White Paper

picoSpin-45 Monitoring of a Transesterification Reaction

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The acid catalyzed transesterification reaction of methanol (MeOH) with ethyl acetate (EtOAc) to produce methyl acetate (MeOAc) and ethanol (EtOH) was monitored using the picoSpin-45 NMR spectrometer (Figure 1).

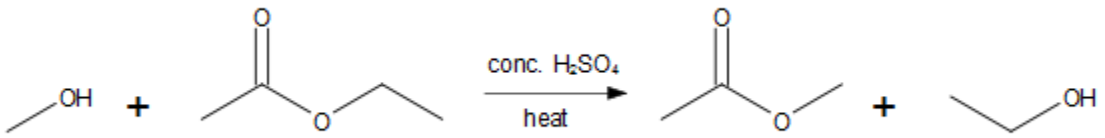

Figure 1. Acid catalyzed transesterification reaction of methanol with ethyl acetate.

A solution of MeOH and EtOAc (6:1 mol/mol) was prepared in a 10 mL HDPE bottle by mixing 5 mL of dry MeOH (Sigma-Aldrich: 99.8%; 24.7 M) with 2 mL of EtOAc (Acros: 99.6%; 10.24 M). To start the reaction 7 drops (0.3 mL) of concentrated H_2SO_4 (18.4 M) was added to the reaction mixture. (The acid concentration after dilution is approximately 0.75 M). The solution was shaken briefly, transferred to a 13 mm (dia.) x 100 mm (L) test tube and placed in a hot water bath preheated to 56 °C - the bath temperature fluctuated between 52-58 °C during the experiment. The reaction vessel was capped with a loose fitting glass stopper to minimize evaporation of reactants and products.

A 40 μL aliquot of the reaction mixture was taken at a sampling interval of 5 minutes, from t = 0 to t = 180 min, and injected into the inlet port of the capillary probe. The magnet temperature was 46 °C and approximates the capillary temperature within the spectrometer's RF coil. After an elapsed time of 180 minutes, the reaction mixture was transferred to a HDPE bottle, sealed, and allowed to react for an additional 40 hours, at which time a final spectrum was acquired. A spectrum of the unreacted MeOH:EtOAc solution, prior to the addition of acid, was also acquired for comparison. The 40 μL sample volume provided sufficient material to purge the capillary of the previous analyte and fill it with the current sample.

Each spectrum is an average of 12 scans acquired using a 90° pulse, 750 ms acquisition time, and 10 s recovery delay. Averaged spectra were processed in MNova first by applying automatic baseline correction, manual phase correction (PH0

and PH1 corrections were both applied as needed), zero filling with 64K points, and finally the spectra were filtered using Sine Bell (26°), exponential (0.10 Hz), and Gaussian (1 Hz) apodization. Spectra are internally referenced against the CH_3 proton signal in MeOH which was set to a chemical shift (δ) of 3.48 ppm. Spectra are presented as unnormalized except where noted. Peak areas were obtained with the line fitting function in MNova using the same spectral width and region for the CH_3 signal in MeOH (3.255 to 3.619 ppm) and the α-CH_3 signal in EtOAc and MeOAc (1.927 to 2.257 ppm).

Figures 2 and 3 show the proton spectrum of unreacted MeOH:EtOAc solution from separate batches (prior to adding the mineral acid catalyst). Methanol produces two singlet peaks, a methyl peak at 3.48 ppm and a hydroxyl peak at 4.7 ppm. Ethyl acetate produces three signals, a triplet (1.36 ppm) arising from the terminal methyl being split by two vicinal protons on the adjacent methylene, a singlet at 2.13 ppm from the α-carbon protons, and a quartet centered near 4.22 ppm (this signal is partially masked by the hydroxyl signal of MeOH where only 3 peaks of this quartet are discernible). Integration of the methyl CH_3 signal from MeOH and that of the α-CH_3 from EtOAc approximates the 6:1 mole ratio of the original solution.

