Student's Instructions

You are provided with an unknown mixture containing are assigned unknown mixture containing 0.18 g nonpolar-neutral component, 0.18 g polar-neutral component, 0.18 g basic component, 0.18 g weakly acidic component and 0.18 g strongly acidic component. You are required to separate each component (using reagents provided in table 1) from the mixture and characterize the component using ^{1}H NMR and melting point. You will determine the identity of each of the component from the list of possible compounds whose Chemical Abstracts Service (CAS) numbers provided in table 2.

Table 1. Other Reagents Needed for the Lab.

Organic Solvents	Extraction Solvents	Drying Agents	Others
- Ethyl acetate For chromatography - 100% hexanes - 20% ethyl acetate in hexanes	- 1M HCl - 1M KOH - Sat. $NaHCO_3$	- Anhydrous Na_2SO_4 - Brine	- Distilled water - pH paper - Erlenmeyer flasks (125 mL) - Separatory funnels (125 mL) - Hirsh funnels and Side arm flasks - Ice/water baths - Beakers - Filter papers - TLC plates

Table 2. CAS numbers of possible compounds in the unknown mixture

86-73-7	99-92-3
92-88-6	100-09-4
92-91-1	103-3-0
97-52-9	108-73-6
99-04-7	120-61-6

Lab meeting 1:

i. Obtain your unknown mixture.
ii. Draw a flow chart outlining how you will separate each of the components in the unknown mixture (your flow chart must be approved by the instructor before you can proceed).
iii. For each extraction steps use no more than 10 mL of the extracting solvent chosen.
iv. Perform the extraction as you outlined in your flow chart and obtain the following solids.
    - Mixture of non-polar and polar neutral compounds
    - Basic compound
    - Weakly acid compound
    - Strongly acidic compound

Lab meeting 2:

i. Develop a TLC plate of the mixture of non-polar and polar neutral compounds in the solvent systems provided for chromatography.
ii. Run a column chromatography to separate the two neutral compounds.
iii. Combine the appropriate fractions and evaporate the solvents to obtain the solids
    - Non-polar neutral compound
    - Polar neutral compound

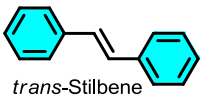
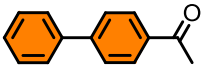
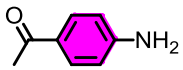
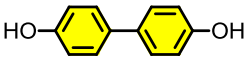
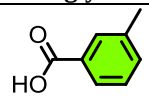
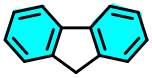
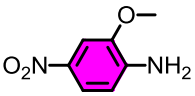
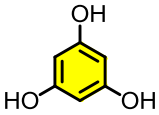
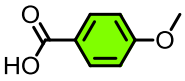
Lab meeting 3:

i. Obtain the mass, melting point ranges and ^{1}H NMR spectrum of each component.
ii. Determine the identity of each component (from list in table 2) based on the melting point and ^{1}H NMR data.

Note to Laboratory Instructors.

1. Preparation of unknowns

Table 1. Examples of Components that can be used to prepare the Unknowns.

	Neutral Non-Polar	Neutral Polar	Basic	Weakly Acidic	Strongly Acidic
Unknown mixture 1	 *trans*-Stilbene mpt. 123-125 °C CAS 103-3-0	 4-acetylbiphenyl mpt. 116-118 °C CAS 92-91-1	 4'-aminoacetophenone mpt. 103-107 °C CAS 99-92-3	 4,4'-dihydroxybiphenyl mpt. 280-282 °C CAS 92-88-6	 3-methylbenzoic acid mpt. 107-113 °C CAS 99-04-7
Unknown mixture 2	 Fluorene mp mpt. 111-114 °C CAS 86-73-7	 Dimethyl terephthlate mpt. 139-141 °C CAS 120-61-6	 2-methoxy-4-nitroaniline mpt. 140-142 °C CAS 97-52-9	 phloroglucinol mpt. 219 °C CAS 108-73-6	 4-methoxybenzoic acid mpt. 182-185 °C CAS 100-09-4

a. Mix about 0.18 g of each of the solid components and use motor and pastel to grind the mixture into a fine powder.
b. Transfer the mixture into a vial labeled for example **unknown mixture 1**.

