Thermo Fisher Scientific
Molecular Spectroscopy
525 Verona Rd, Madison, WI 53711
(608) 276-6100
www.thermoscientific.com

picoSpin™ 45: Microscale Flash Column Chromatography of a Mixture of Ferrocene and Acetylferrocene

Dean Antic, Ph.D., Thermo Fisher Scientific, Boulder, CO, USA

1. Introduction

Column chromatography is one of many basic laboratory techniques taught in organic chemistry. It has widespread application in the organic synthetic lab because of its efficiency for separating and purifying components of a mixture. It can be applied to both liquid and solid samples, and multi-component mixtures. On a small scale, column chromatography is fast and cost effective. It is particularly useful for separating reaction mixtures containing reactants, products and byproducts.

Chromatography takes advantage of differences in polarity and binding strength components of a mixture have for column adsorbents. Adsorbents are high surface area stationary phase materials that bind solute molecules. A mobile phase solvent, or eluent, is used to desorb solute molecules, carrying them along the column to a receiving flask. As the solvent polarity is increased, polar molecules bound more strongly to the column begin to solubilize and are carried down the column in the mobile phase. Equilibrium is established between binding to the stationary phase and solubility in the mobile phase. As the solvent polarity increases, more tightly bound polar molecules, firmly held by the adsorbent, establish equilibrium with the eluting solvent and flow along the column. This process is analogous to thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid-phase chromatography (HPLC).

2. Purpose

The purpose of this experiment is to separate the components of a mixture using column chromatography. The sample is a 50:50 mixture of ferrocene and acetylferrocene. Two fractions will be collected from the column separation of the mixture. Ferrocene (fraction 1) is eluted first using hexanes and appears as a yellow band in the column. Acetylferrocene (fraction 2) is eluted next using a 50:50 mixture of hexanes and diethyl ether solution.

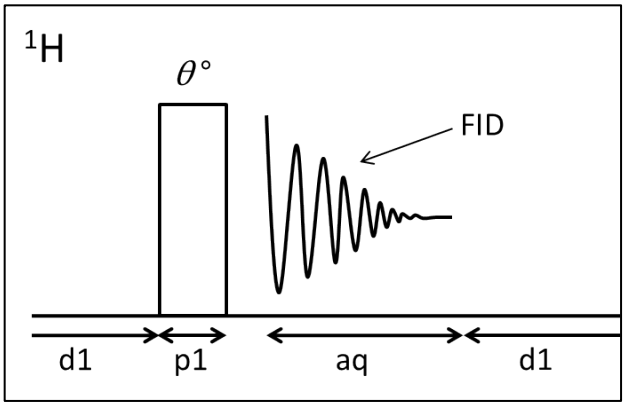
Solutions of pure ferrocene and acetylferrocene, the initial 50:50 mixture, and column separated fractions 1 & 2 will be prepared and analyzed using the Thermo Scientific™ picoSpin™ 45 NMR spectrometer.

3. Literature

Adapted from Williamson, K. L.; Minard, R.; Masters, K. M. *Macroscale and Microscale Organic Experiments*; 5th ed., Houghton Mifflin Co., 2007.

4. Pulse Sequence

In this experiment, we use a standard 90° single pulse experiment. The recycle delay time (d1) is adjusted to maximize signal intensity prior to signal averaging the next FID.



Sequence: d1–[θ°–aq–d1]$_{ns}$
θ°: Pulse rotation angle (flip angle)
FID: Free induction decay
d1: Recycle delay (µs) for spin-lattice relaxation
p1: R.F. transmitter pulse length (µs)
aq: Acquisition time (ms)
ns: # of scans (individual FIDs)

5. Procedures and Analysis

Time requirements: 2 hrs

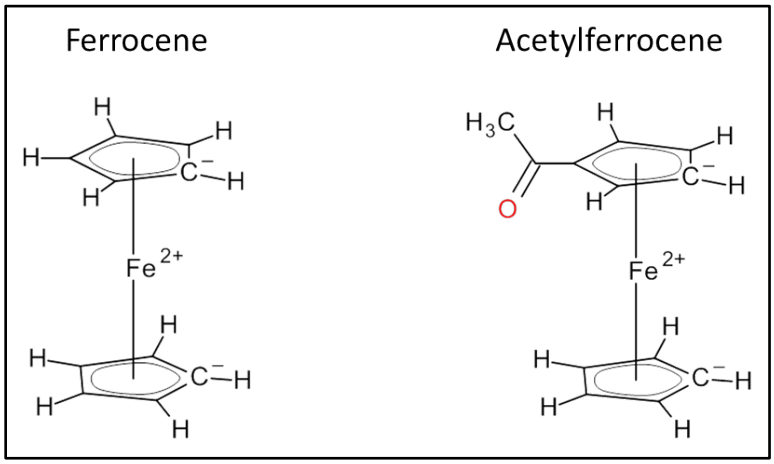
Difficulty: Moderate

Sample: Ferrocene, acetylferrocene, and 50:50 mixture of ferrocene-acetylferrocene

