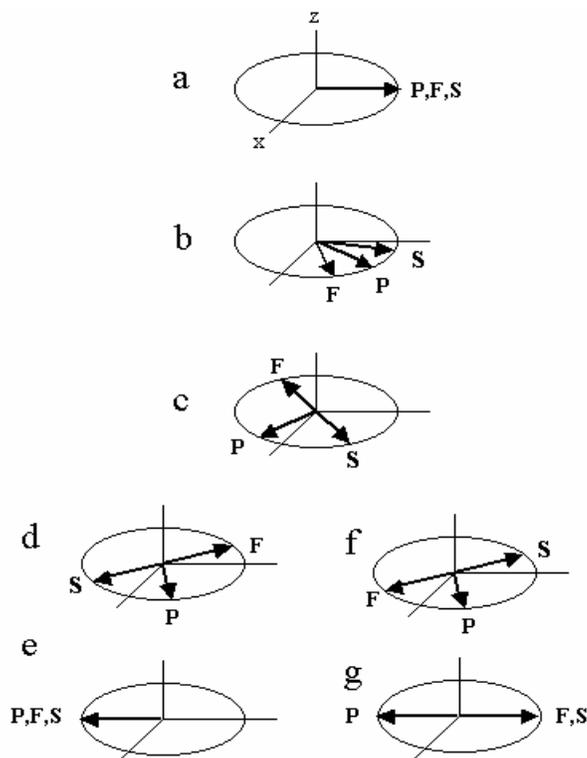


Fig. 2. Heteronuclear spin-echo difference spectroscopy. (a) The strong parent ¹²C-H (P), and the slow (S) and fast (F) component of the ¹³C-H doublet are initially aligned along the +Y axis. (b) They precess for a time τ until F and S lie antiparallel ($1/2J_{CH}$). In the first sequence (a), (b), (c), (d), (e), the proton 180 flips the vectors to mirror-image positions along the -Y axis. In the second sequence (a), (b), (c), (f), (g), the introduction of a carbon-13 180 at time τ interchanges the F and S labels (F), with the result that the F and S vectors become aligned along the +Y axis at time 2τ , whereas the P vector is still returned to the -Y axis. Subtraction of the two removes the strong parent signal.

Single Shot Methods

Difference methods have the advantage that 1) they usually return 100% of the available signal, and 2) nor do they destroy the non-edited signals, which unlike chaff, can be very useful. The disadvantage is that this method is subject to subtraction errors, due to limits in system and patient stability. Single shot methods avoid subtraction error, but lose one and sometimes both of the advantages of difference editing. One way to convert the example shown in Figure 2 into a single shot method is to replace the refocusing with a gradient BIRD pulse as shown in Figure 3. The protons attached to carbon-12 are not refocused and accumulate a phase across the sample equal to the $2G$ and thus cancel. The protons attached to carbon-13 are refocused (coherence order, p goes from 1 to -1) and the effect of the two equal gradient pulses cancels (often referred to as a primer-crusher gradient).

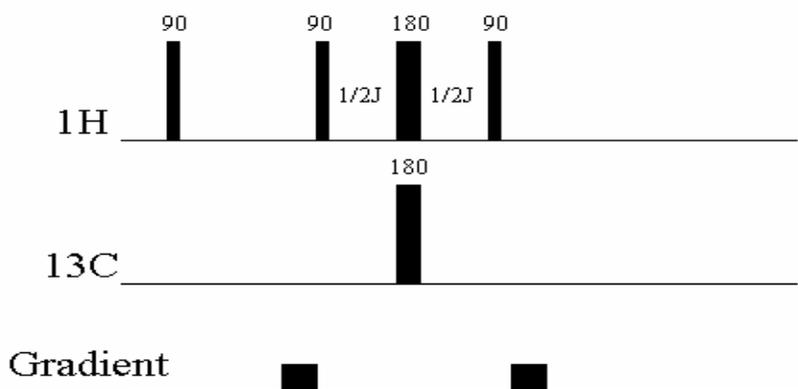


Fig. 3. Gradient-BIRD Spin Echo: Protons attached to carbon-13 are selectively refocused.

Double quantum editing, with gradient selection, is another excellent single shot method that has been used to edit for lactate, taurine, citrate, GABA, and glucose. In general these methods are limited to 50% signal return, but have excellent selectivity. Figure 4 illustrates the basic gradient selective double quantum sequence.

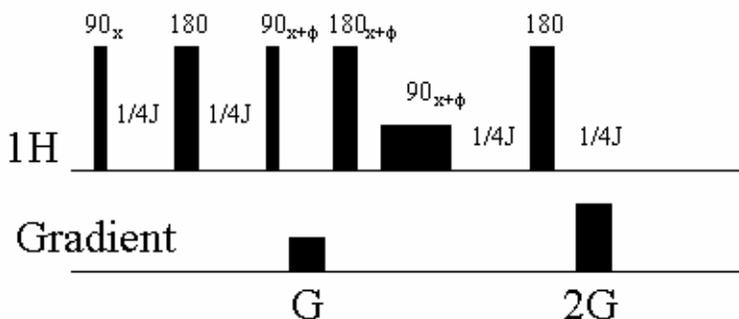


Fig. 4. *Basic frequency-selective double-quantum sequence. The first 90 plus the first and last 180's can be slice selective to make a volume selective version of the sequence. In the case of slice offsets the phase of the selective read pulses must be adjusted by ϕ to optimize signal return.*

T₂ weighting and J-Modulation

While non-coupled spins (e.g. water, or the methyl of NAA) just decay exponentially as TE is increased in a normal spin echo acquisition, coupled spins such as the lactate methyl doublet modulate. The left half of the doublet signal modulates at a frequency of $-J/2$, while the right half of the doublet modulates at a frequency of $+J/2$, resulting in an inverted doublet at $TE = 1/2J = 144\text{ms}$ and returning to a positive doublet at $TE = 1/J = 288\text{ms}$. In neuro applications, T₂ weighting is often sufficient for lactate detection, where the broad lipid and macromolecule signals are removed by difference in scalar relaxivity. The modulation of the lactate signal can also add some specificity.