Table 2. Other Reagents Needed for the Lab.

Organic Solvents	Extraction Solvents	Drying Agents	Others
- Ethyl acetate	- 1M HCl - 1M KOH - Sat. $NaHCO_3$	- Anhydrous Na_2SO_4 - Brine	- Distilled water - pH paper - Erlenmeyer flasks (125 mL) - Separatory funnels (125 mL) - Hirsh funnels and Side arm flasks - Ice/water baths - Beakers - Filter papers - TLC plates
For chromatography - 100% hexanes - 20% ethyl acetate in hexanes			

c. Assign the students (they can work in pairs) the unknown mixture and ask them design a separation procedure using a flow chart.
d. Check and approve/disapprove the students suggested separation procedure before they proceed with the experiment.

Table 3. Suggested tasks to be completed during the laboratory meetings.

Week 1	Week 2	Week 3
- Flow Chart - Successful separation of strongly acidic component, weakly acidic component and basic component (let the solids dry for a week).	- TLC of the mixture of the neural components to determine appropriate solvent system. - Separate the mixture of the neural components using column chromatography.	- Obtain mass, melting point range and ^{1}H NMR spectra of the all the five components.

2. **Separation Procedure for the Five-Component Mixture.**

Flow Chart 1

5 COMPONENT MIXTURE

1M HCl extraction

Organic layer contains 4 components

Aqueous layer contains ammonium salt of basic component

1M KOH

Basic component

Sat. $NaHCO_3$ extraction

Organic layer contains 3 components

Aqueous layer contains benzoate salt of strongly acidic component

1M HCl

Strongly acidic component

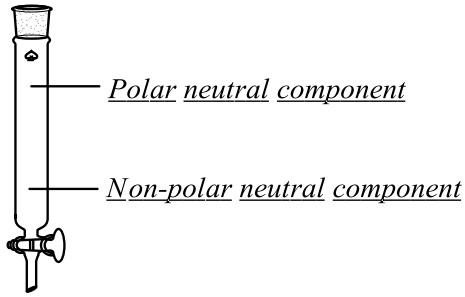
1M KOH extraction

Organic layer contains 2 components

Aqueous layer contains phenoxide salt of weakly acidic component

1M HCl

Weakly acidic component



Polar neutral component

Non-polar neutral component

3. **Example of separation of unknown mixture 1 using procedure outlined in flow chart 1.**

A. Week 1

i. Unknown mixture 1 was placed into a 125 mL Erlenmeyer flask. 25 mL ethyl acetate was added to the flask and the contents of the flask swirled to dissolve the solids. The resulting solution was transferred into a 125 mL separatory flask and extracted twice with 10 mL 1M HCl with the aqueous layers collected in a 125 mL Erlenmeyer flask labeled **HCl wash**. The HCl wash was basified (check with pH paper) with 1M KOH and chilled on an ice bath.*

ii. The remaining organic layer was extracted twice with 10 mL sat. $NaHCO_3$ with the aqueous layers collected in a 125 mL Erlenmeyer flask labeled **$NaHCO_3$ wash**. The $NaHCO_3$ wash was acidified (check with pH paper) with 1M HCl and chilled on an ice bath.

iii. The remaining organic layer was extracted twice with 10 mL 1M KOH with the aqueous layers collected in a 125 mL Erlenmeyer flask labeled **KOH wash**. The KOH wash was acidified (check with pH paper) with 1M HCl and chilled on an ice bath.

iv. The remaining organic layer was washed with 10 mL distilled water followed by 10 mL brine and collected in a 125 mL Erlenmeyer flask labeled **organic layer**. The organic layer was dried using anhydrous Na_2SO_4 and the solvent was evaporated under vacuum.