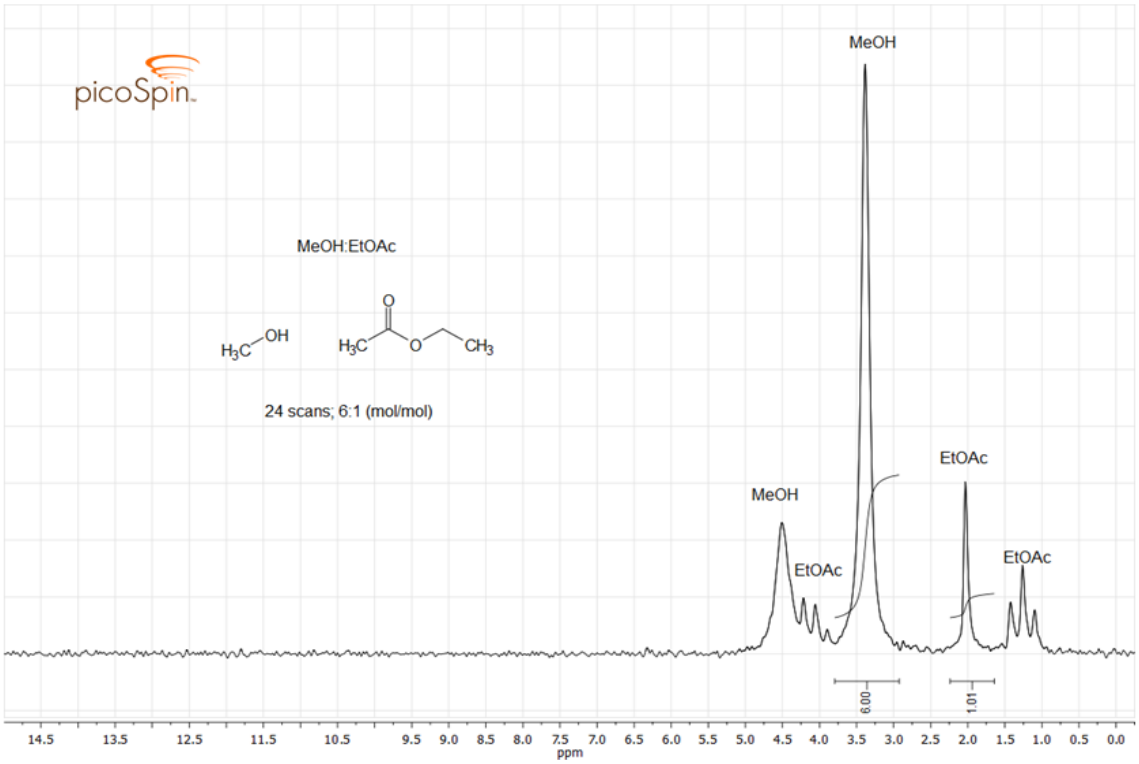

Figure 2. picoSpin-45 spectrum of the initial, uncatalyzed reaction mixture; 6:1 (mol/mol) MeOH and EtOAc.

The spectrum in Figure 3, taken from a separate MeOH-EtOAc solution, differs from the one in Figure 2 in that it shows additional structure in both the methyl and hydroxyl proton signals of MeOH. The doublet signal at 3.48 ppm has a *J* value of 3.9 Hz and suggests the CH_3 protons are split by the adjacent hydroxyl proton. Ordinarily proton exchange is too rapid for spin coupling to be observed in MeOH, however, hydrogen

bonding with EtOAc slows proton exchange, increasing the lifetime of isolated hydroxyl protons sufficiently to allow for spin-spin coupling to occur, which produces the observed fine structure. This behavior is known to occur in methanol solutions containing sufficient concentration of acetone to encourage hydrogen bonding. Addition of even a small amount of acid causes the observed doublets in MeOH to collapse to singlet peaks, as seen in the in the reaction spectra series in Figure 4.

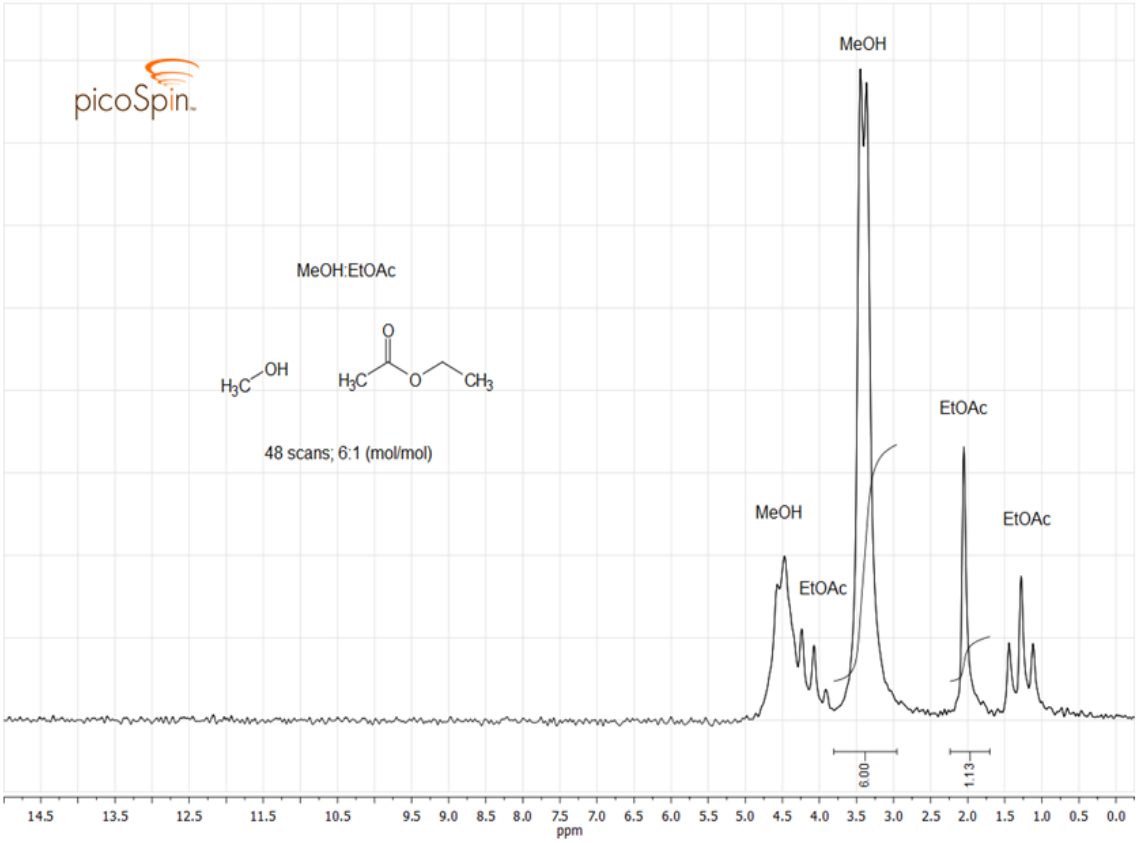

Figure 3. picoSpin-45 spectrum of the initial, uncatalyzed reaction mixture; 6:1 (mol/mol) MeOH and EtOAc exhibiting hydrogen bonding effect on spin coupling in methanol.