2D NMR

Addition of a second dimension can often provide the needed resolution to avoid other forms of editing. For example, a 2D J-Resolved experiment reveals lactate as a $\sim 7\text{Hz}$ doublet in two separate dimensions.

Artifacts and Issues

1. Co-edited signals can present a challenge. In the case of double quantum filter for brain glucose, co-edited lactate is well resolved in chemical shift and can be an advantage. However, in the case of spin-echo difference editing of GABA, a macromolecular component (possibly mobile lysine moieties from the protein content) co-edits at the same chemical shift as the GABA C₄H protons and must be dealt with.

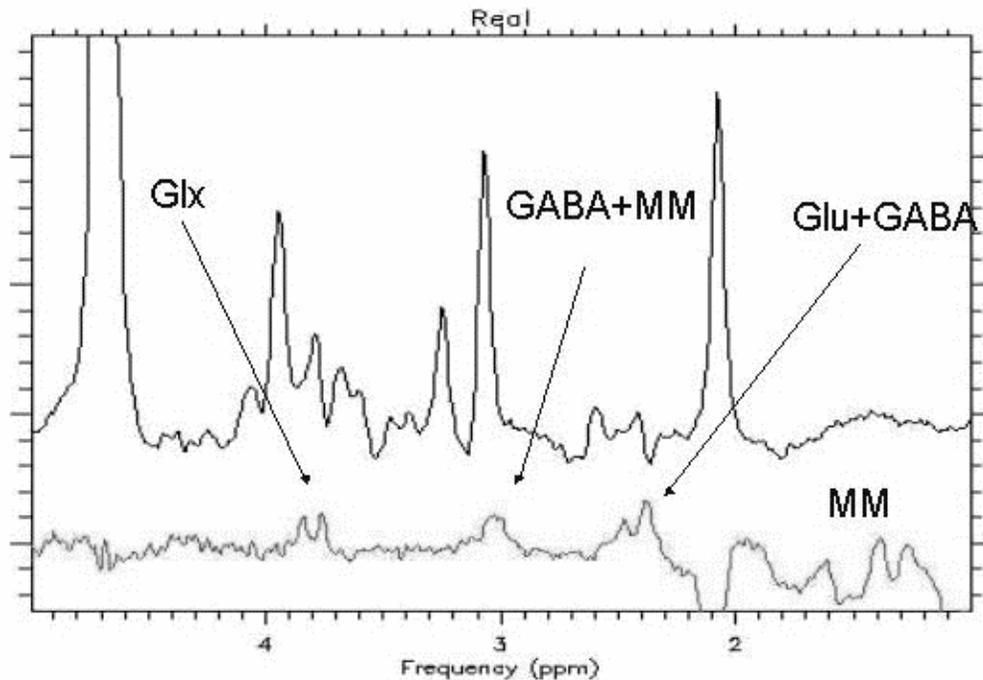


Fig. 5 3T Spin Echo Difference GABA Editing.

2. Chemical Shift Registration Error. In a double spin echo PRESS localization scheme, the selected volume for the lactate methine and the selected volume for the lactate methyl signal are not completely overlapped. J-modulation requires refocusing of both the methine and the methyl groups. Thus, the fraction of the lactate methyl signal coming from a volume where the methine is not excited will not modulate, and will in fact cancel some of the normally modulated signal at $TE = 1/2J = 144\text{ms}$. The amount of registration error is:

$$\text{Chemical Shift Difference/Effective Bandwidth} \quad [1]$$

This can be a significant effect for slice selective refocusing pulses and may typically exceed 10%

3. Symmetric Vs Asymmetric PRESS. Collection of proton spectra at $TE = 1/J$ for lactate ($TE = 288$) is also common, and further discriminates the long T_2 lactate from short T_2 lipid signals. Also, since the lactate methyl signal is fully re-phased, cancellation of signal due to chemical shift registration error is not a problem. However, if a symmetric double spin echo sequence is used, $[90-\tau-180-2\tau-180-\tau-\text{acquire}]$, the non-refocusing portion of the initial $90-\tau-180$ sequence, will generate multiple quantum coherence, leading to a reduction of lactate signal. For the same reason, the lactate methyl signal in long TE data collected using the STEAM sequence $[90-TE/2-90-TM-90-TE/2-\text{acquire}]$, will be compromised and will depend on the mixing time T_M . For this reason

asymmetric PRESS, where a minimum delay $[90-t_{\min}-180-]$ interval is highly recommended.

4. Impact of Water Suppression Methods. One very effective water suppression method used in long TE in vivo spectroscopy (MEGA/BASING) uses an evolution time inversion pulse/gradient spoiler combination as the water stop band. In cases where this stop band also includes the lactate methine at 4.1 ppm, the lactate methyl signal will not modulate. An advantage of this is that no signal will be lost to the chemical shift registration error at any TE. The disadvantage relative to other water suppression methods comes in MRSI studies in which frequency variation (uncorrected in-homogeneity) across the volume studied can place some voxels within the effective modulation stop band, some outside and some in-between.

5. Out of Volume Lipid. One final artifact to look out for is signal from the out of volume subcutaneous lipid as predicted by the spatial response function. This, along with other phase encode errors can effectively leak unwanted signal into the region of interest and ultimately obscure the reliability of lactate estimation.

Selected References

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