v. *No solids were formed in the HCl was so the basic component was isolated as follows; The HCl wash was transferred into transferred into a 125 mL separatory flask and extracted twice with 10 mL ethyl acetate. The combined organic layer was washed with 10 mL distilled water followed by 10 mL brine and collected in a 125 mL Erlenmeyer flask. The organic layer was dried using anhydrous Na_2SO_4 and the solvent was evaporated under vacuum to give the **basic component**.

vi. The precipitate in the flask labeled **$NaHCO_3$ wash** was collected via vacuum filtration, washed with three times with 10 mL of distilled water and allowed to air dry for 15 minutes. The solid was collected onto a watch glass labelled **strongly acidic component** and allowed to air dry for 1 week.

vii. The precipitate in the flask labeled **KOH wash** was collected via vacuum filtration, washed with three times with 10 mL of distilled water and allowed to air dry for 15 minutes. The solid was collected onto a watch glass labelled **weakly acidic component** and allowed to air dry for 1 week.

viii. The **organic layer** mixture was concentered under vacuum for chromatography.

B. Week 2

i. A TLC of the **organic layer** mixture was developed in 100% hexanes (image 1A left) and 20 % ethyl acetate in hexanes (image 1A right).

ii. The solids from the organic layer were separated via column chromatography using silica gel as the stationary phase and 100% hexanes then 20 % ethyl acetate in hexanes as mobile phase.

iii. TLC (in 100% hexanes) of the fractions collected (see image 1 B) shows successful separation of the two neutral components. Fractions 5 and 6 were combined and the solvent evaporated to give the **non-polar neutral component**. Fractions 16-18 the solvent evaporated to give the **polar neutral component.**

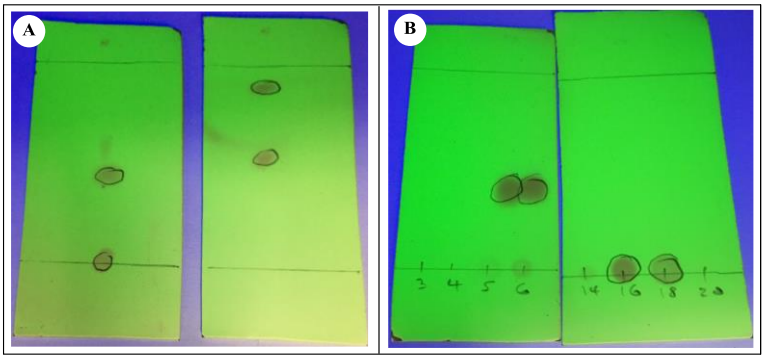


Image 1: TLC plates showing separation of the two neutral components.

