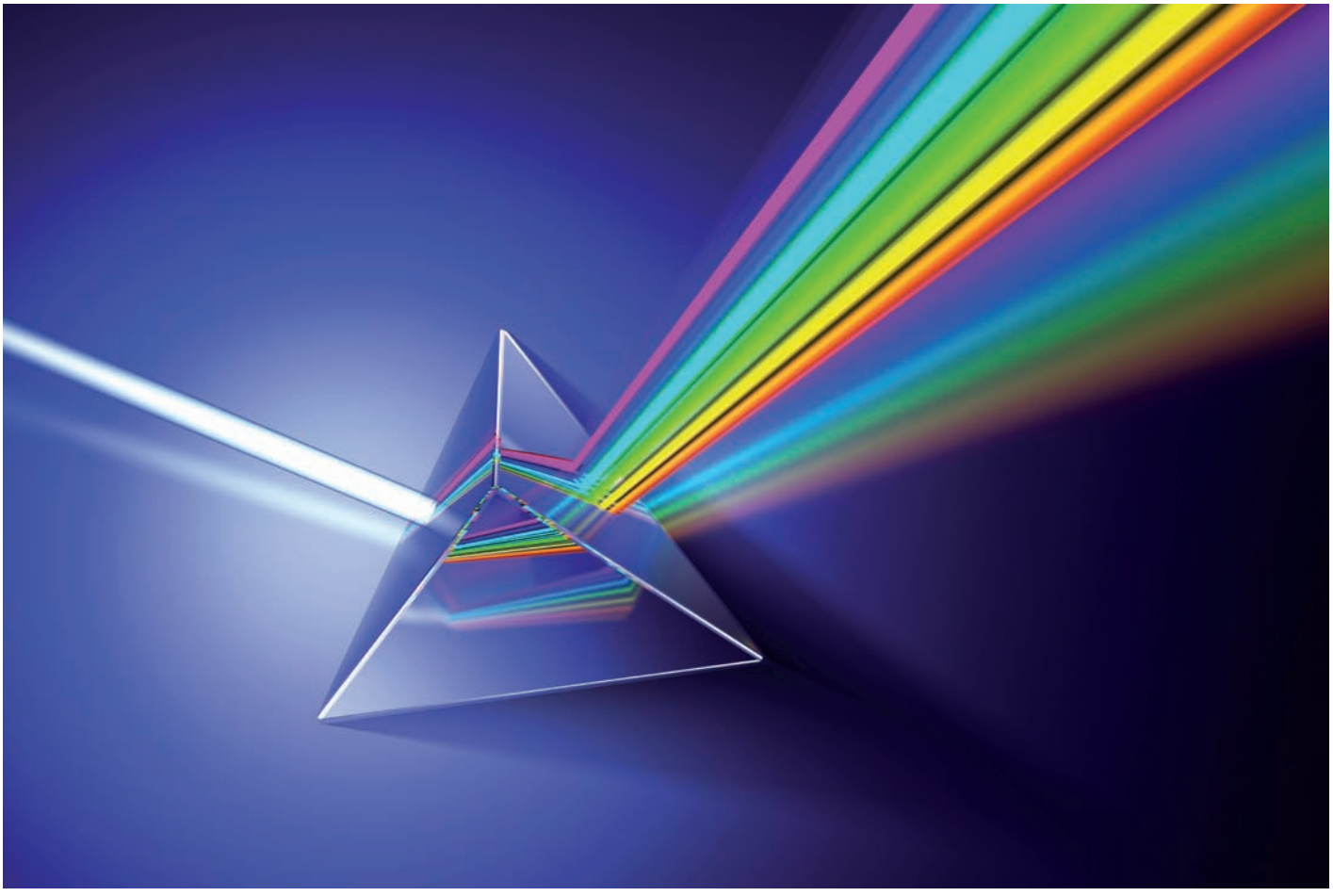
Spectroscopy

Student Notes



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Activity 1: Spectrophotometric measurement of concentration using colorimeters

This experiment uses colorimeters and is reliant upon Beer-Lamberts law whereby there is a proportional, straight line relationship between absorbance and concentration (for low concentration solutions). In this experiment you will be using solutions of potassium manganate VII.

This experiment follows the following steps:

1. Prepare the solutions
2. The stock solution provided contains 120 mg dm^{-3}. You are going to use this as your highest concentration. You will also need to dilute this with distilled water in order to complete the following table.

Conc. mg dm^{-3}	0	20	40	60	80	100	120
Absorbance A							

3. Finding the wavelength of maximum absorbance
4. Using the 60 mg dm^{-3} solution (a mid range solution) change the filter on the colorimeter to find out which wavelength gives the highest reading for A.

Measuring absorbance

At the wavelength from 2 record the absorbance for your blank (the solvent) by placing a cuvette containing the solvent into the colorimeter and pressing R. Replace the blank cuvette with all of your solutions, pressing T on each occasion and recording the reading.

