

**NATIONAL UNIVERSITY OF LESOTHO**  
**FACULTY OF HEALTH SCIENCES**  
**DEPARTMENT OF PHARMACY**  
**BACHELOR OF PHARMACY (HONOURS)**  
**PHA 3406 – INSTRUMENTATION IN PHARMACEUTICAL ANALYSIS**  
**SUPPLEMENTARY EXAMINATION**

**AUGUST 2023**

**TIME 3 HOURS**

**TOTAL MARKS 100**

---

**INSTRUCTIONS:**

- **THIS PAPER CONSISTS OF 5 QUESTIONS, EACH CARRYING 20 MARKS**
- **ANSWER ALL QUESTIONS**
- **START EACH QUESTION ON A NEW PAGE**
- **MARKS ARE SHOWN IN PARENTHESIS AT THE END OF EACH QUESTION**

**Question 1****[20 marks]**

- a. Define the following terms [10]
- Spectroscopy
  - Chromophore
  - Wave number
  - Base peak
  - Molecular ion
  - Chemical ionisation
  - Electron ionisation
  - Electromagnetic radiation
  - Specific absorbance
  - Beer's law
- b. State one application IR spectroscopy in pharmaceutical analysis. [1]
- c. Explain the major difference in instrumentation between UV/Vis and IR [2]
- d. List any 4 applications of UV-Vis in pharmaceutical analysis [2]
- e. State Beer's Law and define each term that is characteristic of its equation [5]

**Question 2****[20 marks]**

In an effort to synthesise coumaranone derivatives, a medicinal chemist uses thin layer chromatography (TLC) to monitor the progress of a reactions.

- a. Describe chromatography as a separation technique (1), and outline step by step how to monitor the progress of the reaction using TLC (8). [9]
- b. Mention any four techniques that can be used to visualise the spots on the TLC plate. [4]
- c. In what class of chromatography does TLC fall? Explain your answer. [2]
- d. Mention one other importance of chromatography in pharmaceutical analysis. [1]
- e. Mention 2 advantages and 2 disadvantages of column chromatography [4]

**Question 3****[20 marks]**

Potassium sorbate is a popular preservative in liquid pharmaceutical preparations. However, ingestion of large amounts of potassium (K) leads to toxicity. A pharmaceutical preparation was analysed to determine the amount of potassium, given that its only source is the preservative. Reference standard potassium solutions were prepared and their signals

measured. An analyte sample whose potassium concentration was unknown was also measured. The data was recorded in the table below.

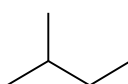
K Conc (ng/ml)	K signal response
20	64
30	130
40	171
50	186
60	287
Analyte sample	182

- Which calibration technique was employed in this problem? [2]
- Construct an equation of the straight line from this data. [10]
- Calculate the concentration of potassium in the unknown sample. [2]
- Calculate the standard deviation of the data. [2]
- Mention and describe two other methods of calibration. [4]

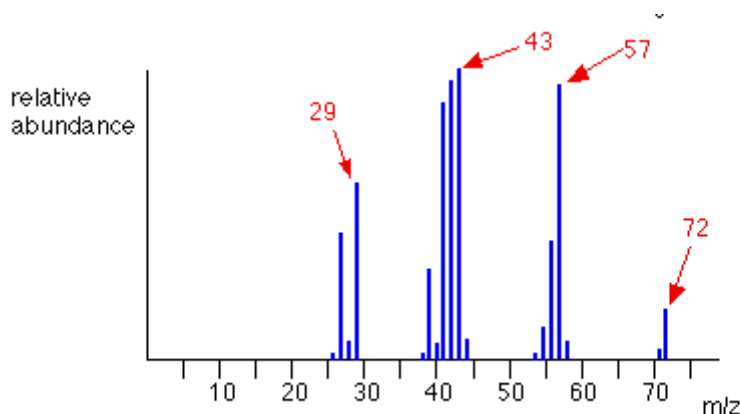
#### **Question 4**

**[20 marks]**

- State the major difference in instrumentation or technique between AES and AAS. [2]
- Mention any one limitation each of AAS and AES. [2]
- Describe homolytic cleavage (1) and mention any 5 functions of MS in pharmaceutical analysis (5) [6]
- The given MS spectrum belongs to 3-methylbutane.