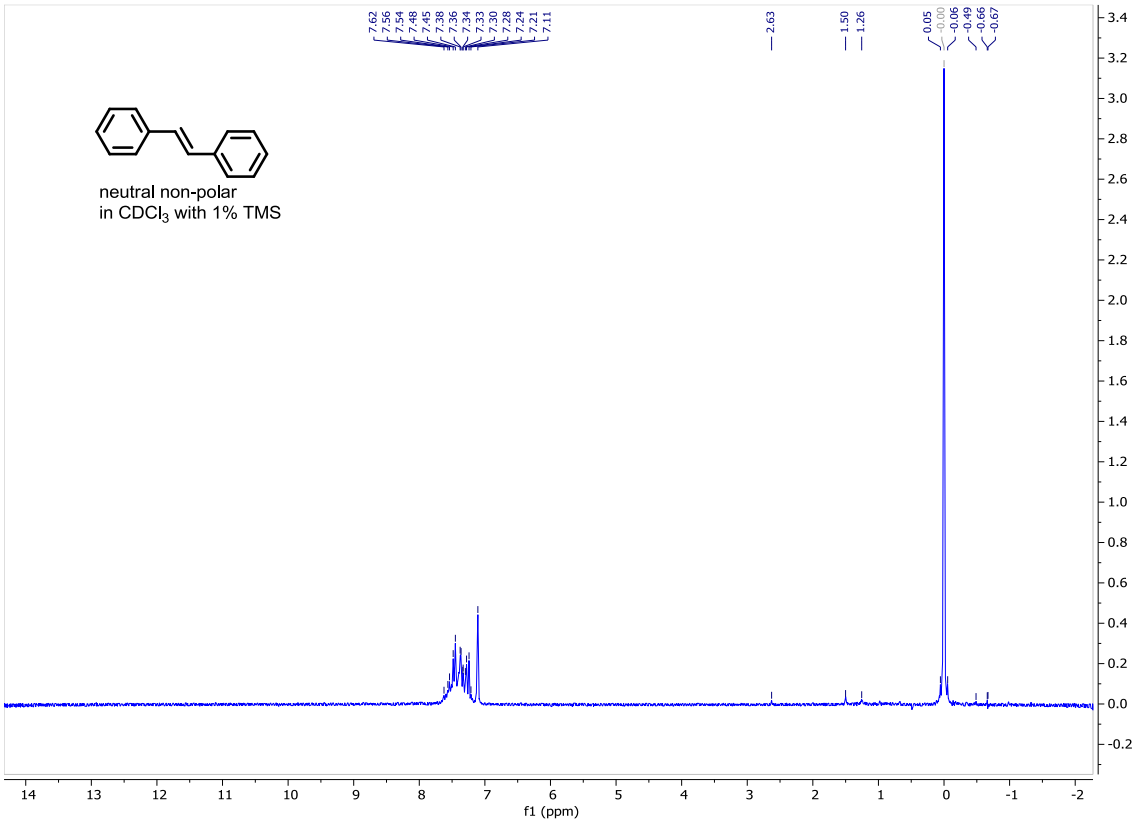
C. Week 3

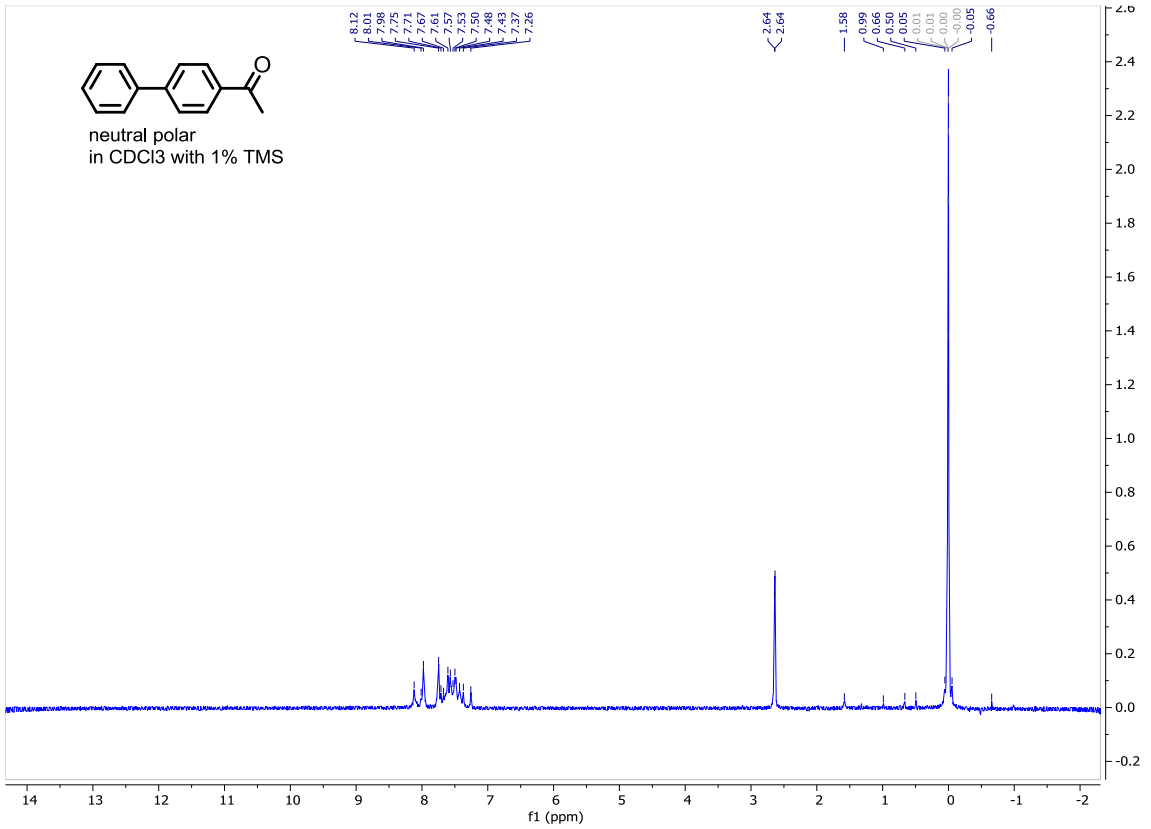
i. The mass and melting point ranges (average of three trials) of the five components were obtained (see table 4).