In Figure 4 spectra acquired during the reaction taken at 5 minute intervals, from *t* = 0 to *t* = 180 min, are stacked for comparison. Several regions of the composite spectrum are expanded to provide clarity and to emphasize changes occurring in the α-CH_3, alkoxy ethyl and methyl CH_3, methylene CH_2, and hydroxyl OH signals.

The peak centered at δ2.13 is attributable to resonances of the α-CH_3 group protons in both the reactant EtOAc and product MeOAc. Since chemical transformation occurs on the ester side of EtOAc and the reaction does not create any new keto-methyl groups the chemical shift of these α-carbon protons, which are very closely spaced in both reactant and product, is not expected to change during the course of reaction.

Initially, the triplet structure at δ1.35 is due to splitting of the terminal alkoxy ethyl CH_3 group in EtOAc being split by two vicinal protons on the adjacent methylene

group. As the reaction progresses the triplet shifts upfield to δ1.29 and is attributable to splitting of the alcoholic CH_3 group in EtOH.

At first glance the signal centered on δ3.76 appears to be a triplet but is actually two overlapping signals; one is a quartet signal due to splitting of the methylene group by three vicinal protons of the terminal methyl group of the product EtOH, and the second signal comes from the alkoxy methyl group of the product methyl acetate. Only three of the four peaks of the EtOH methylene quartet are resolved, with the peak farthest upfield buried under the leading edge of the large CH_3 signal from MeOH (δ3.48). The alkoxy methyl proton resonance overlaps nearly perfectly with the third downfield peak of the methylene quartet giving the appearance of the triplet structure. There are no triplet resonances expected to appear in this region of the spectrum.

The progression of the reaction is best seen in the downfield 'walk' of the OH signal which indicates a change in the mole fraction of MeOH and EtOH as the reaction proceeds. Initially the reaction contains only MeOH, with a MeOH:EtOH mole fraction of 6:1. As EtOH product is generated, the time-averaged OH signal arising from a rapid exchange of hydroxyl protons shifts to higher frequency as the MeOH:EtOH mole ratio changes. The OH signal reaches its maximum chemical shift in this reaction at t = 180 min. A comparison of a spectrum taken of the reaction mixture at t = 40 hrs (Figure 5) to that obtained at t = 180 min suggests the reaction has already finished by this time.

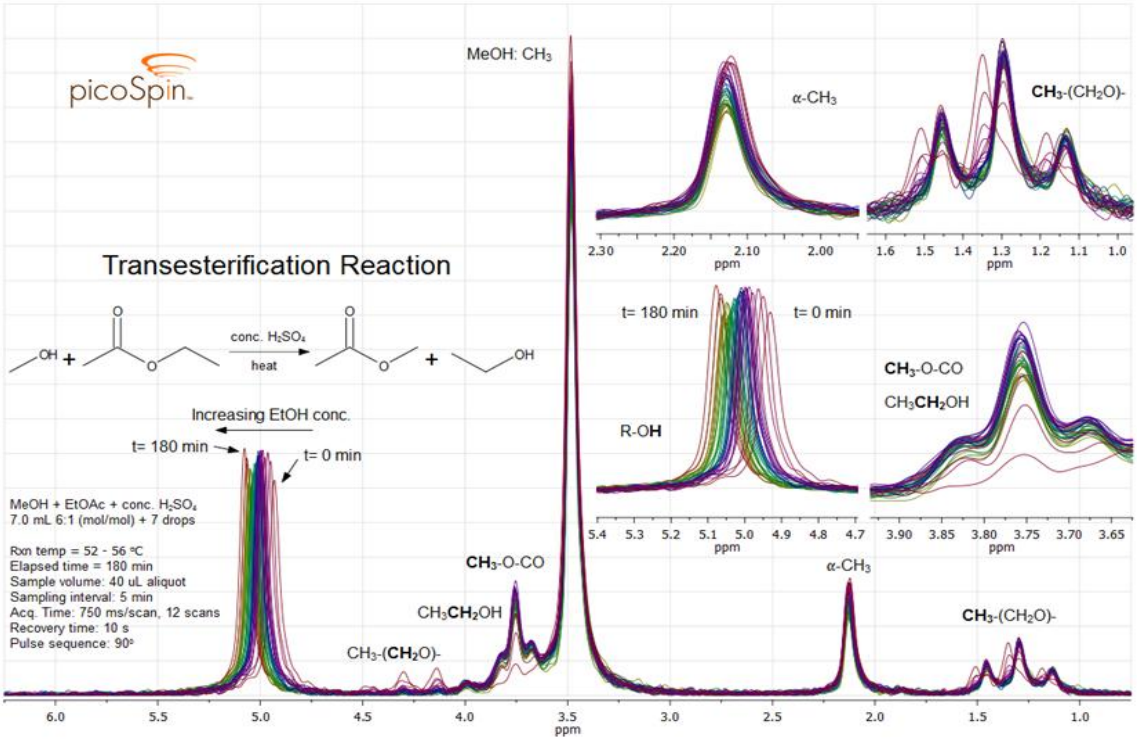

Figure 4. picoSpin-45 spectra of the reaction mixture sampled at 5 min intervals.