3-methylbutane



(C; 12.011 g/mol), (O; 15.999 g/mol), (H; 1.00784 g/mol)

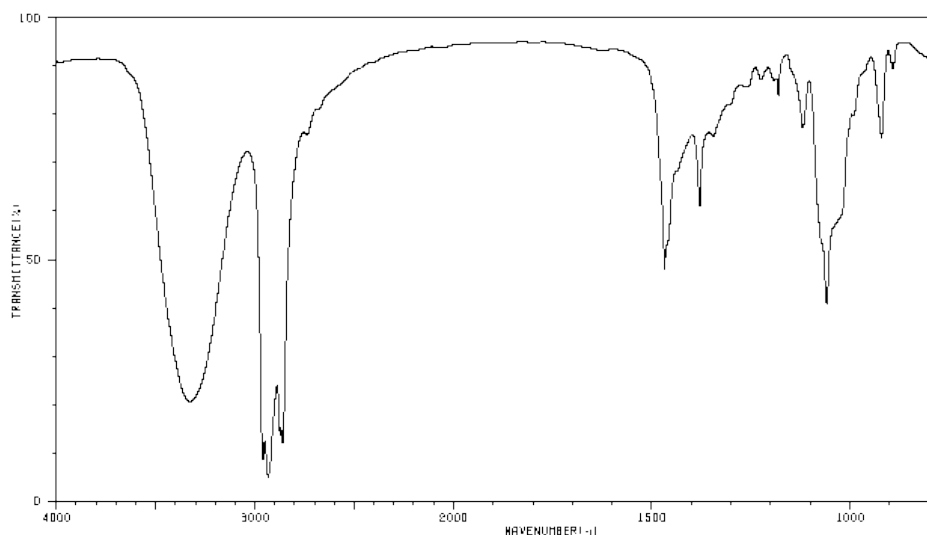
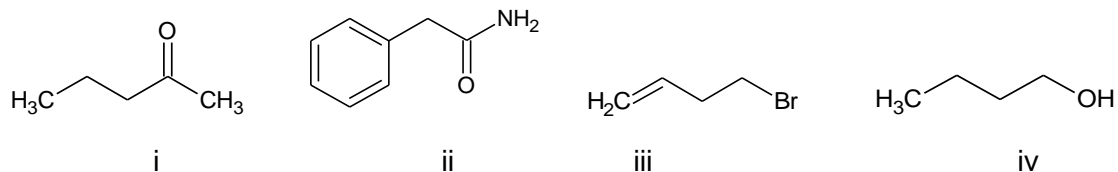
From the above data:

- i. Identify the molecular ion and the base peak [2]
- ii. Assign all characteristic fragments. [8]

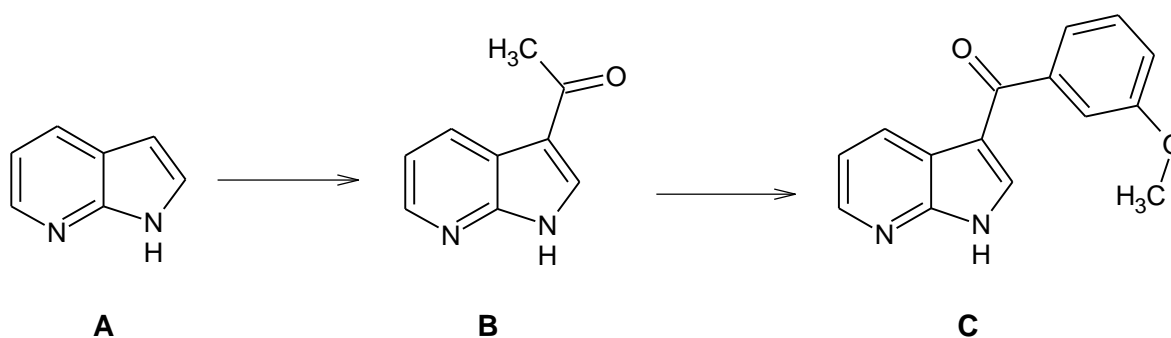
**Question 5**

**[20 marks]**

- a. The spectrum below belongs to one of the following compounds. Identify the compound to which this spectrum belongs (1). Support your answer with reasoning (4). [5]