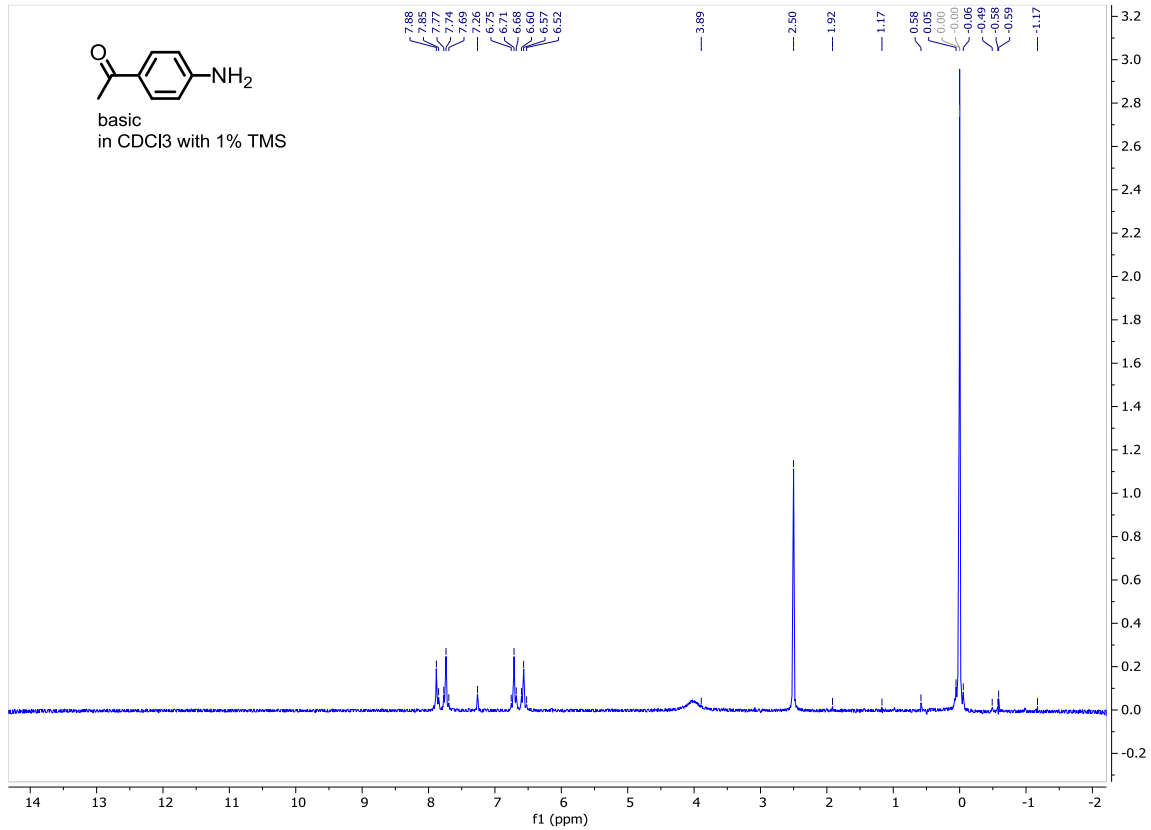
Table 4. Mass and Melting Point (mpt.) Data of the Five Components.