Equipment/materials:

- Thermo Scientific™ picoSpin™ 45
- Ferrocene ($C_{10}H_{10}Fe$)
- Acetylferrocene ($C_{12}H_{12}Fe$)
- Ferrocene/acetylferrocene (50:50 mix)
- Hexanes
- Diethyl ether
- Dichloromethane
- NMR solvent: $CDCl_3$ w/ 1% TMS
- NMR solvent: Acetone-d_6 w/ 1% TMS
- Silica gel (230-425 mesh), or alumina
- Tapered collar neoprene filter adapter
- Several 7 mL vial with PTFE cap liner
- 1 mL polypropylene syringes
- 22 gauge blunt-tip dispensing needles
- Pasteur pipet
- Pipet bulb
- Polypropylene funnel
- Weighing paper/boat
- Cotton swab/ball
- Hirsch funnel
- Filter paper
- pH paper or litmus paper
- Ring stand, ring clamp, iron ring
- 25 mL vacuum flask
- Several 10, 25 and 50 mL beakers
- Mnova NMR Processing Suite
- picoSpin accessory kit:
  - Port plugs
  - Syringe port adapter
  - Drain tube assembly

Molecular Structures:



Physical data:

Substance	FW (g/mol)	Quantity	MP (°C)	BP (°C)	Density (g/mL)
ferrocene	186.04	50 mg	172.5	-	1.107
acetylferrocene	228.07	50 mg	81-83	-	-
ferrocene/acetylferrocene		150 mg			
hexanes	86.18	10-15 mL	-95	68-69	0.655
diethyl ether	74.12	10-15 mL	-116.3	34.6	0.7134
dichloromethane	84.93	2 mL	-96.7	41	1.33
chloroform-d ($CDCl_3$) w/1%TMS	120.384	1 mL	-64	61	1.50
chloroform*	119.38	1 mL	-82.3	61.2	1.48
acetone*	58.08	1 mL	-95	56	0.791
acetone-d_6 (Ac-d_6) w/ 1%TMS*	64.12	1 mL	-94	56	0.872

*Optional NMR solvents

Experimental procedure

Prior to packing the column, label and tare (weigh) several 7 mL vials and 2-25 mL flasks for receiving eluting fractions.

Preparing Samples

Several samples will be prepared for analysis. These solutions can be prepared in chloroform ($CHCl_3$), chloroform-d ($CDCl_3$) or acetone-d_6 (Ac-d_6). If $CHCl_3$ is used then its proton NMR signal, at 7.24 ppm, can be used to shift reference the spectrum, otherwise the TMS signal (0 ppm) in $CDCl_3$ or Ac-d_6 is used. The sample preparation guide and spectra presented are for $CDCl_3$ solutions.

- Prepare about 10 mL of a 50:50 mixture of hexanes and diethyl ether in a flask. Stopper the flask to prevent diethyl ether from evaporating.

- Prepare in a tared, labeled vial about 150 mg of a 50:50 ferrocene and acetylferrocene mixture. Record the weights of each component.

- Sample 1: To a tared, labeled vial measure about 30 mg of ferrocene and dissolve in 200 µL of $CDCl_3$. Record the sample weight. Cap and save for NMR analysis.

- Sample 2: To a tared, labeled vial measure about 30 mg of acetylferrocene (*Caution: toxic)* and dissolve in 200 µL of $CDCl_3$. Record the sample weight. Cap and save for NMR analysis.

- Sample 3: To a tared, labeled vial measure about 30 mg of 50:50 ferrocene and acetylferrocene mixture and dissolve in 200 µL of $CDCl_3$. Record the sample weight. Cap and save for NMR analysis.

- Chromatography Sample 4: To a tared, labeled vial measure about 120 mg of 50:50 ferrocene and acetylferrocene mixture. Record the sample weight. Dissolve in a minimum amount of dichloromethane. Once dissolved, add about 300 mg of silica gel (pre-weighed) and thoroughly mix the solution. Evaporate off the solvent using a hot water bath being careful not to cause bumping. (*Reminder: dichloromethane boils at 41°C.*). This sample is used for chromatography.

Chromatography fractions

Prior to preparing these samples, the eluting solvent must be evaporated off. A quick way to evaporate the solvent is under vacuum. Use a filter flask connected to a faucet aspirator fitted with a tapered collar neoprene (or 1-hole rubber stopper) as shown in Figure 1. Using your thumb control the vacuum pressure. Under reduced pressure, the boiling point of the eluting solvents lower to where the warmth of your hand is sufficient to cause boiling. Alternatively, in the absence of a 25 mL vacuum flask a similar apparatus can be made with a regular flask (or vial) using a 1-hole stopper and a Y-connector.