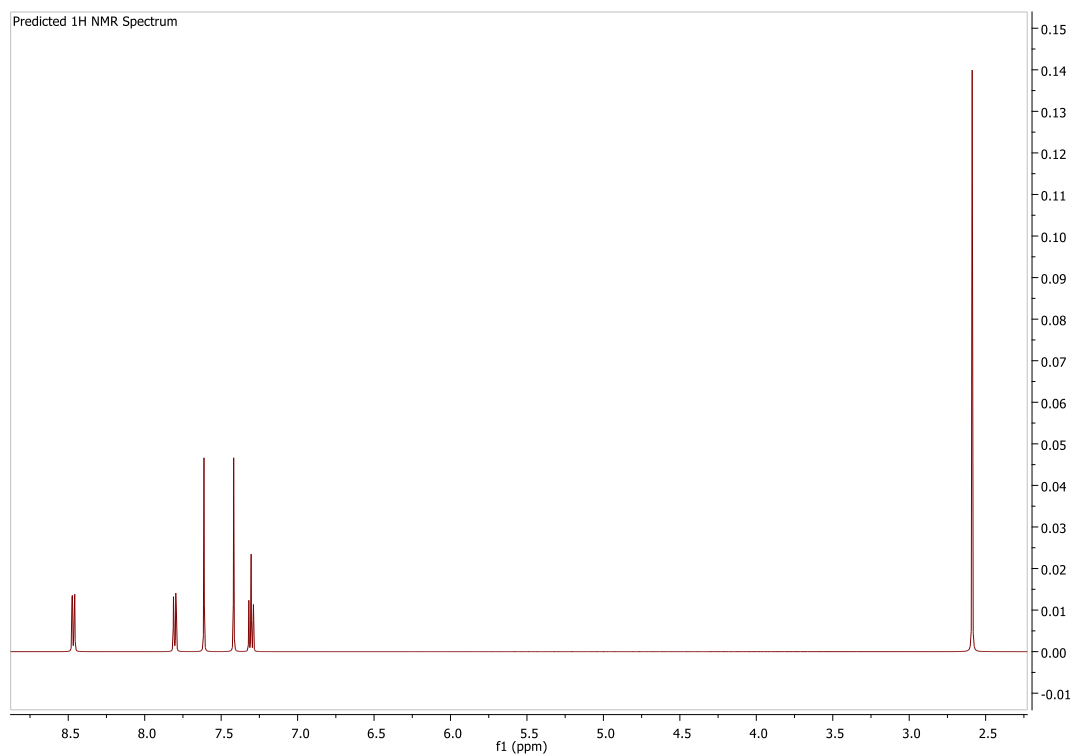
- b. A potential anti-TB drug (C) was synthesised from a series of reactions including synthesising B from A.



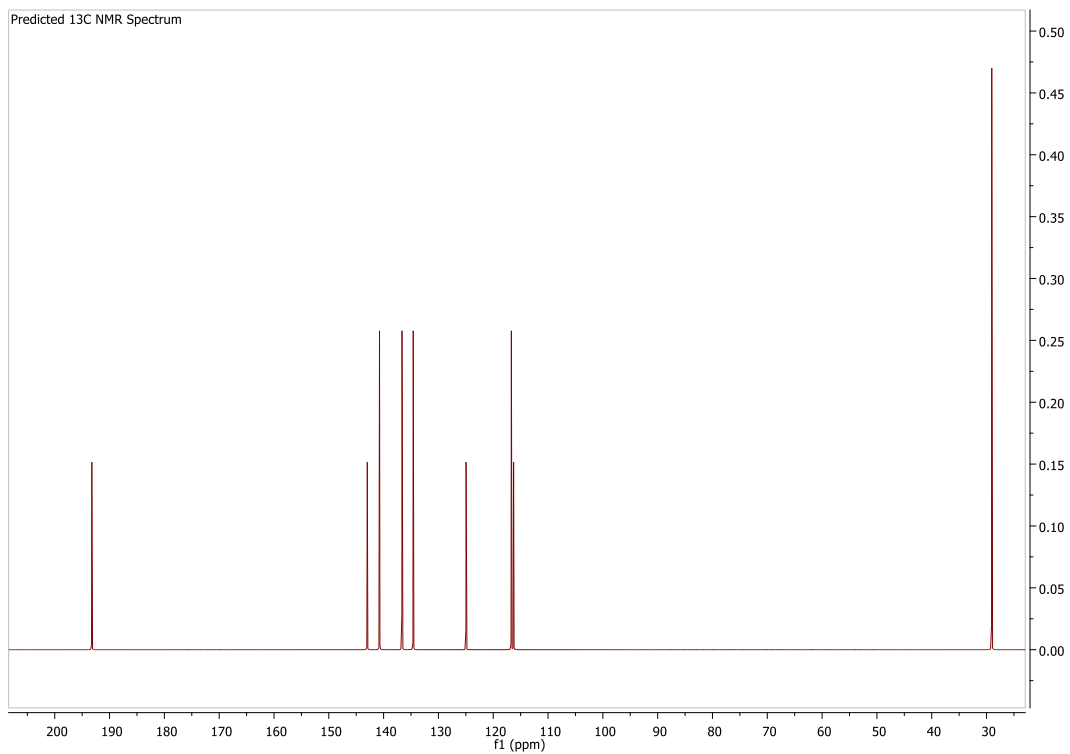
- i. Identify the spectral set that corresponds to C, and support your answer with reasoning. [3]
- ii. Using the structural formulae provided above, assign all of the characteristic H-NMR and C-NMR peaks to the spectra set that corresponds to compound C. [12]