Plotting a calibration graph

Using the same wavelength from 3 record the absorbance for all of your solutions. Plot this information on the graph paper. Concentration on X axis. Plot a line of best fit through the origin.

Concentration of the unknown solution

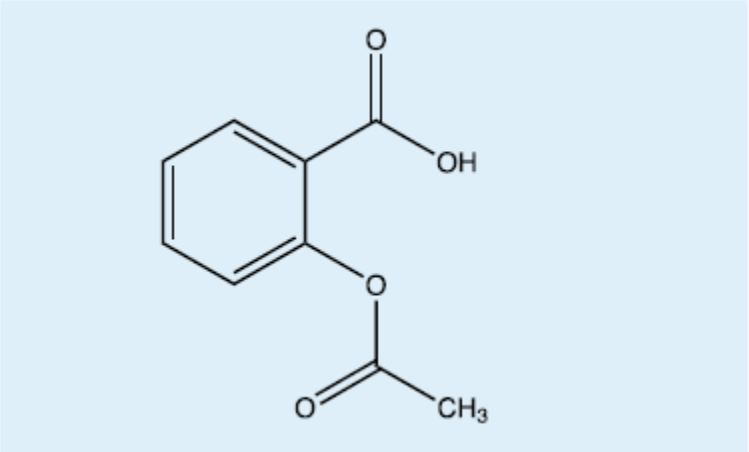
Using the same wavelength measure the absorbance of the unknown solution. Now use your calibration graph to determine the unknown concentration.

Concentration of unknown = _____ mg dm^{-3}

Activity 2: Body in a Lab: Aspirin Overdose

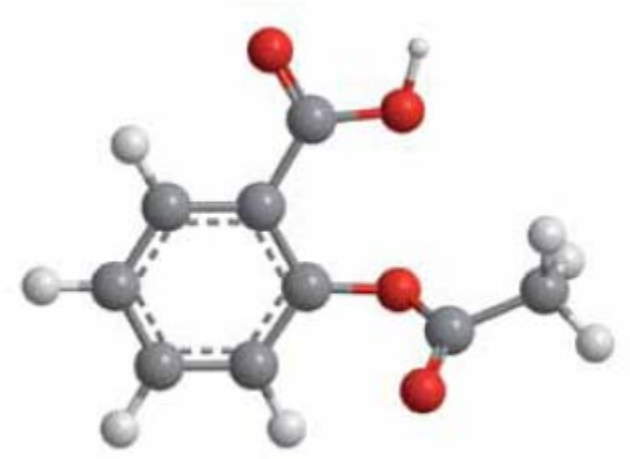
Introduction

A body has been found in the Lab! The deceased, Mr Blue, was known to be taking aspirin and a sample of his blood plasma has been sent for analysis. Use UV spectroscopy to determine the concentration of aspirin in the body and ascertain if the amount present was enough to be the cause of death.



Analysis of Salicylate in Blood Plasma by UV-Visible Spectroscopy

Aspirin or acetyl salicylic acid is a widely available drug with many useful properties. It was one of the first drugs to be commonly available and it is still widely used with approximately 35,000 tonnes produced and sold each year, equating to approximately 100 billion aspirin tablets.



Aspirin is prepared by the acetylation of salicylic acid using acetic anhydride. Its many properties as a drug include its uses as an analgesic to reduce pain, anti-inflammatory to reduce inflammation, antipyretic to reduce temperature, and platelet aggregation inhibitor to thin the blood and stop clotting.

Therapeutic levels taken after a heart attack are typically 150 – 300 mg/L and for post by-pass operations 75 mg/L. The levels of salicylate present in blood plasma can be analysed using UV-visible spectroscopy to indicate if the subject has taken a therapeutic dose or an overdose (see following table).

Therapeutic	< 300 mg/L
Moderate Overdose	500 – 750 mg/L
Severe Overdose	>750 mg/L

Most adult deaths occur when the measured plasma level is greater than 700 mg/L. (Note: the maximum salicylate plasma levels usually occur approximately 4-6 hrs after ingestion).

Method

This method involves measuring the absorbance of the redviolet complex of ferric and salicylate ions at about 530 nm using a UV/Visible spectrometer. It is possible to use a colorimeter should a UV/Visible spectrometer is not available.

A 5% iron (III) chloride solution has been prepared for you (5g iron (III) chloride in 100 ml of de-ionised water).

1. Preparation of Salicylate Calibration Standards

A stock solution of 2000 mg/L salicylate in 250 ml of de-ionised water has been prepared for you by dissolving 580 mg sodium salicylate in a 250 ml volumetric flask.

Make up Standard Calibration Solutions (if this has not already been done for you)

In 100 ml standard volumetric flask dilute appropriate volumes of the stock solution to give 100, 200, 300, 400, and 500 mg/L salicylate calibration standards using the dilutions given below.

