

4.4: Wet Lab

Wet Lab Procedure:

Part A. Preparation of standard solutions

1. Prepare a solution of the reference standard by weighing dry KHP and dissolving in an appropriate volume of deionized water or D₂O (Your instructor will indicate which solvent should be used in your experiments). Exact knowledge of the mass and volume of the KHP solution is important because the concentration of this solution controls the accuracy of the analysis.
2. Prepare a stock solution of malic acid by weighing dry malic acid and dissolving in an appropriate volume of deionized water or D₂O (Your instructor will indicate which solvent should be used in your experiments). Adjust these stock solutions to around pH 1 with HCl

Note: if deionized water is used as the solvent you will need to add some D₂O (usually around 10%) as a lock solvent. You also may want to consider using a chemical shift reference standard.

Part B. NMR parameters and quantitative analysis of standard solutions

1. Combine aliquots of the malic acid and KHP solutions you made in part A.
2. Determine and record the values for the each parameter listed below. Be prepared to justify your choice in each case. Obtain an NMR spectrum of the solutions you prepared in part B1.

Acquisition time =

Relaxation delay* =

Pulse width =

Spectral width =

Receiver gain =

Temperature =

Number of scans =

* The appropriate value of the relaxation delay can be determined from an inversion recovery experiment. If this is not feasible, your instructor may help you decide on the appropriate value of this parameter.

3. After the experiment is completed, process the spectra using appropriate line broadening, zero filling, phasing, and baseline correction.
4. Assign the KHP and malic acid resonances.
5. Calculate the S/N for this measurement by dividing the integrals measured for resolved malic acid resonances by the rms (root mean square) noise over a region of the spectrum that free of resonances and has a flat baseline.
6. Determine the concentrations of malic acid in your stock solution relative to KHP. How does the concentration you determined by Q-NMR compare with the value you would calculate from the mass and volume used in the preparation of the stock solutions?

Part C. Determination of the malic acid content of unknown fruit juice samples

1. If necessary clarify the juice sample by centrifugation. Prepare a 5-fold dilution of the juice sample with deionized water, adjusting the pH to around 1 with HCl.
2. Prepare at least 3 replicate solutions for quantitative analysis using KHP as an internal standard.
3. Acquire NMR spectra for the juice solutions starting from the optimized parameters used for the standard solutions in part B.
4. Process the spectra and calculate the average concentration of malic acid in the fruit juice. Determine the relative standard deviation of your measurements.

Wet Lab Report:

1. Report the amount of malic acid in your sample along with the relative standard deviation. How does the amount of malic acid you determined in the juice sample compare with the amount reported in the table in the background section of this lab experiment?
2. Using the S/N values calculated for the standard malic acid solution, estimate the limit of quantitation and detection.

3. Is the splitting pattern for any of the resonances of the compounds studied different than what you might predict from the simple rules you learned in organic chemistry? To what do you attribute these differences?

Reference

del Campo, G.; Berregi, I.; Caracena, R.; Santos, J. I. *Analytica Chimica Acta*. **2006**, 556, 462-468.

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