### Spectra set 1

#### HNMR spectrum

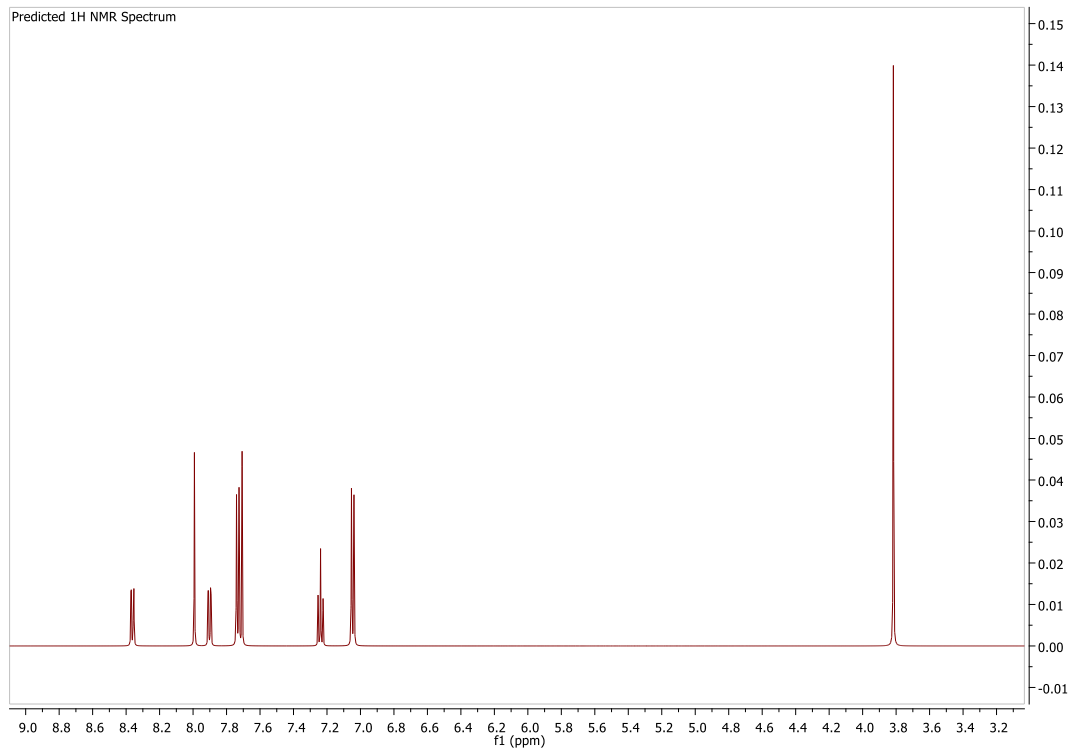


#### CNMR spectrum

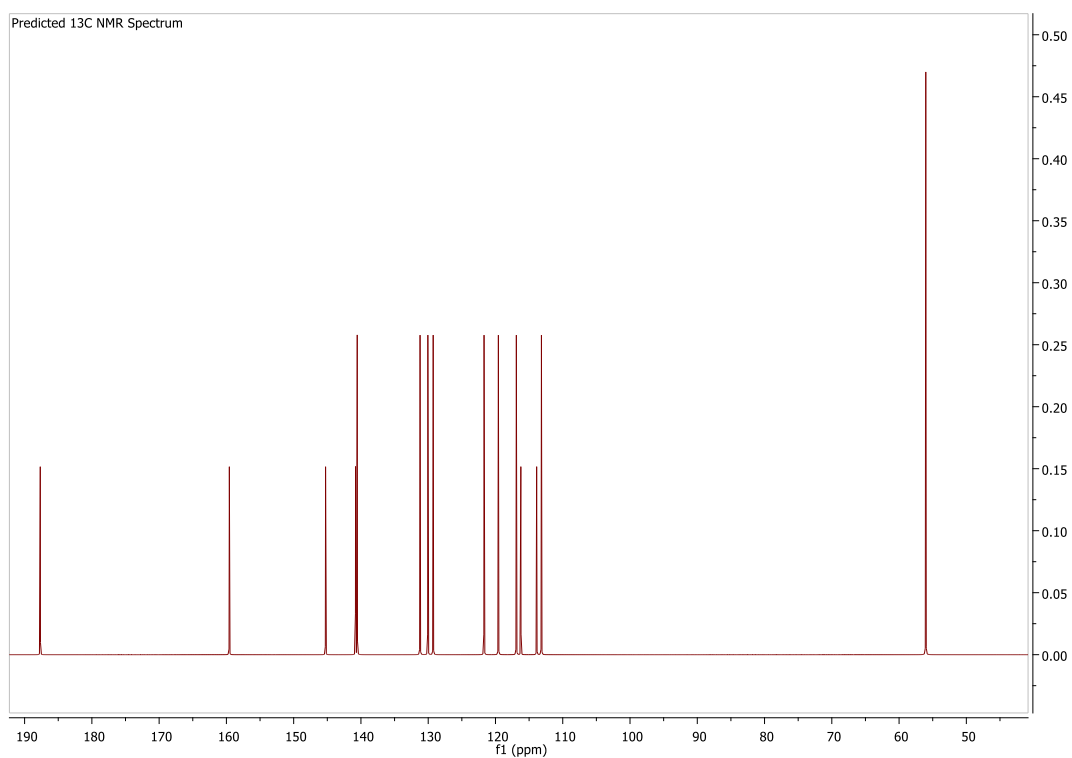


## Spectra set 2

### HNMR spectrum

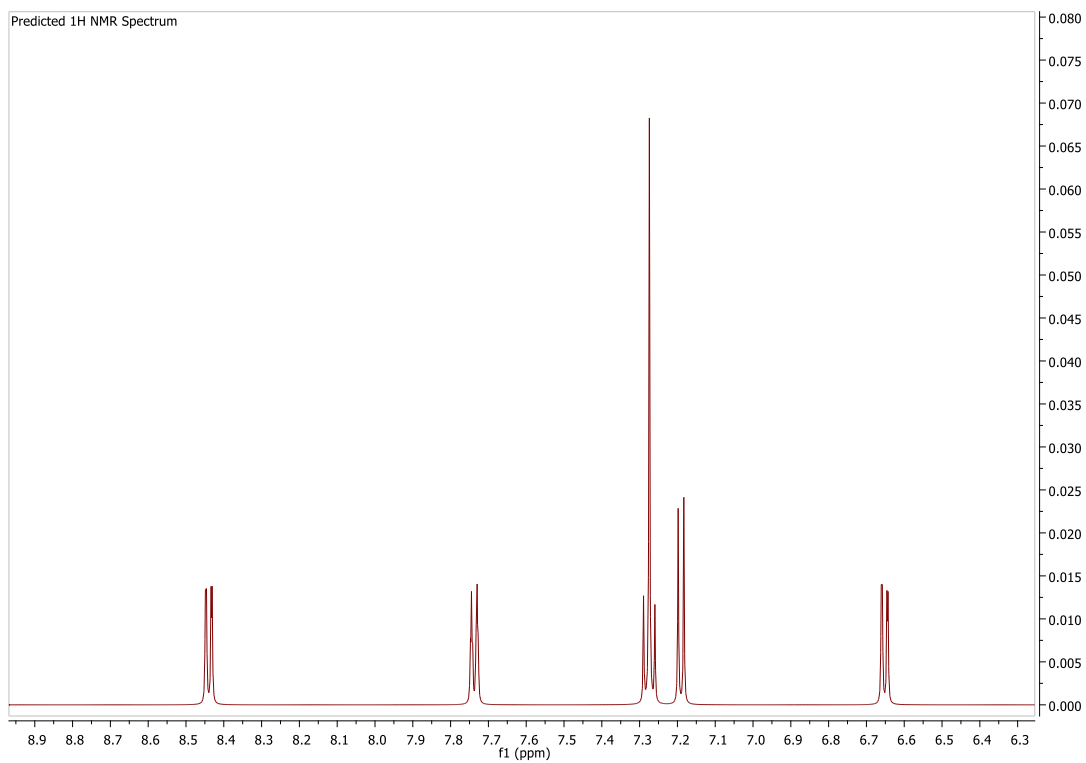