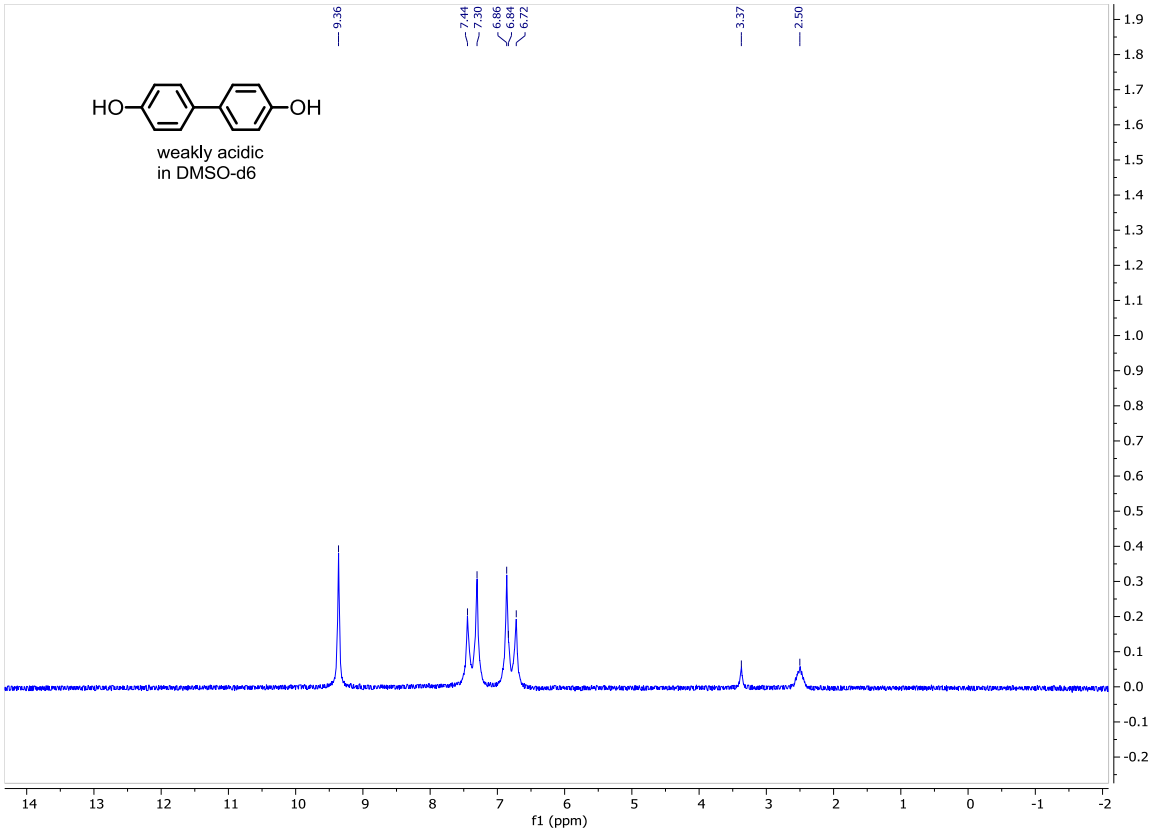
	Neutral Non-Polar	Neutral Polar	Basic	Weakly Acidic	Strongly Acidic
Mass Recovered (g) (% recovery)	0.093 (51%)	0.110 (61%)	0.119 (66%)	0.121 (67%)	0.107 (59%)
Experimental mpt. (°C)	123-125	120-123	106-109	284-287	112-114