CONCENTRATION	DILUTION
100 mg/L	5 ml Stock Salicylate Solution in 100 ml De-ionised water
200 mg/L	10 ml Stock Salicylate Solution in 100 ml De-ionised water
300 mg/L	15 ml Stock Salicylate Solution in 100 ml De-ionised water
400 mg/L	20 ml Stock Salicylate Solution in 100 ml De-ionised water
500 mg/L	25 ml Stock Salicylate Solution in 100 ml De-ionised water

2. Prepare a Blank

In a test tube prepare a blank solution by taking 1 ml of de-ionised water and adding 4 ml of 5% iron (III) chloride solution.

3. Prepare Standards and Unknown Plasma Sample for UV/Vis Analysis

Prepare each of the standards and the unknown plasma sample by pipetting 1 ml into a separate test tubes and adding 4 ml of 5% iron (III) chloride solution to each (making sure each is carefully mixed).

4. Record the Absorbance

Transfer the calibration solutions, blank and unknown sample to separate cuvettes to record the absorbance. For each sample record the absorbance in the visible region between 400 – 600 nm. A peak should be observed at about 530 nm (see your demonstrator for instructions on using the UV/Visible spectrometer).

Analysis of results

1. Using the Beer-Lambert law plot the absorbance versus concentration calibration graph for the standards and using this find the unknown concentration of the salicylate present in the plasma.

2. Use this result to decide if the subject had taken a therapeutic or life threatening dose.

References

1. *Encyclopaedia of Analytical Science*, ed. Paul Worsfold, Alan Townshend, Colin Poole, Worsfold, Paul J. (2005), 543.003 ENC
2. *The Aspirin Foundation*
http://www.aspirin-foundation.com/what/index.htm
3. *Article by Professor A.N.P. van Heijst/ Dr A. van Dijk*
http://www.inchem.org/documents/pims/pharm/aspirin.htm
4. *Analysis of Analgesics*
http://faculty.mansfield.edu/bganong/biochemistry/aspirin.htm
5. *TLC of Analgesic Drugs*
http://www2.volstate.edu/msd/CHE/122/Labs/TLC.htm

Activity 3: Body in a Lab: Compound determination

Background

A body has been found in the lab!

The victim, Mr Blue, was known to have a heart condition but on the bench at the scene where the victim had been working a large bottle of concentrated acid had been upturned and spilt. All around this acid were different chemical bottles which also had been knocked over and may have mixed with the acid. A medicine bottle was also present with unknown tablets inside (Sample X - the tablets have been ground ready for analysis).

Objective

Try to establish cause of death by using infrared analysis to discover the functional groups present in the chemical samples collected. Decide if any of these are likely to be toxic or may have formed a lethal toxic gas on contact with the spilt acid. Establish the identity of the medicine found by library comparison of the spectra and suggest possible implications.

Method

You are provided unknown samples A – H and medicine sample X

1. Run a liquid film on all liquid samples.

2. Use the ATR attachment to run all solid samples.

(Note: Care must be taken with this expensive and fragile equipment, use only when supervised by a demonstrator).

Interpretation of Spectra

To interpret the spectra obtained from a sample it is necessary to refer to correlation charts and tables of infrared data. There are many different tables available for reference, one of which has been provided.

3. Using the correlation chart provided interpret your spectra and identify the functional groups present in the chemical.

Record your results in the table provided.

Identification of Unknown Compound

While IR spectroscopy is a very useful tool for identifying the functional groups in an unknown compound, it does not provide sufficient evidence to confirm the exact structure. Chemists make use of a variety of techniques in order to piece together the structure of a molecule.

4. Use your interpreted IR spectra and the mass spectra provided to determine the structure of all unknown compounds.

5. Identify any chemicals that you think may be toxic or where the functional group may possibly release a toxic gas on contact with an acid.

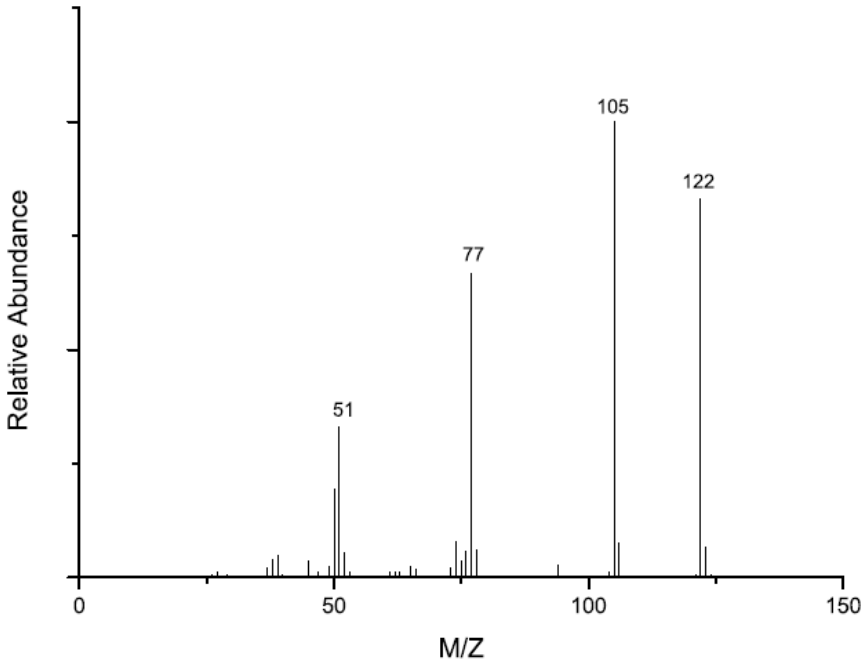
6. Suggest what other instrumental technique or techniques would be required to confirm the identity of the chemicals. (Your demonstrator will then be able to provide you with additional data for confirmation of analysis).