### CNMR spectrum

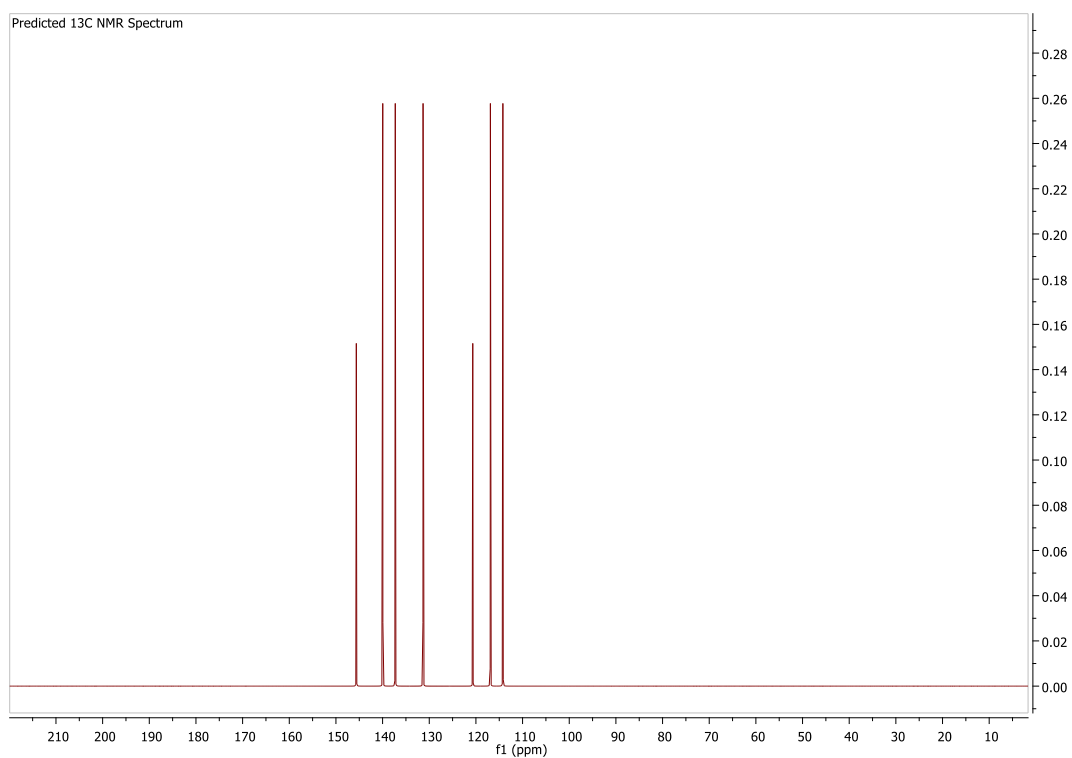


### Spectra set 3

### HNMR spectrum



# CNMR spectrum





## APPENDIX

### Formulas

$$y = mx + c$$

$$m = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sum(x_i - \bar{x})^2}$$

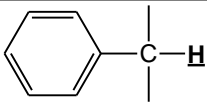
$$c = \bar{y} - m\bar{x}$$

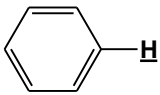
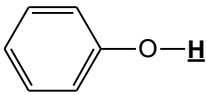
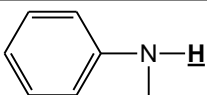
**Table 1: Characteristic infrared absorption peaks**