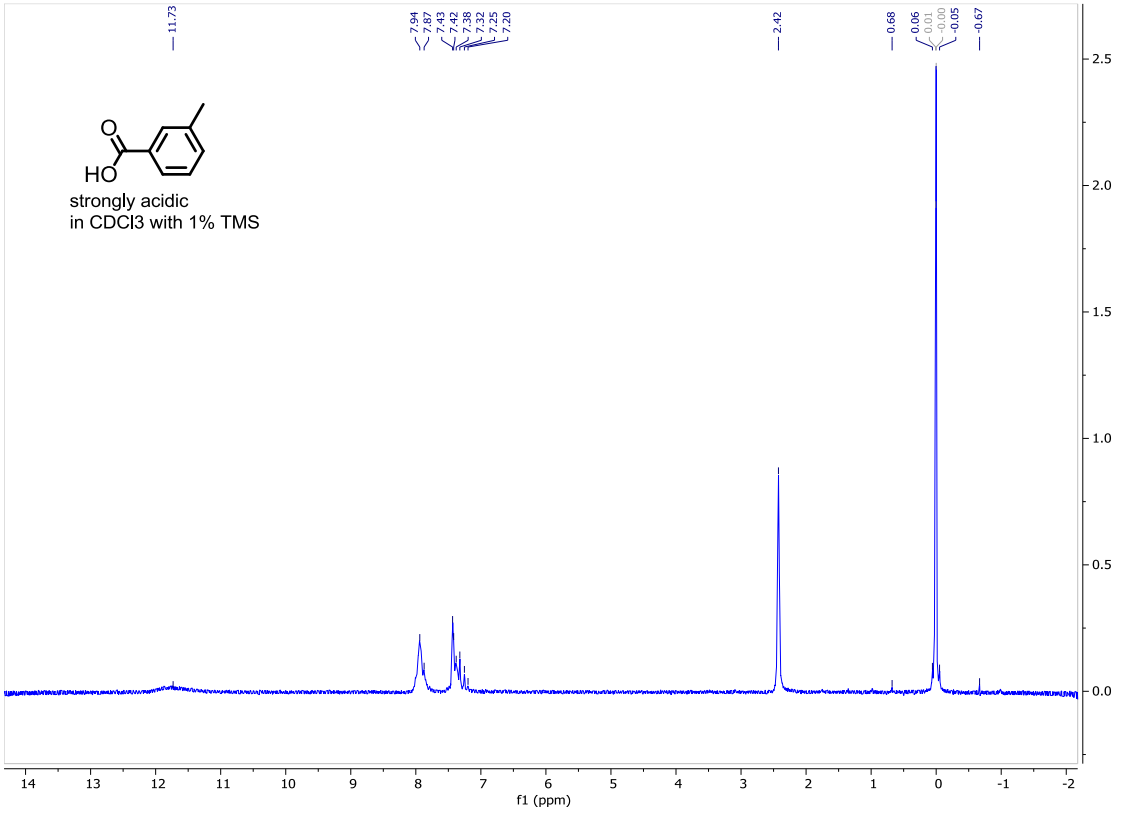
ii. ^{1}H NMR spectra (60 MHz) were obtained and are provided below.




neutral polar
in CDCl3 with 1% TMS
f1 (ppm)


basic
in CDCl3 with 1% TMS
f1 (ppm)


9.36
7.44
7.30
6.86
6.84
6.72
3.37
2.50
HO
OH
weakly acidic
in DMSO-d6
f1 (ppm)


11.73
7.94
7.87
7.43
7.42
7.38
7.32
7.25
7.20
2.42
0.68
0.06
0.01
0.00
-0.05
-0.67
O
HO
strongly acidic
in CDCl3 with 1% TMS
f1 (ppm)