7. Identify sample X by using the library spectra.

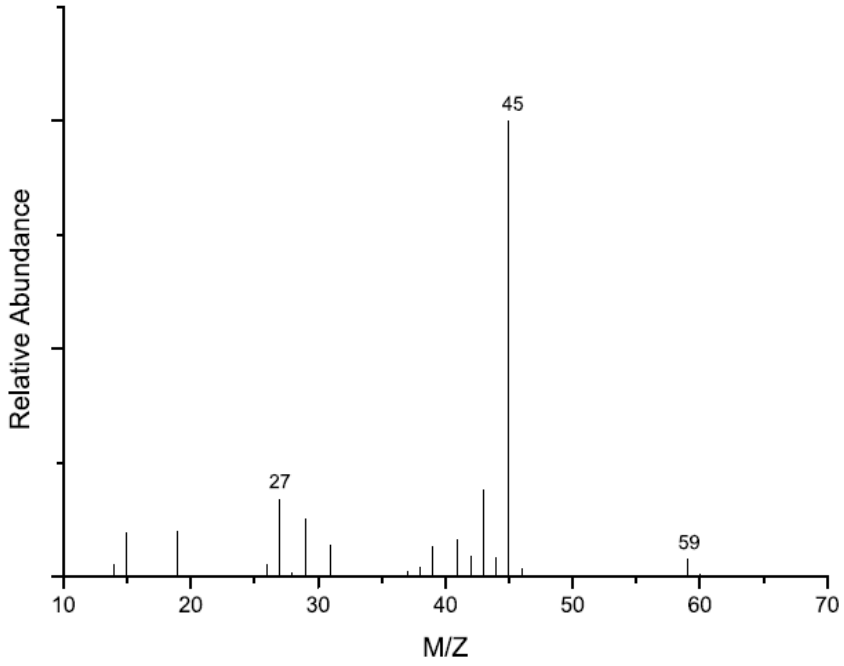
Identification of unknown compounds

Use interpreted IR spectra and mass spectra below to determine the structure of the unknown compounds