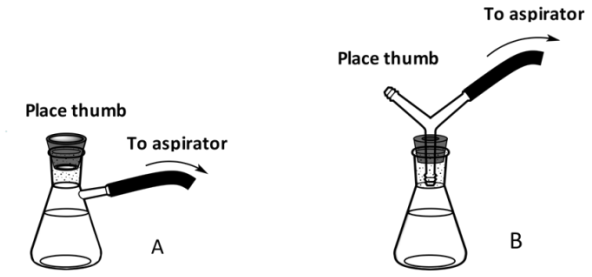


Figure 1. A. Evaporation flask with filter adapter. B. Flask (or vial) with Y-connector.

- Fraction 1: Collect fraction 1 in a tared, labeled 25 mL vacuum flask, 25 mL flask or 7 mL vial. Dry, weigh and record the amount of sample recovered. Add 200 µL of $CDCl_3$. Cap and save for NMR analysis.

- Fraction 2: Collect fraction 2 in a tared, labeled 25 mL vacuum flask, 25 mL flask or 7 mL vial. Dry, weigh and record the amount of sample recovered. Add 200 µL of $CDCl_3$. Cap and save for NMR analysis.

Packing the Column

Whether using a microscale or macroscale technique, it is important, and critical to the success of column chromatography, to pack the column uniformly such that it is free of air pockets and gaps. Two methods for column packing are used: dry packing and slurry packing. In dry packing, the adsorbent, silica gel or alumina, is added directly to the column and 'tapped' into place to remove cracks, gaps and air pockets, then the first eluting solvent is added to wet the column. With slurry packing, a slurry mixture of adsorbent and first eluting solvent is prepared and then poured into the column. The preferred method is slurry packing as it has a higher chance of minimizing gaps and air bubbles, but can be a difficult technique to perfect since adsorbent must stay suspended during transfer to the column.

Dry Packing Method

For microscale columns using a Pasteur pipet, the dry packing method is preferred.

- Remove the cotton end of a cotton swab, or an equivalent pea-sized cotton ball, and plug a Pasteur pipet. Use a wooden stick or stir rod to tamp it into place. Do not pack it too tightly.
- Measure about 5 mL of adsorbent using a 10 mL beaker. Add dry adsorbent to a height of about 5-6 cm. Leave ~3 cm at the top for the pre-adsorbed. (Folded weighing paper is helpful in transferring adsorbent to the column).
- Pack the column by alternately tapping gently the side of the column with a stir rod or spatula, and tamping it on the bench top. Add more column pack material as need.
- Once *chromatography sample 4* is prepared, pre-elute the column with hexanes (the first eluent). Do not let the column dry out; allow a small volume of eluent to remain above the column pack.
- Use a disposable syringe or pipet to add additional solvent.

Slurry Packing Method

Slurry packing a Pasteur pipet column is difficult because the pipet opening is small and the slurry needs to be introduced rapidly. This technique is better suited to macroscale chromatographic column preparation, such as with a burette.

- Fill a 25 mL beaker to about the 5 mL line with adsorbent. Add hexanes to initially wet the adsorbent, then while swirling add additional hexanes to produce a slurry. (*Note:* Increase the quantity of adsorbent and solvent accordingly to accommodate larger

columns. With a burette, close the stopcock, and add solvent about a third of the way up the column.)

- When ready, swirl the mixture to produce a suspension, simultaneously removing air bubbles, and then quickly pour the slurry into the column.
- Fill to a height of about 5-6 cm. Leave ~3 cm at the top for the pre-adsorbed.
- Tap the side of the column to cause the adsorbent to settle.
- Do not let the column dry out; allow a small volume of eluent to remain above the column pack.
- Use a disposable syringe or pipet to add additional solvent.

Adding the Sample

- To the top of the column, add all of *chromatography sample 4*, gently tapping to settle the dry powder.

Chromatography of a Mixture of Ferrocene and Acetylferrocene

Eluting the Column

Two fractions will be collected. The first fraction will be eluted using hexanes. The second fraction will be eluted with a 50:50 mix of hexanes and diethyl ether.