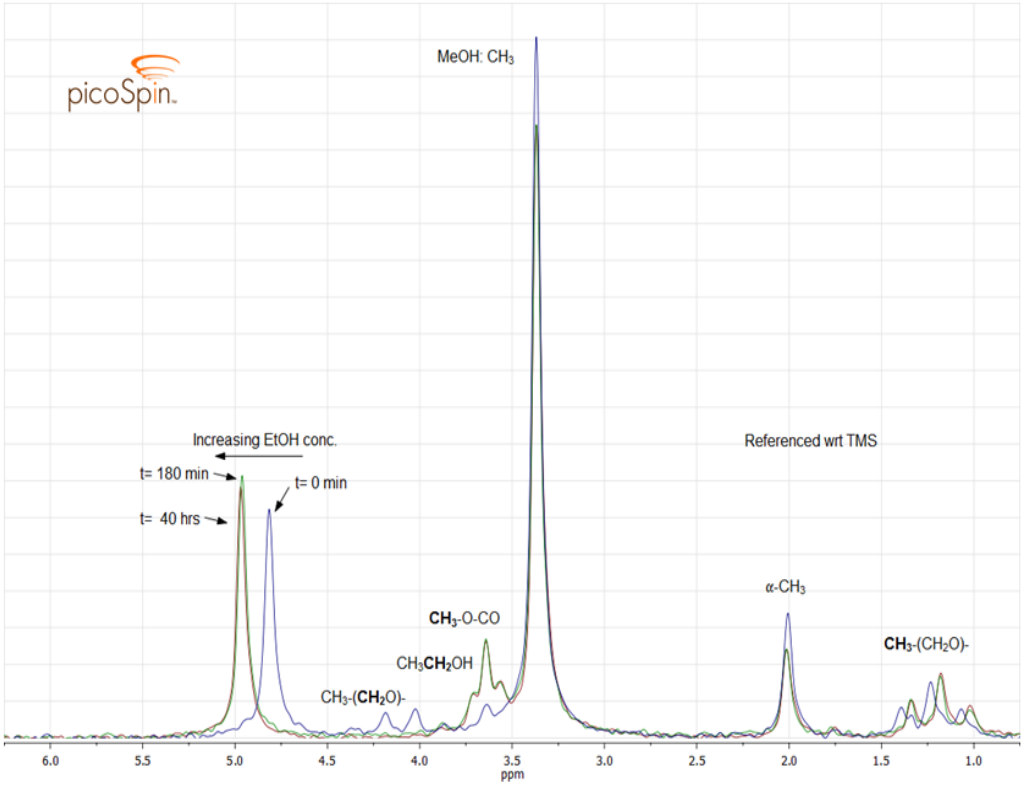

Figure 5. picoSpin-45 spectra of the reaction mixture measured at *t* = 0 min, 180 min, and 40 hrs.

However, closer examination of the composite spectrum (Figure 4) suggests the reaction is nearly complete in the first 20-30 mins of reaction. The first six spectra taken at time intervals *t* = 0 to *t* = 25 mins are shown in Figure 6. At *t* = 0 the alkoxy methylene proton resonance (-CH_2-O_2C) in EtOAc, centered near δ4.11, is nearly completely absent by *t* = 25 mins. Keeping in mind that even though the first spectrum is labeled *t* = 0 min approximately 5 minutes had elapsed since the addition of acid to when NMR analysis of this extracted sample was completed and, thus, it is not surprising that changes in the initial reaction mixture have already occurred. This is most evident in the weak but discernible signal emerging at δ3.76, which belongs to the alkoxy methyl protons of the MeOAc product. As the reaction progresses this signal continues to grow, and the methylene signal from EtOH, which overlaps this alkoxy methyl signal, also begins to appear and grow. Further evidence of the extent of reaction can be seen in the triplet signal at δ1.20 where two overlapping triplets, one from the reactant EtOAc and the other from the product EtOH, are apparent already at *t* = 0 and coalesce into one triplet signal by *t* = 25 mins.

The continual downfield shift of the hydroxyl peak up to *t* = 180 mins and the comparison of this last spectrum to the one taken at *t* = 40 hrs, which at first suggests the reaction is still progressing up to the 3 hr mark, can be partially explained by evaporation. The position of the hydroxyl peak is sensitive to the mole fraction of alcohols in solution, and as the reaction vessel was only loosely capped and the reaction temperature was kept close to the boiling point of MeOH (65 °C), the MeOH:EtOH mole ratio would favor EtOH as MeOH continued to evaporate, shifting the OH signal further

downfield as the mole fraction of MeOH decreases. At *t* = 180 mins the reaction mixture was placed in a tightly sealed bottle preventing further evaporation and thus the *t* = 180 mins and *t* = 40 hrs spectra look identical.

