# Improved Tissue Metabolite Quantification in 1H HR-MAS Spectroscopy using the ERETIC Method

**T. N. Butler[1], M. J. Albers[1,2], K. Keshari[1], M. G. Swanson[1,2], and J. Kurhanewicz[1,2]**

[1]Department of Radiology, University of California San Francisco, San Francisco, CA, United States, [2]Bioengineering Department, UC Berkeley / UC San Francisco, San Francisco, CA, United States

## INTRODUCTION:

Measurement of absolute concentration in intact tissue permits quantification of changes in individual metabolites and can provide important biochemical information. High resolution magic angle spinning (HR-MAS) spectroscopy allows solution-like NMR spectra to be obtained from tissue, which can yield quantitative data under the correct experimental conditions. The reference standard 3-(trimethylsilyl)propionic-2-2-3-3-d4 acid (TSP) has previously been used as a quantitative standard in tissue HR-MAS spectra [1]. Alternatively, the ERETIC (Electronic REference To access In vivo Concentrations) method uses a synthesized RF pulse to provide an internal electronic standard which has been shown to provide robust concentration measurements in solution and solid state NMR [2,3]. The purpose of this study was to evaluate the reproducibility of ERETIC and TSP in HR-MAS spectroscopy of solution and tissue samples in order to establish their accuracy and reliability as quantitative reference signals.

## MATERIALS AND METHODS:

Spectral data were acquired at 11.7T (500 MHz), 1°C, and a 2,250 Hz spin rate using a Varian INOVA spectrometer equipped with a 3 channel, 4 mm gHX nanoprobe. A decaying exponential was generated using PBOX and synthesized using the waveform generator for the third channel of a three channel system. The 1H signal and the ERETIC signal were phase locked to one another by connecting the waveform generators for these two channels to the same frequency source. The ERETIC signal was injected into a detuned X-nucleus RF coil (tuned for $^{13}C$) contained in the probe using a directional coupler to combine the X channel and ERETIC channel inputs to the probe. 1D spectra were acquired with a calibrated 90 degree pulse, 40,000 complex points, 20,000 Hz spectral window, 2 s delay, 2 s presaturation delay, 2 s acquisition time (6 s repetition time, fully relaxed), 4 steady state pulses, and 64 transients. For stability testing, solutions of 3 µl aliquots of $D_20$ containing 0.75 wt% TSP ($D_20$+TSP) weighed to ±0.01 mg and added to 27 µl of $D_20$ were prepared and analyzed daily for three separate weeks and monthly for five months. Prostate biopsy samples (n=15, 5.90± 1.15 mg) and surgical samples (n=12, 18.7±3.44 mg) were harvested and frozen on dry ice. The ERETIC reference was validated in these samples using an analogous presat sequence with a repetition time of 4 s and 128 transients.

## RESULTS:

Figure 1 demonstrates the addition of the ERETIC signal to a standard prostate surgical tissue spectrum at a frequency upfield from any metabolites to ensure reliable integration. The resulting peak had the expected Lorenztian character and was observed to be consistent in its frequency and linewidth. ERETIC peak area measurements had a relative standard deviation (RSD) of 3.94% in solution samples analyzed daily over three weeks and 3.83% in solution samples analyzed monthly over five months (Figure 2), compared to RSDs of 4.90% and 3.01% over the same periods for the ratio of TSP peak area to TSP mass. In both tissue types, the relative standard deviation of the ERETIC peak area was significantly lower than that of the ratio of TSP peak area to TSP mass (4.53% compared to 20.1% in surgical, $p < .01$; 4.47% vs. 19.0% in biopsy, $p < .01$), while no significant difference was found between ERETIC and TSP RSDs in solution (Table 1).

**Figure 1:** Representative ERETIC HR-MAS spectrum of prostate surgical tissue

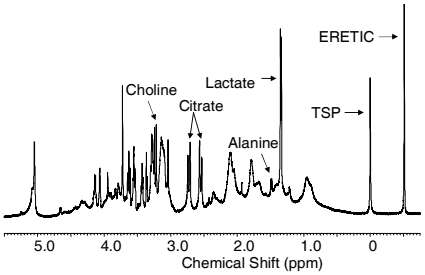


**Figure 2:** Plot of ERETIC peak area in solution over a period of five months

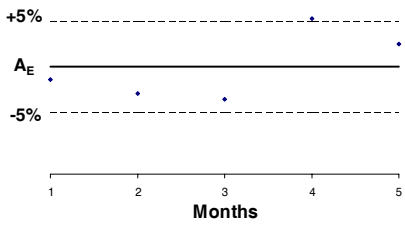


**Table 1:** Relative standard deviations in peak area of ERETIC vs TSP in solution and in tissue

| | TSP peak area / TSP mass | ERETIC peak area |
|---|---|---|
| **Standard solution (n=15)** | 4.90% | 3.94% |
| **Prostate Surgical (n=12)** | 20.1% | 4.53% |
| **Prostate Biopsy (n=15)** | 19.0% | 4.47% |

## DISCUSSION AND CONCLUSIONS:

The ERETIC signal's frequency, linewidth, and peak area were found to be reproducible in solution and in tissue using HR-MAS. While TSP is a suitable choice as a quantitative reference standard in solution, it is less robust in tissue due to increased variability in peak area, which leads to greater uncertainty when calculating metabolite concentrations. These shortcomings may be due to errors in measurement of TSP as well as TSP becoming undetectable due to differential absorption into tissue. The externally generated ERETIC signal offers greatly reduced peak area variation in tissue and thus represents an improvement over TSP as a quantitative reference standard in HR-MAS spectroscopy of tissue samples.

## REFERENCES:

[1] Swanson, M., Zektzer, A. Magn Reson Med. 2006; 55(6), 1257-64.
[2] Akoka, S., Barantin, L. Anal. Chem. 1999; 71, 2554-2557.
[3] Ziarelli F., Caldarelli S. SSNMR 2006; 29: 214-218.

**ACKNOWLEDGEMENTS:** National Health Institute, R01-CA102751, K01-CA96618