To make this column chromatography a 'flash' technique:

- Use a pipet to add eluting solvent to the fill the remaining available column space.
- Use a pipet bulb to 'push' the solvent through the column. Allowing only for gravity to percolate solvent through the column is very slow. The flash technique speeds up the process.
- Be careful not to aspirate the solvent and column pack to into the pipet bulb. Create a light seal when squeezing, and break the seal before releasing pressure on the bulb.
- Even while pushing the solvent, always maintain the solvent level just above the column pack. Add additional solvent as needed.
- Collect solvent in a small waste beaker as it passes through the column until the yellow band approaches the top of the cotton plug.
- Ferrocene (fraction 1) is eluted first with hexanes and is visible as a yellow band.
- Collect fraction 1 in the tared, labeled vial. Cap and set the vial aside.
- Replace the waste beaker under the column. Switch to the next eluting solvent.
- Acetylferrocene is eluted next (fraction 2) with a 50:50 mix of hexanes and diethyl ether, and is visible as an orange band.
- Maintain a solvent level just above the column pack.
- Continue collecting solvent in the waste beaker until the orange band approaches the top of the cotton plug, and then swap the beaker and collect this fraction in the tared vial labeled fraction 2.
- Cap and set the vial aside.
- Evaporate the solvent from the two vials (or flasks) and determine the weights of the crude residue.

- *Optional*: Recrystallize fractions from a minimum quantity of hot hexanes.
- *Optional:* Isolate and dry the crystals. Determine their weights and melting points.
- Calculate the percent recovery of the crude and recrystallized products based on the weight of each component in the original 50:50 mixture.

Instrumental procedure

The general procedure for sample analysis using a picoSpin NMR spectrometer is as follows:

Shim

- Ensure the NMR spectrometer is shimmed and ready to accept samples.

Pre-sample preparation

- Displace the shim fluid from the picoSpin capillary cartridge with air.
- Flush the cartridge with 0.1 mL of chloroform, and then displace the solvent with an air push. A small signal in your sample spectrum may appear at 7.24 ppm due to residual $CHCl_3$, it can be used to shift reference the spectrum.
- Set up the *onePulse* script according to parameters listed in the Pulse Script table.

Injection

- Using a 1 mL disposable polypropylene syringe fitted with a 1.5” long, 22 gauge blunt-tip needle, withdraw a 0.2 mL aliquot of sample.
- Inject about half the sample. Ensure all air bubbles have been displaced from the cartridge by examining the drain tube.
- Seal both the inlet and outlet ports with PEEK plugs.

Acquire

- Execute the onePulse script according to the values in the table of parameters provided.
- Once the onePulse script has finished, prepare the cartridge for the next user by displacing the sample from the cartridge according to the following protocol: air, solvent, air.

Pulse Script: onePulse

Parameter	Value
tx frequency (tx)	proton Larmor frequency (MHz)
scans (ns)	16
pulse length (p1)	Instrument specific 90° pulse length
acquisition time (aq)	750 ms
rx recovery delay (r1)	500 µs
T1 recycle delay (d1)	6 s
bandwidth (bw)	4 kHz
post-filter atten. (pfa)	10 (11)[a]
phase correction (ph)	0 degrees (or any value)
exp. filter (LB)	0 Hz
max plot points	400
max time to plot	250 ms
min freq. to plot	-200 Hz
max freq. to plot	+1000 Hz
zero filling (zf)	8192
align-avg. data	✓
live plot	✓
JCAMP avg.	✓
JCAMP ind.	Unchecked

[a] Choose the instrument's default pfa values

6. Processing

Download the experimental JCAMP spectra files and open them by importing into Mnova. The free induction decay (FID) will undergo automatic Fourier transformation and a spectrum will be displayed.

To each spectrum, apply the following processing steps using the given settings:

Function	Value
Zero-filling (zf) & Linear Predict (LP)	16 k
Forward predict (FP)	From aq → 16 k
Backward predict (BP)	From -2 → 0
Phase Correction (PH)	PH0: Manually adjust
	PH1: 0
Apodization	
Exponential (LB)	0.6 Hz
First Point	0.5
Shift reference (CS)	Manually reference

Peak Picking (pp)	Manually Select Peaks
Integration (I)	Automatic Selection
Multiplet Analysis (J)	-

- Import each data file into the same workspace in Mnova. Manually apply Ph0 phase correction to each spectrum.
- Manually shift reference each spectrum using Mnova's TMS tool. Assign the TMS signal (0 ppm) or $CHCl_3$ signal (7.24 ppm), whichever is present.
- Identify and assign each signal in the spectra.
- Save the Mnova document, print each spectrum and paste into your lab notebook.

7. Results

The ^1H NMR spectrum of a 0.5 M solution of ferrocene in $CDCl_3$ is presented in Figure 2. The spectrum is dominated by one signal centered at 4.14 ppm, arising from the target sample. Due to high symmetry of this metallocene, the singlet arises from excitation of 10 chemically and magnetically equivalent aromatic bis-cyclopentadienyl protons.