Sample A **Empirical Formula C7H6O2**

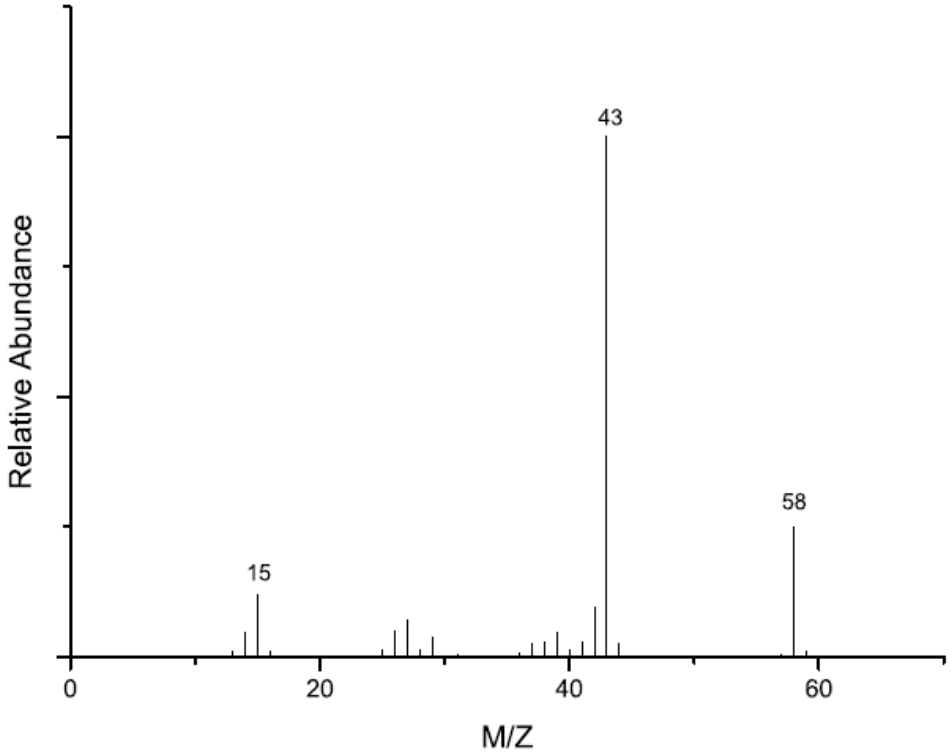


Sample B **Empirical Formula C3H8O**



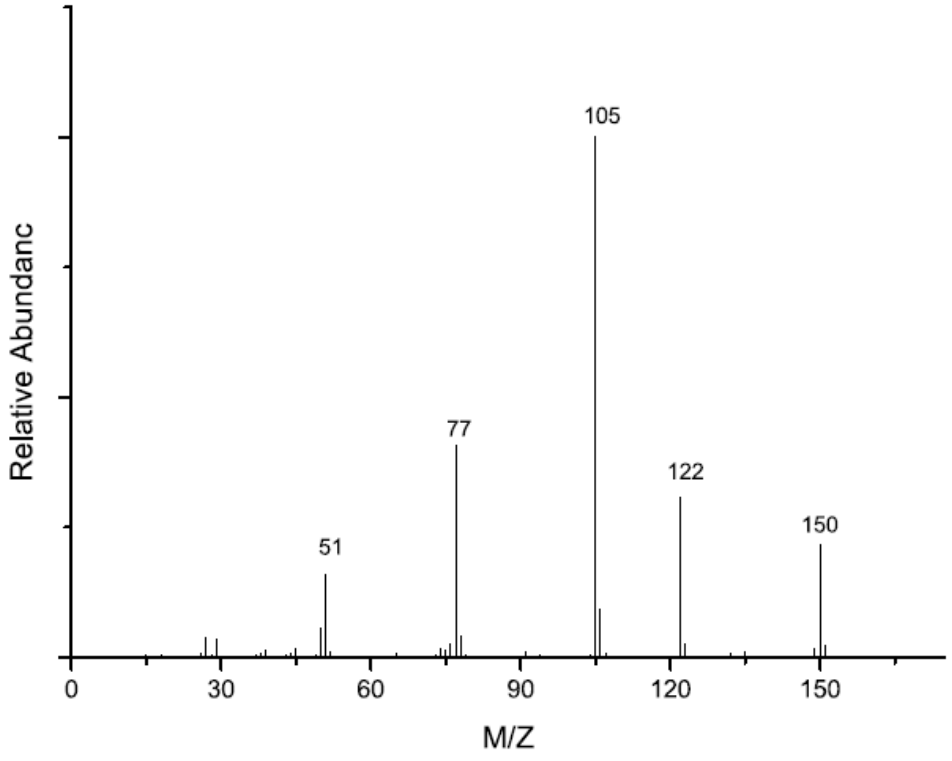
Sample C

Empirical Formula C3H6O



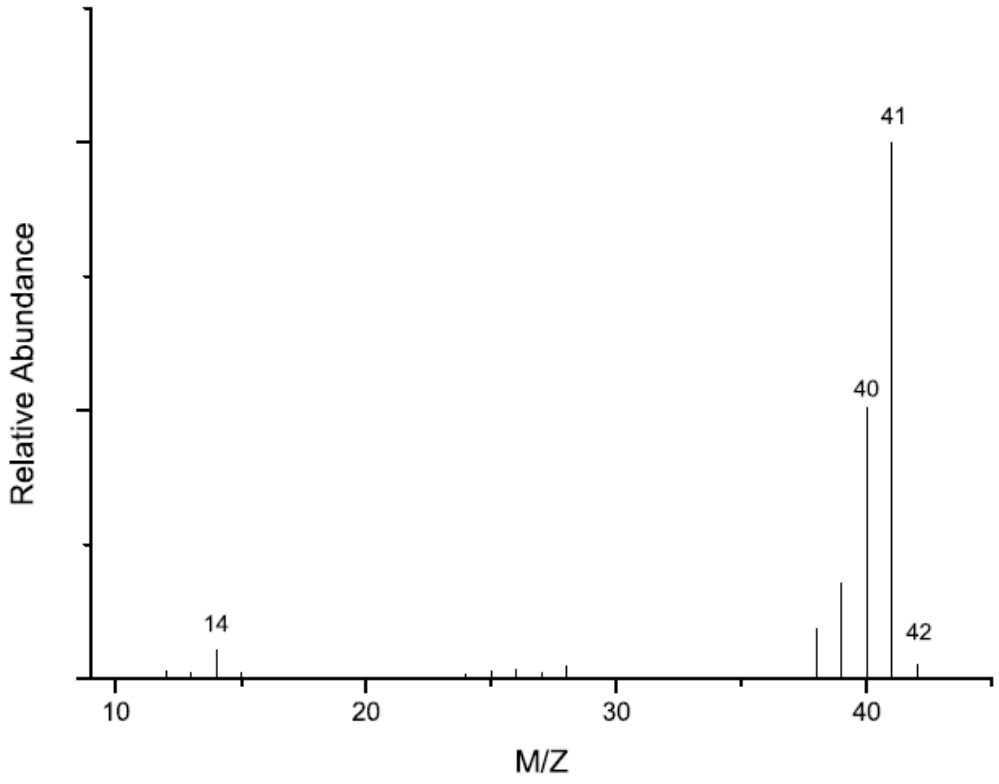
Sample D

Empirical Formula C9H10O2



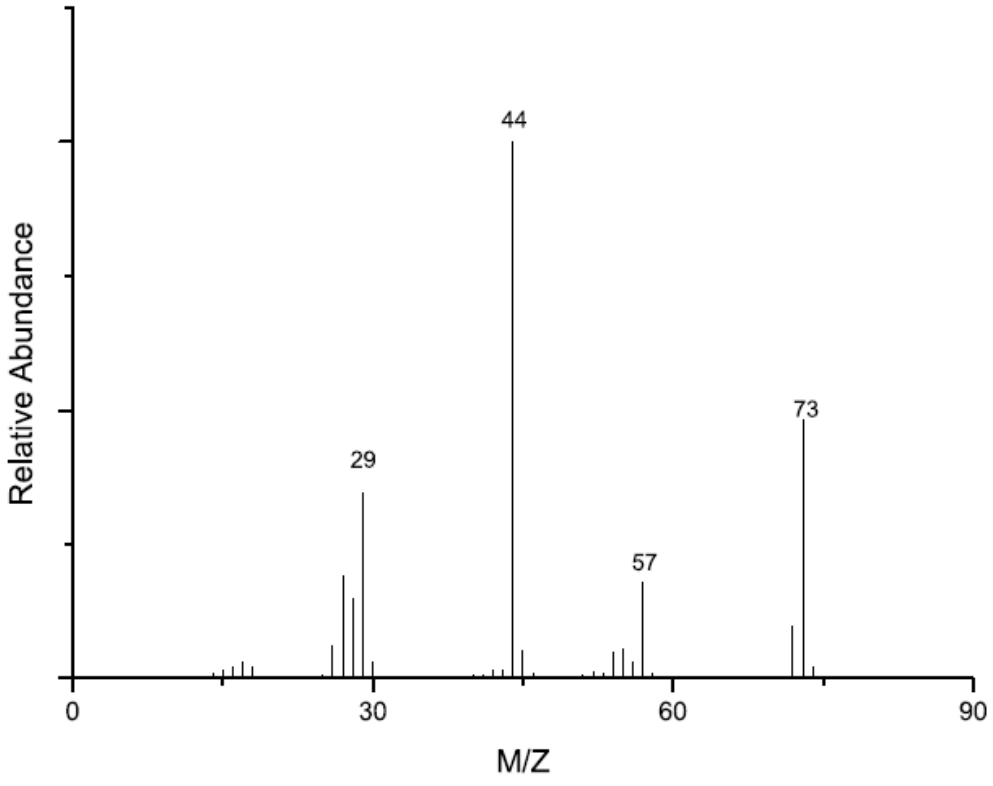
Sample E

Empirical Formula C2H3N



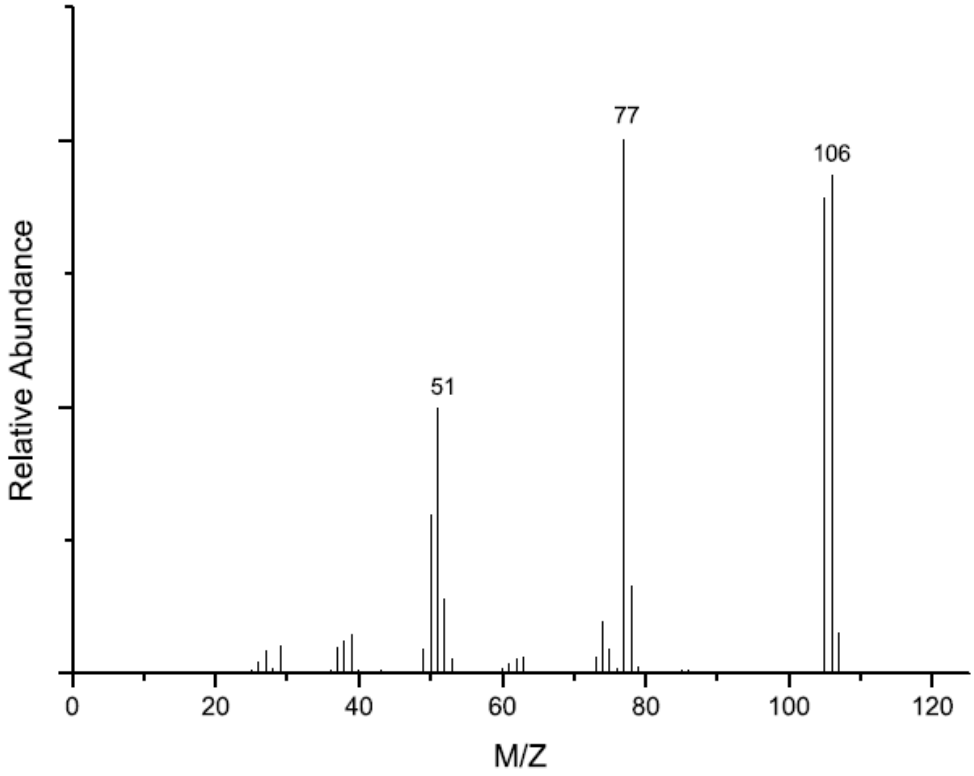
Sample F

Empirical Formula C3H7NO



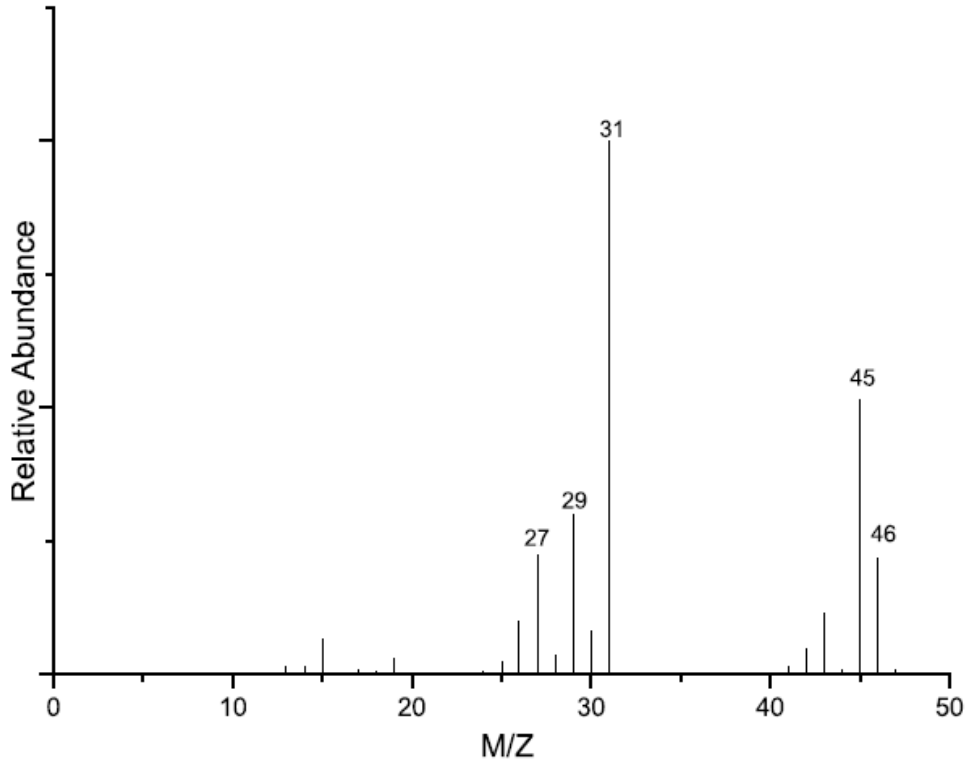
Sample G

Empirical Formula C7H6O



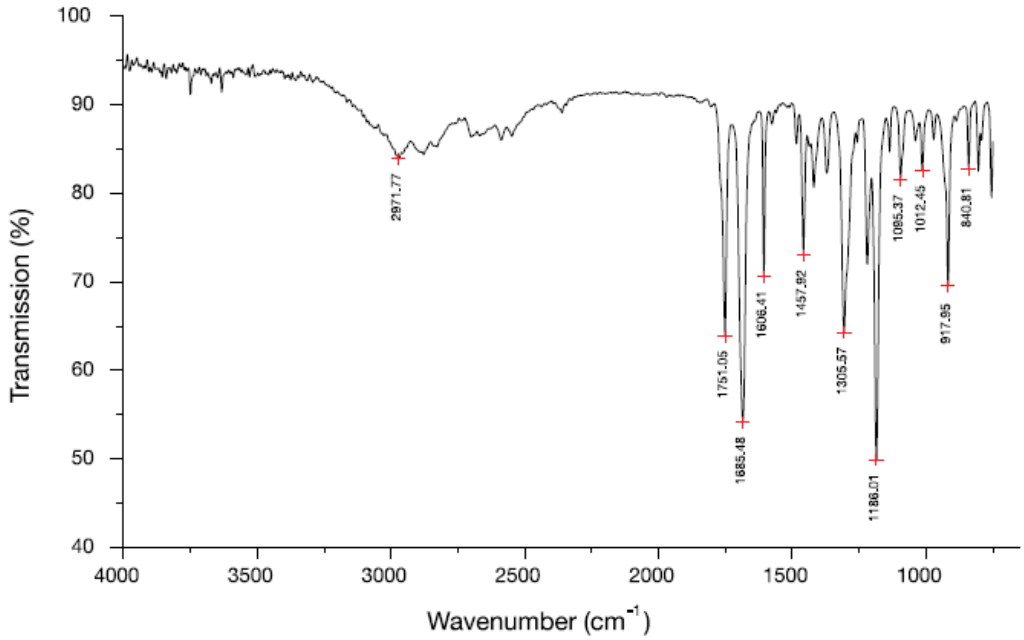
Sample H

Empirical Formula C2H6O



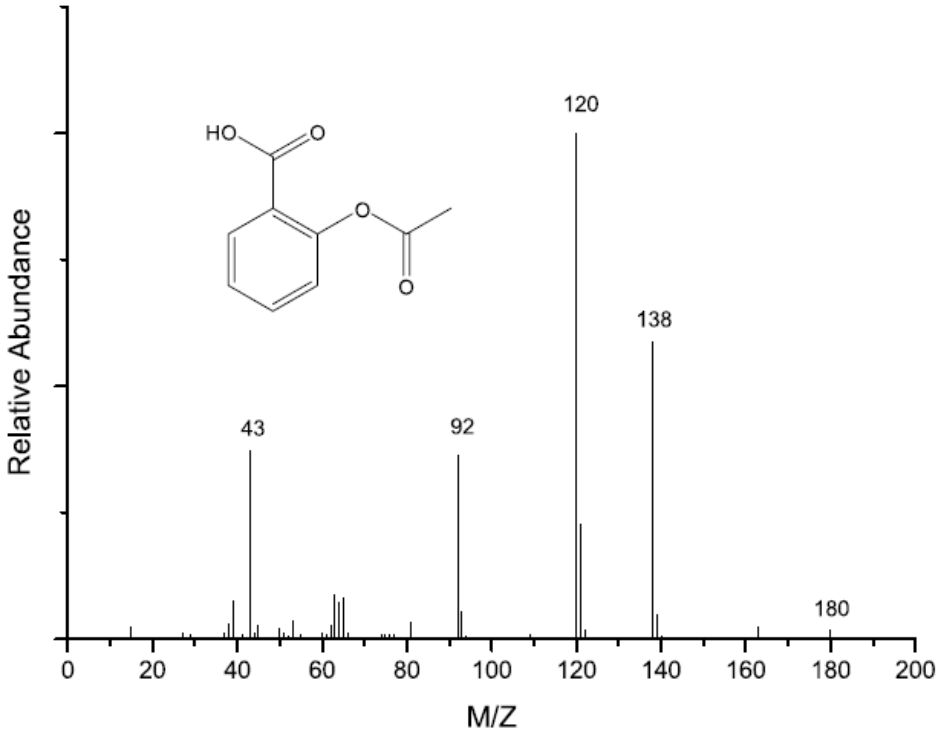