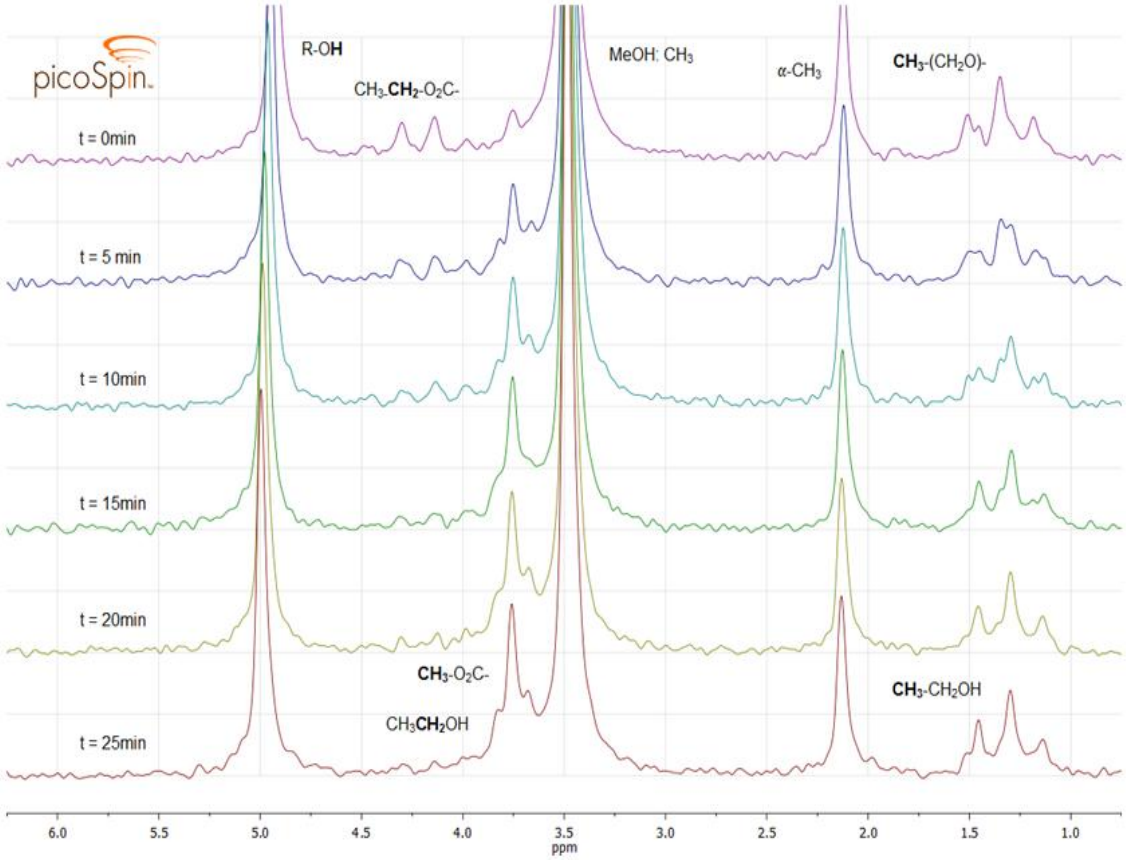

Figure 6. picoSpin-45 spectra measured during the first 25 min of reaction at 5 min intervals.

Slowing Down a Reaction

To approximate more closely a real-time analysis of the composition of the system while the reaction is in progress we changed the conditions of this reaction. By slowing down the reaction we can observe chemical transformations occurring on the timescale of the NMR experiment. In the above series of spectra (Figure 4) the reaction was catalyzed by concentrated sulfuric acid and conducted at 52-56 °C. In the following series of NMR spectra (Figures 7 and 8) we make several modifications: 1) run the reaction at room temperature, 2) reduce the amount of catalytic acid added, 3) shorten the T1 recovery delay and number of scans per sample, or 4) increase the sampling rate by shortening the time delay between sample injections.

The series of spectra in Figure 7 were obtained from a reaction mixture held at room temperature and with a reduced quantity of catalytic acid added. In this series, individual spectra were normalized with respect to the peak maximum of the α-CH_3 signal (δ2.13). This choice is justified since this keto-methyl group does not undergo chemical transformation during the reaction and therefore its signal intensity should remain constant throughout the experiment. As the reaction proceeds we see a downfield shift in the hydroxyl peak position as the EtOH product concentration. Since the reactant MeOH is in large excess its concentration is effectively constant during the reaction. Focusing on the hydroxyl signal, we see that at *t* = 0 min the signal intensity is at a minimum and increases in intensity as EtOH is produced. We also clearly see the ethyl triplet signal from the reactant EtOAc transform into the ethyl triplet signal of the product EtOH as the reaction proceeds. The ethyl group signal in EtOAc (δ1.35) is shifted 0.05 ppm downfield relative to the ethyl signal in EtOH (δ1.30) and diminishes more slowly under these reaction conditions, making its transformation easier to follow. Similarly, the methyl ester signal (δ3.77) of the product methyl acetate appears to still be growing even as the last spectrum is acquired.