The ^1H NMR spectrum of a 0.5 M solution of acetylferrocene in $CDCl_3$ is presented in Figure 3. Acylation of one of the aromatic cyclopentadienyl rings disrupts the previous symmetry of ferrocene, resulting in three cyclopentadienyl proton signals and one acetyl proton signal. The singlet appearing at 2.38 ppm, assigned as 'c', arises from excitation of 3 acetyl ($-CH_3$) protons. The singlet appearing at 4.19 ppm, assigned as 'b', arises from excitation of 5 protons on the unsubstituted cyclopentadienyl ring. The two multiplet signal groups appearing at 4.48 and 4.76 ppm, assigned as 'a', arise from excitation of 4 protons on the substituted cyclopentadienyl ring. The 2 ring protons closest to the acetyl group are more strongly deshielded and shift further downfield at 4.76 pm, while the other two ring protons, being more shielded, appear at 4.46 ppm. The multiple structures derive from non-first order coupling of adjacent protons on the substituted ring.

Figure 4 shows the ^1H NMR spectrum of a roughly equi-molar solution of ferrocene and acetylferrocene in $CDCl_3$. The characteristic acetyl singlet and double multiplet structures of the substituted ring of acetylferrocene is evident in this solution mixture spectrum. The unsubstituted ring proton signal appears as a shoulder at 4.19 ppm on the downfield side of the large ferrocene signal at 4.14 ppm. These signal groups, the double multiplet structures or acetyl group protons, aid in distinguishing acetylferrocene from ferrocene in the NMR spectrum of the mixture.

The NMR spectra of crude fractions (1 & 2) eluted from the chromatographic column are shown in Figure 5. The top spectrum displays the distinctive singlet of ferrocene, while the bottom spectrum shows the characteristic double multiplet structures of acetylferrocene and the acetyl singlet at 2.38 ppm.