Sample X

Infra Red Spectrum



Mass Spectrum

Empirical Formula C9H8O4



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Glossary

Absorbance	The amount of incident light at a set wavelength absorbed as it passes through a sample.
ATR	Attenuated total reflectance – a sample preparation system for FTIR
Fourier transform	A mathematical operation that decomposes a signal into its constituent frequencies.
Resonance	Resonance is the tendency of a bond to oscillate at a greater amplitude at some frequencies than at others.
Transmittance	The amount of incident light at a set wavelength that passes through a sample.

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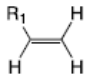
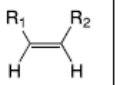
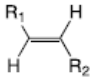
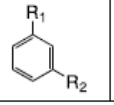
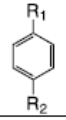
BASIC ORGANIC FUNCTIONAL GROUP REFERENCE

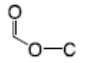
	C—H	C=C	C≡C	C—O	O—H	C=O	N—H	C≡N
Aliphatic								
Methyl	2960							
Methylene	2930							
Unsaturated								
Alkenes	3050	1640						
Vinyl	910	1640						
Vinylidene	890	1640						
Cis	700	1640						
Trans	965	1670						
Alkynes	3200		2200					
Aromatics								
Mono	750		700					
Ortho	750		-					
Meta	782		700					
Para	817		-					
Oxygen Group								
Ether				1100				
Alcohol				1100	3100			
Carbonyl Compounds								
Aldehyde	2700					1750		
Ketone						1730		
Ester				1200		1700		
Carboxlic Acid					3100	1740		
Nitrogen Groups						1720		
Amide						1640	3200	
Amine							3300	
Nitrile								2250

Methyl	Methylene
CH_3—	—CH_2—

Vinyl	Vinylidene	Cis	Trans	Alkene
				C=C
				Alkyne
				C≡C

Mono	Othro	Meta	Para
			

Ether	Alcohol
C—O—C	—OH

Aldehyde	Ketone	Ester	Carboxylic Acid
			

Amide	Amine	Nitrile
	C—N	C≡N

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