Bond	Functional group	Wavenumber	Intensity
<b>C-H</b>	Alkanes (stretch)	3000–2850	s
	-CH <sub>3</sub> (bend)	1450 and 1375	m
	-CH <sub>2</sub> - (bend)	1465	m
	Alkenes (stretch)	3100–3000	m
	(out-of-plane bend)	1000–650	s
	Aromatics (stretch)	3150–3050	s
	(out-of-plane bend)	900–690	s
	Alkyne (stretch)	ca. 3300	s
	Aldehyde	2900–2800	w
	<b>C-C</b>	Alkane	Not interpretatively useful
<b>C=C</b>	Alkene	1680–1600	m-w
	Aromatic	1600 and 1475	m-w
<b>C≡C</b>	Alkyne	2250–2100	m-w
<b>C=O</b>	Aldehyde	1740–1720	s
	Ketone	1725–1705	s
	Carboxylic acid	1725–1700	s
	Ester	1750–1730	s
	Amide	1700–1640	s
	Anhydride	1810 and 1760	s
	Acid chloride	1800	s
	<b>C-O</b>	Alcohols, ethers, esters, carboxylic acids, anhydrides	1300–1000
<b>O-H</b>	Alcohols, phenols		
	Free	3650–3600	m
	H-bonded	3400–3200	m
	Carboxylic acids	3400–2400	m
<b>N-H</b>	Primary and secondary amines and amides (stretch)	3500–3100	m
	(bend)	1640–1550	m-s
<b>C-N</b>	Amines	1350–1000	m-s
<b>C=N</b>	Imines and oximes	1690–1640	w-s
	Nitriles	2260–2240	m


<b>X=C=Y</b>	Allenes, ketenes, isocyanates, isothiocyanates	2270–1940	m-s
<b>N=O</b>	Nitro (R-NO <sub>2</sub> )	1550 and 1350	s
<b>S=O</b>	Sulfoxides	1050	s
	Sulfones, sulfonyl chlorides, sulfates, sulfonamides	1375–1300 and 1350– 1140	s
<b>C-X</b>	Fluoride	1400–1000	s
	Chloride	785–540	s
	Bromide, Iodide	< 667	s

**Table 2: Proton NMR Chemical shifts**

Proton type	Chemical shift (ppm)	Proton type	Chemical shift (ppm)
R-CH <sub>3</sub>	0.7 – 1.3	$\begin{array}{c}   \\ \text{R}-\text{N}-\text{C}-\text{H} \\   \end{array}$	2.2-2.9
R-CH <sub>2</sub> -R	1.2 – 1.4	$\begin{array}{c}   \\ \text{R}-\text{S}-\text{C}-\text{H} \\   \end{array}$	2.0-3.0
R <sub>3</sub> CH	1.4 – 1.7	$\begin{array}{c}   \\ \text{I}-\text{C}-\text{H} \\   \end{array}$	2.0-4.0
$\begin{array}{c}   \quad   \quad   \\ \text{R}-\text{C}=\text{C}-\text{C}-\text{H} \\   \end{array}$	1.6 – 2.6	$\begin{array}{c}   \\ \text{Br}-\text{C}-\text{H} \\   \end{array}$	2.7-4.1
$\begin{array}{c} \text{O} \quad   \\    \quad   \\ \text{R}-\text{C}-\text{C}-\text{H} \end{array}, \quad \begin{array}{c} \text{O} \quad   \\    \quad   \\ \text{H}-\text{C}-\text{C}-\text{H} \end{array}$	2.1 – 2.4	$\begin{array}{c}   \\ \text{Cl}-\text{C}-\text{H} \\   \end{array}$	3.1-4.1
$\begin{array}{c} \text{O} \quad   \\    \quad   \\ \text{OR}-\text{C}-\text{C}-\text{H} \end{array}, \quad \begin{array}{c} \text{O} \quad   \\    \quad   \\ \text{OH}-\text{C}-\text{C}-\text{H} \end{array}$	2.1-2.5	$\begin{array}{c} \text{O} \quad   \\    \quad   \\ \text{R}-\text{S}-\text{O}-\text{C}-\text{H} \end{array}$	ca. 3.0
$\begin{array}{c}   \\ \text{R}-\text{C}\equiv\text{C}-\text{C}-\text{H} \\   \end{array}$	2.1-3.0	$\begin{array}{c} \text{O} \quad   \\    \quad   \\ \text{OR}-\text{C}-\text{H} \end{array}, \quad \begin{array}{c} \text{O} \quad   \\    \quad   \\ \text{OH}-\text{C}-\text{H} \end{array}$	3.2-3.8
	2.3-2.7	$\begin{array}{c} \text{O} \quad   \\    \quad   \\ \text{OR}-\text{C}-\text{O}-\text{C}-\text{H} \end{array}$	3.5-4.8
$\begin{array}{c}   \\ \text{R}-\text{C}\equiv\text{C}-\text{H} \end{array}$	1.7-2.7	$\begin{array}{c}   \\ \text{O}_2\text{N}-\text{C}-\text{H} \\   \end{array}$	4.1-4.3
R-S-H var	1.0-4.0	$\begin{array}{c}   \\ \text{F}-\text{C}-\text{H} \\   \end{array}$	4.2-4.8
$\begin{array}{c}   \\ \text{R}-\text{N}-\text{H} \\   \end{array}$ var	0.5-4.0	$\begin{array}{c}   \quad   \\ \text{R}-\text{C}=\text{C}-\text{H} \end{array}$	4.5-6.5