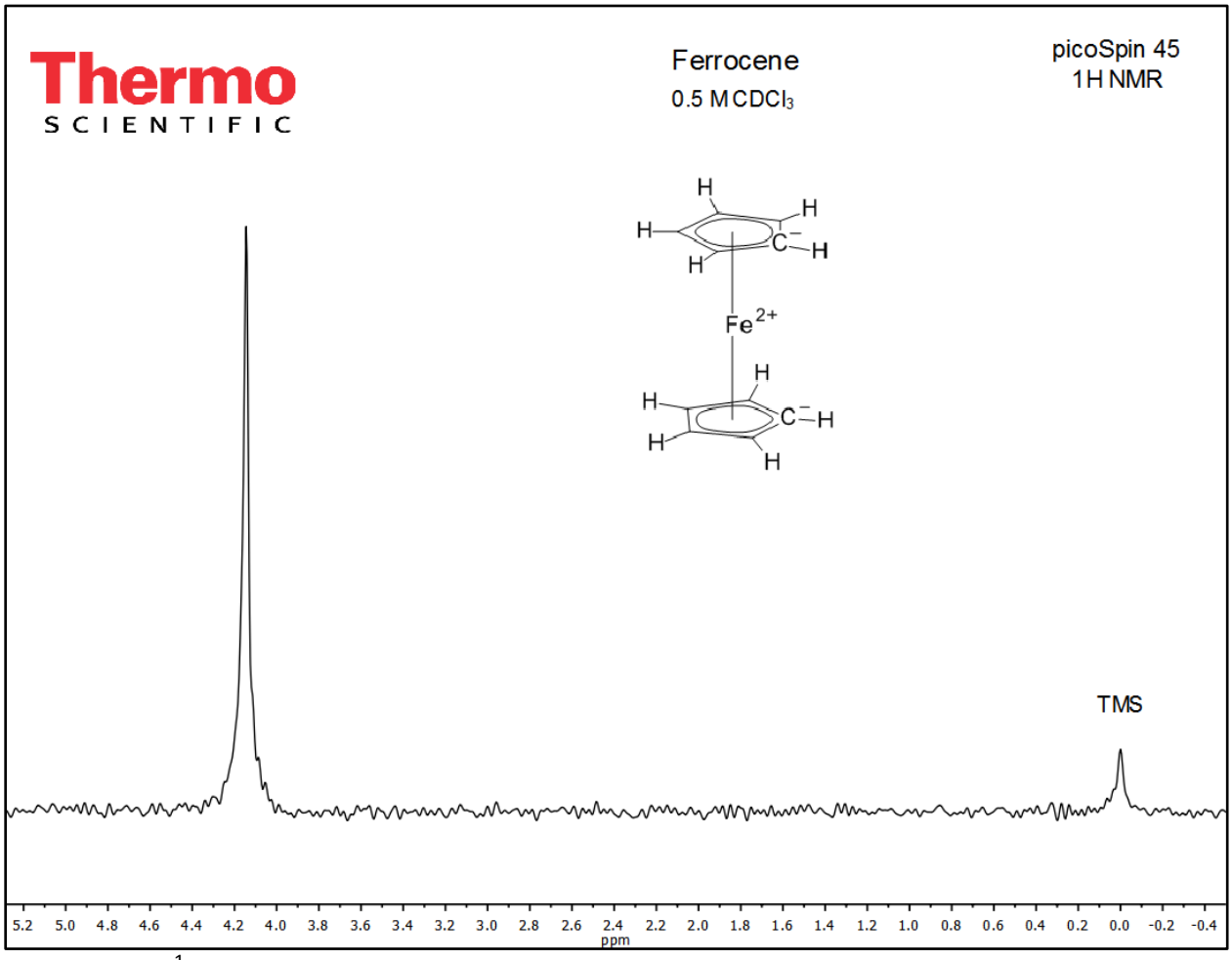


Figure 2. Full ^{1}H NMR (45 MHz) spectrum of a 0.5 M solution of ferrocene in $CDCl_3$.

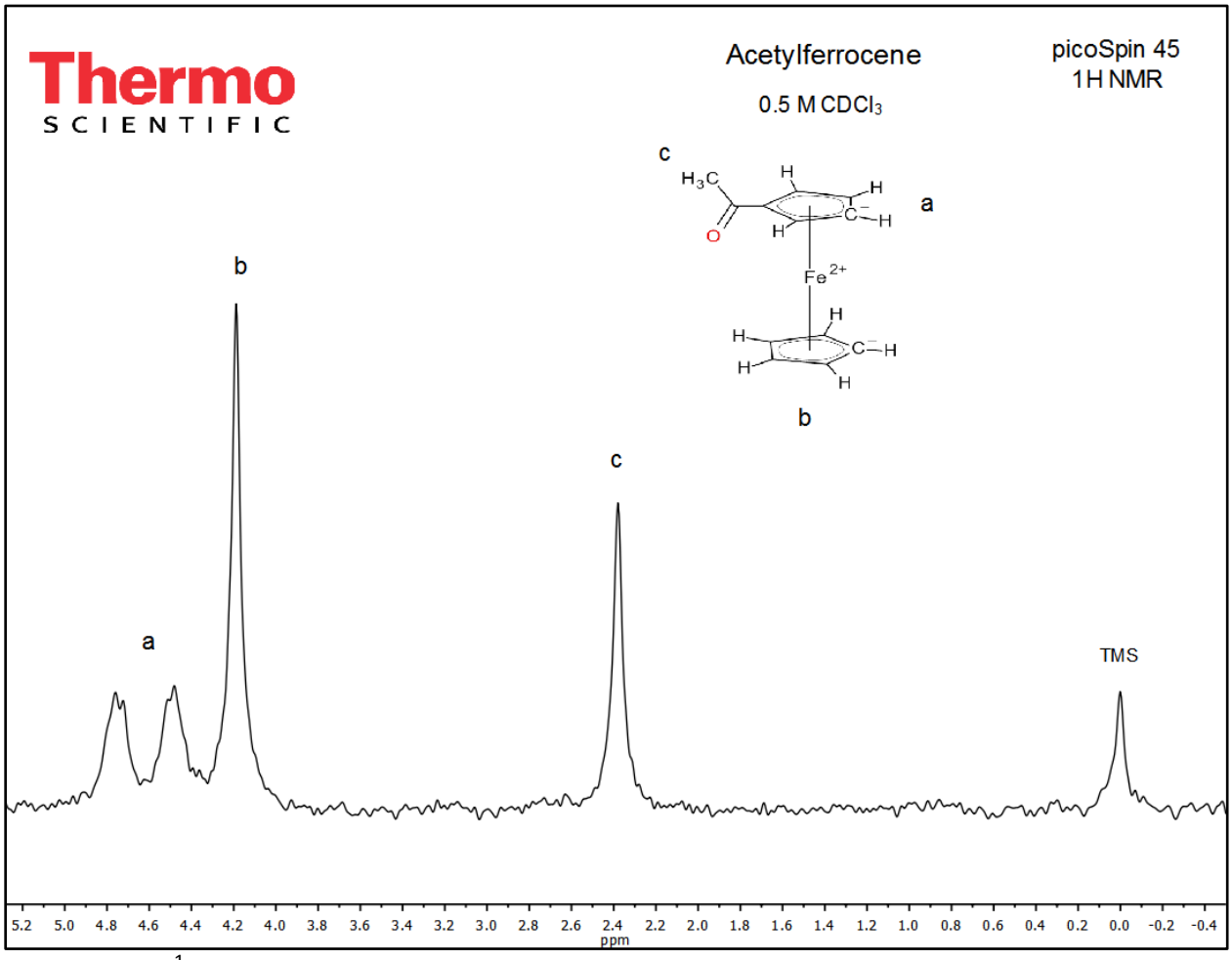


Figure 3. Full ^{1}H NMR (45 MHZ) spectrum of a 0.5 M solution of acetylferrocene in $CDCl_3$.