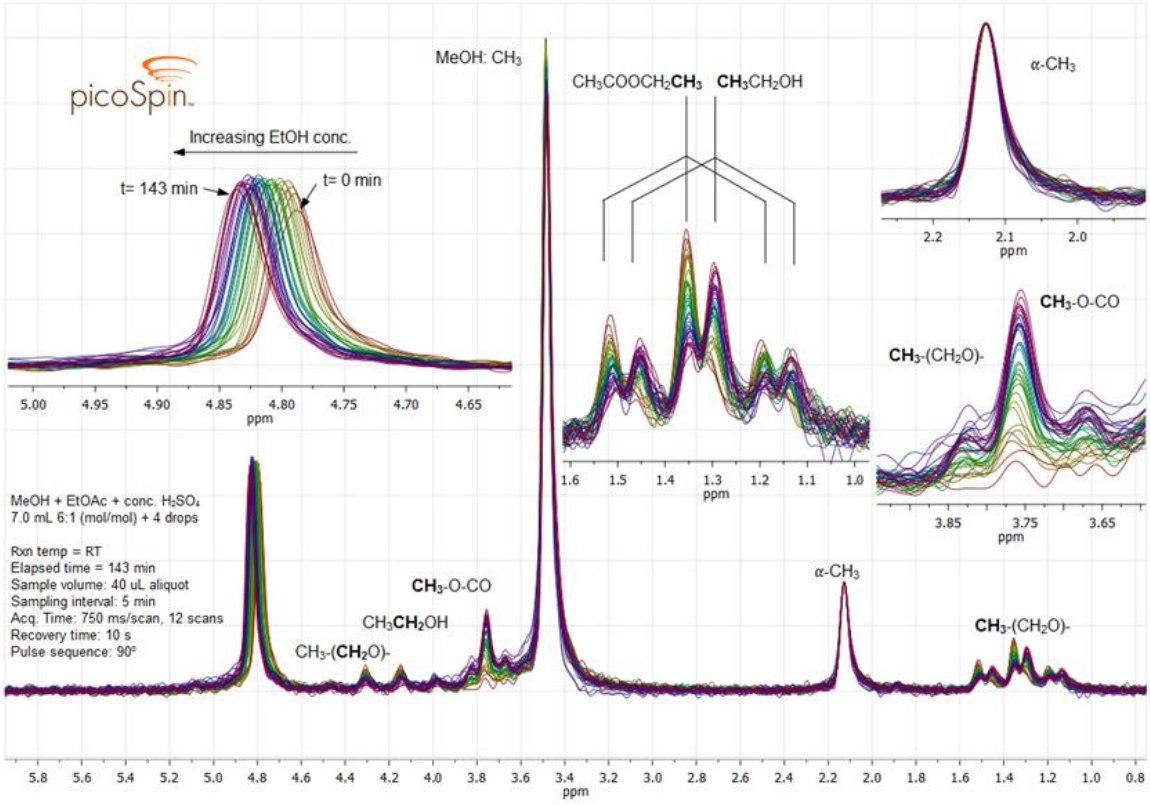

Figure 7. picoSpin-45 spectra of the reaction mixture sampled at 5 min intervals from a reaction conducted at room temperature and reduced amount of catalytic acid added.

In Figure 8 we modified the reaction conditions by substituting methanol-d (MeOD) for methanol, running the reaction at room temperature and further reducing the quantity of catalytic acid added to the reaction mixture. Like Figure 7 above, spectra in

Figure 8 are normalized relative to the peak maximum of the α-CH_3 signal (δ2.13). Samples were acquired and spectra measured every 2-3 min. Each spectrum is an average of 10 scans measured using a 90° pulse, 750 ms acquisition time and a T1 recovery delay of 3s - each average of 10 scans required 30s to acquire.

The most notable difference between the composite spectra in Figures 4, 7, and 8 is the absence of a hydroxyl signal near 4.8 ppm due to the use of MeOD as a reactant in place of MeOH. As such, both the react MeOH and product EtOH hydroxyl signal are not observed. Otherwise the spectra in this figure look similar except for the slower progression of growth of the methyl ester signal at δ3.77, and the change in ethyl groups signals at δ1.30 and δ1.35.

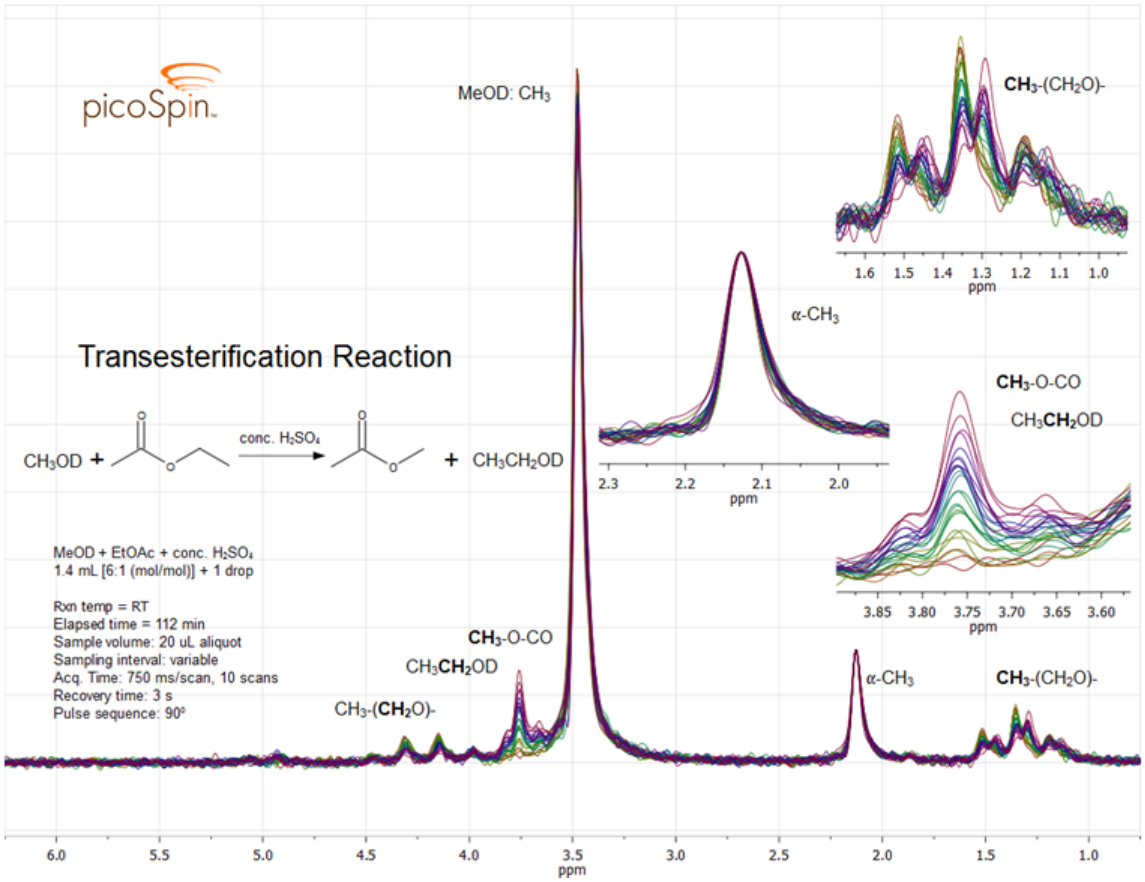

Figure 8. picoSpin-45 spectra of the reaction mixture MeOD and EtOAc sampled at 3 min intervals from a reaction conducted at room temperature and reduced amount of catalytic acid added.