R-O-H var	0.5-5.0		6.5-8.0
 var	4.0-7.0	$\text{R}-\overset{\text{O}}{\parallel}{\text{C}}-\text{H}$	9.0-10.0
 var	3.0-5.0	$\text{R}-\overset{\text{O}}{\parallel}{\text{C}}-\text{OH}$	11.0-12.0
$\text{R}-\overset{\text{O}}{\parallel}{\text{C}}-\text{N}-\text{H}$ var	5.0-9.0		

**Table 3: Carbon NMR Chemical shifts**

Carbon type	Chemical shift (ppm)	Proton type	Chemical shift (ppm)
R- $\underline{\text{C}}\text{H}_3$	8-30	$\underline{\text{C}}\equiv\text{C}$	65-90
R- $\underline{\text{C}}\text{H}_2\text{-R}$	15-55	$\underline{\text{C}}=\text{C}$	100-150
R <sub>3</sub> $\underline{\text{C}}\text{H}$	20-60	$\underline{\text{C}}\equiv\text{N}$	110-140
$\underline{\text{C}}\text{-I}$	0-40		110-175
$\underline{\text{C}}\text{-Br}$	25-65	$\text{R}-\overset{\text{O}}{\parallel}{\underline{\text{C}}}-\text{OR}, \text{R}-\overset{\text{O}}{\parallel}{\underline{\text{C}}}-\text{OH}$	155-185
$\underline{\text{C}}\text{-N}$	20-65	$\text{R}-\overset{\text{O}}{\parallel}{\underline{\text{C}}}-\text{NH}_2$	155-185
$\underline{\text{C}}\text{-Cl}$	35-80	$\text{R}-\overset{\text{O}}{\parallel}{\underline{\text{C}}}-\text{R}, \text{R}-\overset{\text{O}}{\parallel}{\underline{\text{C}}}-\text{H}$	185-220
$\underline{\text{C}}\text{-O}$	40-80		

**Table 4: Common losses from molecular ions**

Loss amu	Radicals/neutral fragments lost	Interpretation	Loss amu	Radicals/neutral fragments lost	Interpretation
1	H•	Often a major ion in amines, alcohols and aldehydes	42	CH <sub>2</sub> =C=O	Acetate
2	H <sub>2</sub>		43	C <sub>3</sub> H <sub>7</sub> •	Readily lost if isopropyl group present
15	CH <sub>3</sub> •	Most readily lost from a quaternary carbon	43	CH <sub>3</sub> CO•	Methyl ketone
17	OH• or NH <sub>3</sub>		43	CO +CH <sub>3</sub>	

<b>18</b>	H <sub>2</sub> O	Readily lost from secondary or tertiary alcohols	44	CO <sub>2</sub>	Ester
<b>19/20</b>	F•/HF	Fluorides	45	CO <sub>2</sub> H•	Carboxylic acid
<b>28</b>	CO	Ketone or acid	46	C <sub>2</sub> H <sub>5</sub> OH	Ethyl ester
<b>29</b>	C <sub>2</sub> H <sub>5</sub> •		46	CO + H <sub>2</sub> O	
<b>30</b>	CH <sub>2</sub> O	Aromatic methyl ether	57	C <sub>4</sub> H <sub>9</sub>	
<b>31</b>	CH <sub>3</sub> O•	Aromatic methyl ether	59	CH <sub>3</sub> CONH <sub>2</sub>	Acetamide
<b>31</b>	CH <sub>3</sub> NH <sub>2</sub>	Secondary amine	60	CH <sub>3</sub> COOH	Acetate
<b>32</b>	CH <sub>3</sub> OH	Methyl ester	73	(CH <sub>3</sub> ) <sub>3</sub> Si•	Trimethylsilyl ether
<b>33</b>	H <sub>2</sub> O + CH <sub>3</sub> •		90	(CH <sub>3</sub> ) <sub>3</sub> SiOH	Trimethylsilyl ether
<b>35/36</b>	Cl•/HCl	Chloride			