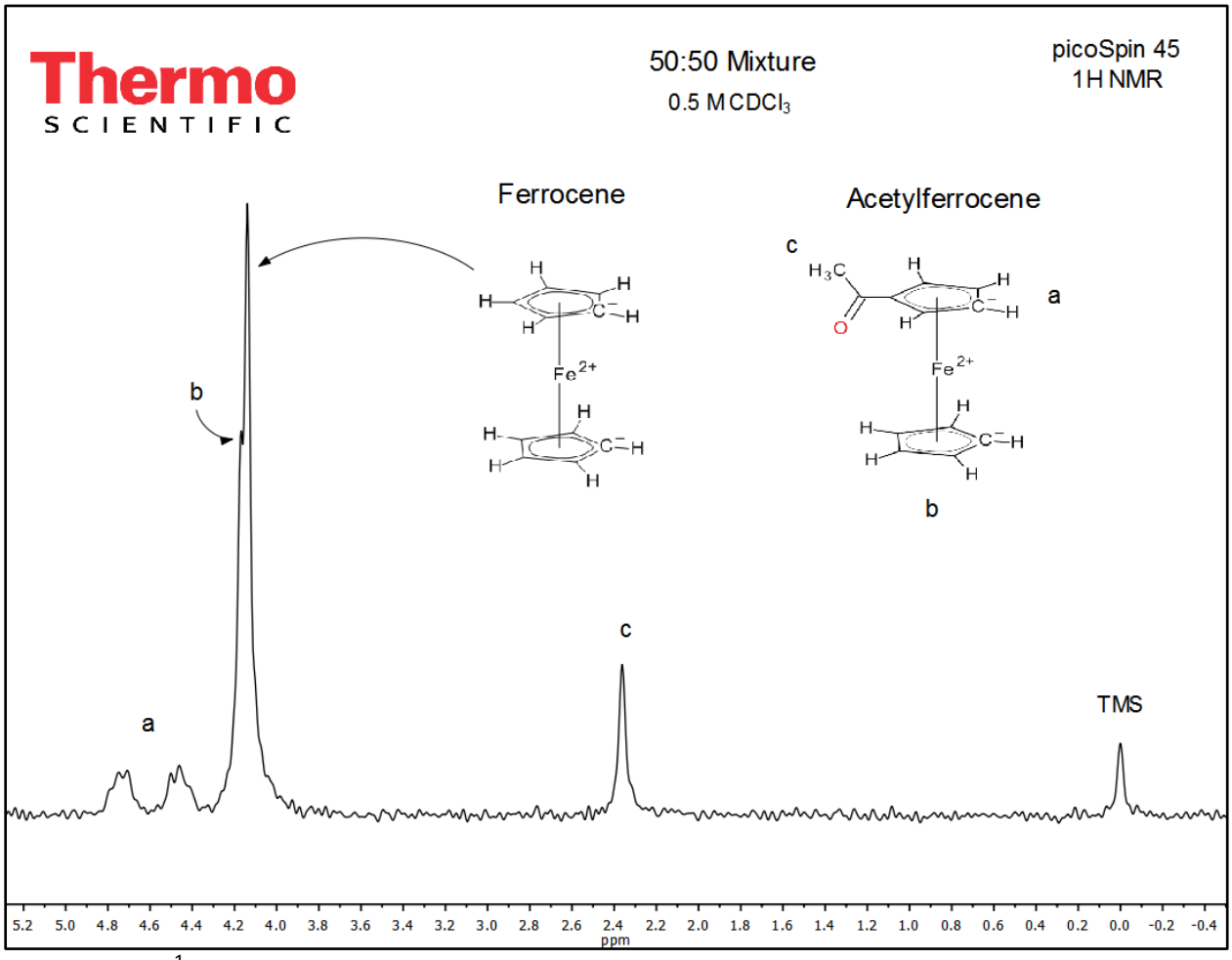


Figure 4. Full ^{1}H NMR (45 MHz) spectrum of a 0.5 M solution of a 50:50 mixture of ferrocene and acetylferrocene in $CDCl_3$.