The growth of the methyl ester signal as the reaction proceeds is clearly observed in Figure 9, where initial (t = 0 min), intermediate (t = 25 min) and final (t = 112 min) spectra of the reaction mixture are presented. In Figure 10 the progression of the ethyl signal in the reactant EtOAc and product EtOD is tracked.

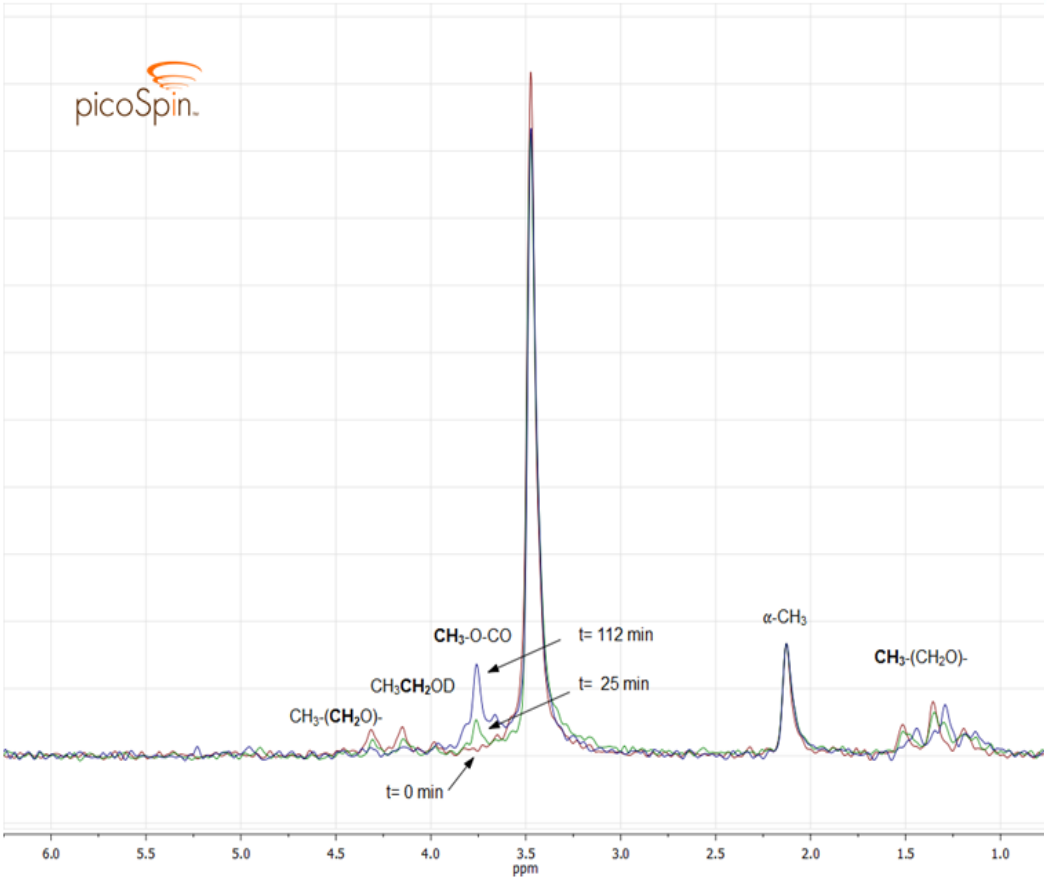

Figure 9. picoSpin-45 spectra of the reaction mixture measured at t = 0 min, 25 min, and 112 min.

In Situ **Monitoring**

As a final test we follow the reaction as it proceeds within the RF coil region of the spectrometer's capillary probe. Figure 11 presents averaged spectra measured at elapsed times Δt = 9, 19, 28, and 41 mins during the course of reaction. Individual, single pulse (90°) spectra were measured from the same sample every 5s (recovery delay), 750 ms acquisition time, for 41 mins. Spectra are normalized relative to the peak maximum of the α-CH_3 signal (δ2.13). The initial sample injection was a 20 µL aliquot of a 1.4 mL reaction mixture of MeOH (1 mL) and EtOAc (0.4 mL) with 2 drops of concentrated H_2SO_4 added as a catalyst. The reaction temperature was held fixed a capillary probe temperature of 46°. These spectra show a similar conversion of EtOAc to MeOAc as in Figures 6 and 10, but in this case we eliminate the possibility of MeOH reactant evaporation, which can account for changes in both the methyl and hydroxyl signal intensity. As the MeOH:EtOH mole fraction changes so too will the chemical shift of the hydroxyl signal, and reducing or eliminating evaporation will allow for higher precision in chemometric analysis.