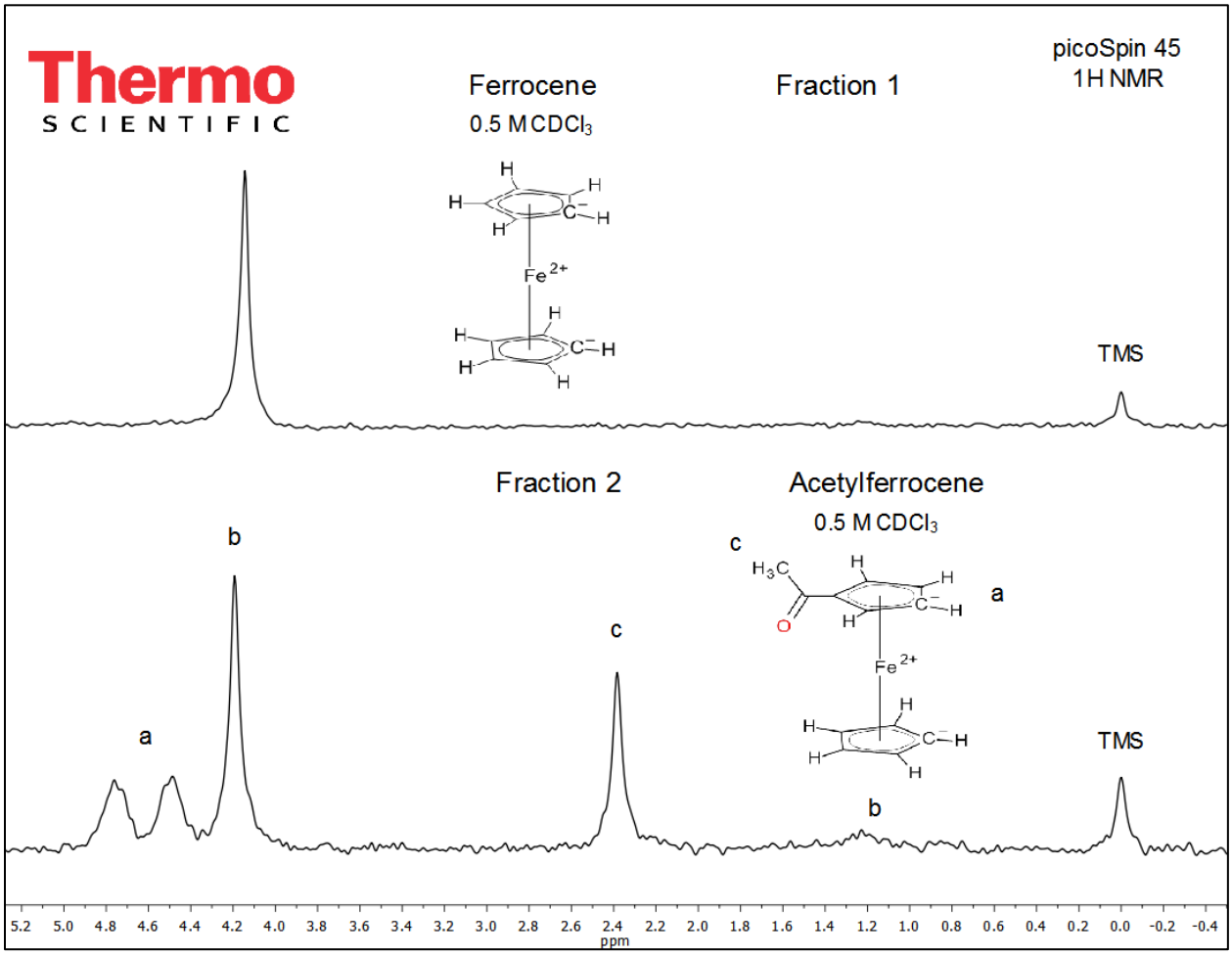


Figure 5 Full, stacked ^{1}H NMR (45 MHz) spectra of crude, chromatographic fraction 1 (top; ferrocene) and fraction 2 (bottom; acetylferrocene) after column separation.

Table 1. ^{1}H NMR Spectral Data

Figure	Compound	Signal Group	Chemical Shift (ppm)	Nuclides	Multiplicity
2-5	TMS	$Si(CH_3)_4$	0	12 H	singlet
2, 4, 5	ferrocene	$Fe[C_5H_5]_2$	4.14	10 H	singlet
3-5	acetylferrocene	$Fe[C_5H_5]C_5H_4C(O)CH_3$	2.38	3 H	singlet
		$Fe[C_5H_5]C_5H_4C(O)CH_3$	4.19	5 H	singlet
		$Fe[C_5H_5]C_5H_4C(O)CH_3$	4.48	2 H	singlet
		$Fe[C_5H_5]C_5H_4C(O)CH_3$	4.76	2 H	singlet
	water	HOD	4.65	1 H	singlet
	chloroform	$CHCl_3$	7.24	1 H	singlet
	acetone	$O=C(CH_3)_2$	2.05	6 H	singlet

8. Comments

The challenges of this lab experiment are manifold and largely related to preparing and using a microscale column.

1. Preparation of a microscale column in a Pasteur pipet – dry packing is preferred.
2. Maintaining adequate solvent volume above the column pack while simultaneously applying pressure with a pipet bulb.
3. Small headspace above the column pack after adding the pre-adsorbed mixture of alumina and then solvent.
4. Evaporating off relatively large quantities of eluting solvent.

9. Own Observations

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