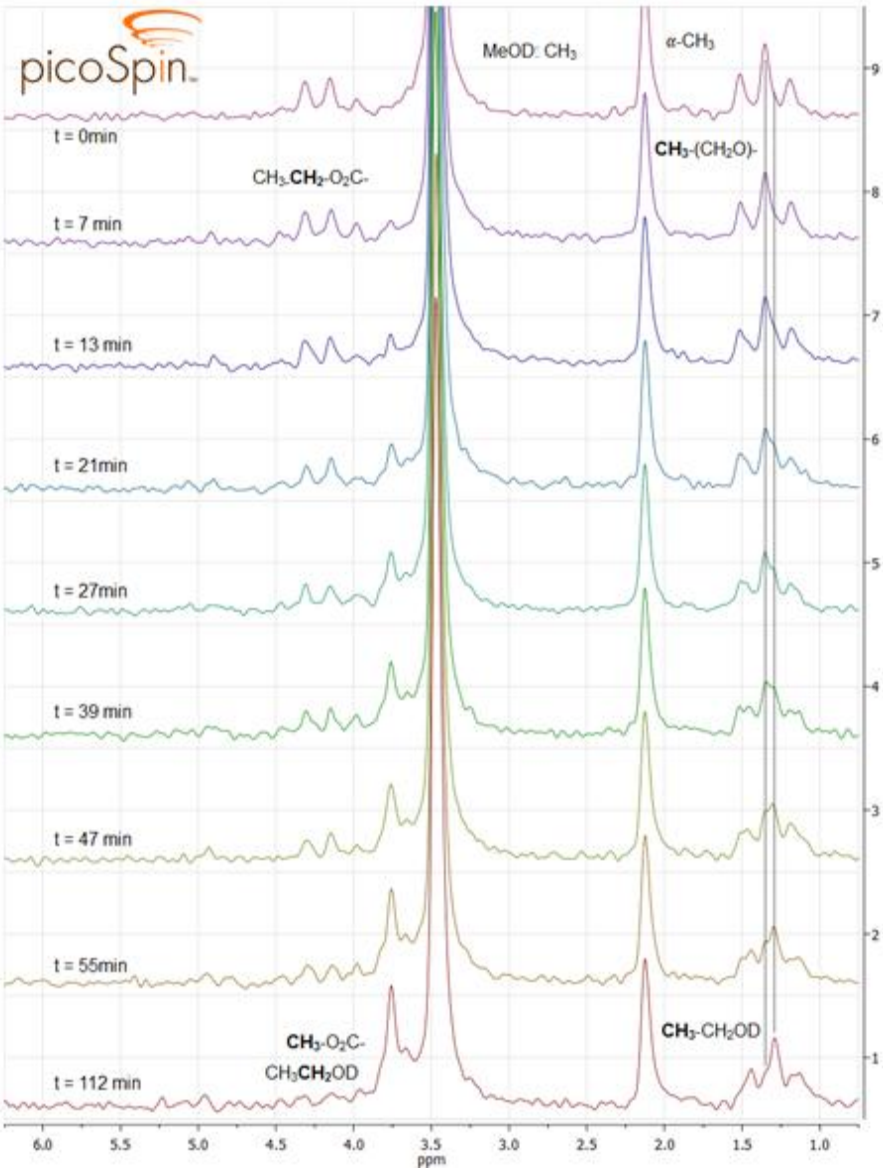

Figure 10. picoSpin-45 spectra measured at various times during the course of the reaction.

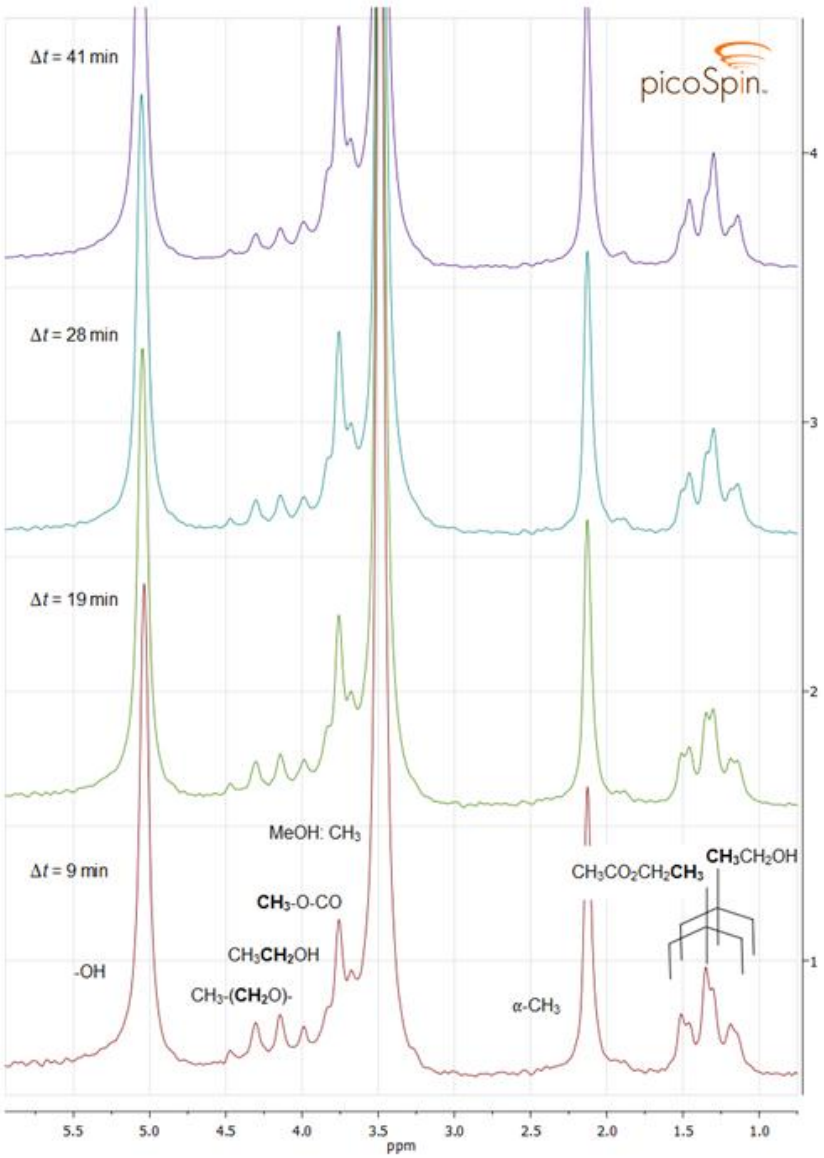

Figure 11. picoSpin-45 averaged spectra of the reaction mixture measured *in situ* at elapsed times 9, 19, 28, and 41 mins.