Midlands Regional Heat
Schools Analyst Competition 2017

Active Compounds in Commercial Medicine Tablets

LABORATORY HANDBOOK
Royal Society of Chemistry
Analytical Division

Introduction

You are supplied with tablets that are currently being marketed by a pharmaceutical company. Your job is to isolate the physiologically active organic compound from them, to purify it, and then to work out its structure from the analytical and spectroscopic data. Your role is to carry out the following investigations:

- Isolate the active ingredient, material X.
- Record the infrared (IR) spectrum of X, with the help of a demonstrator.
- Run a thin layer chromatography (TLC) analysis of X.
- Measure the melting point of X and record it on the report sheet.
- Investigate the solubility of X by adding small samples to various solvents.

NOTE: The last four tasks are analytical exercises. Precision and accuracy are of vital importance.

Background

In this section, the theory of each analytical method is explained. Enough detail is provided to allow you to understand what you are doing and why you are doing it.

Safety

Although the branded products are medicinal items you must remember that you are in a laboratory and therefore they must not be ingested. Laboratory coats and safety spectacles are mandatory and must be worn for the entire duration in the laboratory. Disposable gloves must be worn when necessary and this will be clearly stated in the lab script. Long hair must be tied back.

Isolation of material X

The tablets contain one or more active ingredients combined with a water-soluble binder that holds the tablet together. A number of laboratory techniques are used in sequence to isolate the active organic compound.

Spectroscopic determination of material X

Infrared radiation cannot be seen by the human eye. This is because it lies outside the visible spectrum – it has a lower energy. Although it is invisible, infrared radiation makes a highly visible contribution to analytical chemistry. Infrared spectroscopy (IR) is one of the most important analytical techniques available to chemists and it is one of the most familiar. It can be used to study how fats crystallise in margarines and low fat spreads, and it can also be used for categorising or identifying fibres and paint chips in forensic analysis, and for the screening of illicit substances. (Many illicit drugs are ‘cut’ by adding other powders and many of these powders contain carbohydrates that are identified easily using infrared spectroscopy).

The physical property that is measured in IR is the ability of some molecules to absorb infrared radiation. Atoms in molecules are not static, but rather they vibrate about their equilibrium positions. The frequency of these vibrations depends on the mass of the atom and the length and strength of the bonds. Molecular vibrations are stimulated by bonds absorbing radiation of the same frequency as their natural vibrational frequency (usually in the infrared region). For each molecule a variety of vibrations is possible.

By using characteristic absorption frequencies it is easy to identify the presence of functional groups in unknown compounds but more information is required for a full structural determination.

Thin Layer Chromatography for the detection of organic components

You will perform a solvent extraction of material X and then use thin layer chromatography and UV visualisation to determine its Rf value.

- Chromatography is used to separate mixtures of substances into their components.

- A chromatogram consists of a stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates.
- As the solvent begins to soak up the plate, it first dissolves the compounds in the spot that you have put on the base line. The compounds present will then tend to get carried up the chromatography plate as the solvent continues to move upwards.
- How fast the compounds get carried up the plate depends on two things:
    - How soluble the compound is in the solvent. This will depend on how much attraction there is between the molecules of the compound and those of the solvent.
    - How much the compound sticks to the stationary phase - the silica gel, for example. This will depend on how much attraction there is between the molecules of the compound and the silica gel (on the TLC plate).

Melting point of material X
Determining the melting point of a compound is one way to test if the substance is pure. A pure substance generally has a melting range (the difference between the temperature where the sample starts to melt and the temperature where melting is complete) of one or two degrees. Impurities tend to depress and broaden the melting range so the purified sample should have a higher and smaller melting range than the original, impure sample.

Solubility of material X
Solubility is a measurement of how much of a substance will dissolve in a given volume of a liquid. The liquid is called the solvent. Solubility tests can suggest the size and polarity of an unknown compound and the presence of basic or acidic functional groups. A compound's solubility in aqueous acid or base involves ionization of the compound and, therefore, a chemical reaction. The salts produced are water-soluble.

What is required?

In your team of three decide who is going to do each analysis once the isolation procedure is complete; then read the experiment you are going to do. Each experiment has a section explaining the hazards of the chemicals you will be using; you should read this section carefully. Once you are happy with what you are going to do, you should begin the practical work. Pool the results of the experiments in your 'neat copy' of the answer booklet and hand this in together with any spectra. Make sure your names are correctly and clearly spelled as there is nothing worse than a participation certificate with your name spelled incorrectly!

Good luck and enjoy the challenge.

There are marking guidelines alongside the tasks to guide you in the depth of answer required. Good communication is very important so make sure your calculations are laid out well.

This resource is based on an activity run by Prof Helen Aspinall, University of Liverpool.

Experiment 1: Isolation procedure

Safety:

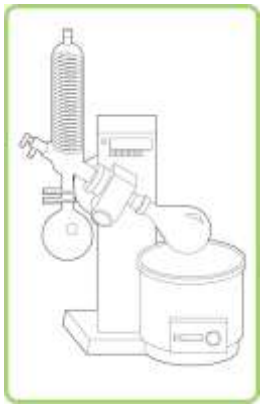
- Ethyl ethanoate (also known as ethyl acetate) is flammable and **must** be handled in the fumecupboard ONLY.
- Wear gloves when using ethyl ethanoate; if you get any on your skin wash off with water.
- Anhydrous magnesium sulfate is a powder, take care not to inhale dust.
- Spillages must be mopped up immediately.

Materials and Apparatus:

- Four medicine tablets
- Ethyl ethanoate (labelled as ethyl acetate)
- Deionised water
- Anhydrous magnesium sulfate
- 50ml beaker
- 25 ml measuring cylinder
- Stirring rod
- 100ml separating funnel with stopper
- Clamp stand, boss and retort ring
- 100ml conical flask
- 100ml round bottomed flask
- Cork ring
- Filter paper
- Funnel
- Rotary evaporator

Procedure:

1. Weigh out four tablets and note down the mass. Place them in a beaker containing 20ml of deionized water. Observe what happens.
2. Stir the tablets so they dissolve as much as possible. Transfer the contents of the beaker to a separating funnel and add 20ml of ethyl ethanoate.
3. Stopper the separating funnel and shake it vigorously until everything has dissolved; take care to let the pressure out of the funnel by inverting the funnel and opening the tap to allow any gas to escape! Allow the solvents to separate and run off the lower aqueous layer (including any emulsion at the interface) into the beaker. Keep this fraction to one side.
4. Run off the remaining ethyl ethanoate layer into a dry 100ml conical flask.
5. Pour the aqueous layer back into the separating funnel and extract it once more with a fresh 20ml of ethyl ethanoate. Combine this ethyl ethanoate extract with the previous one to give a combined organic extract. Keep the aqueous layer in the beaker.
6. Dry the ethyl ethanoate solution by adding a heaped spatula of anhydrous magnesium sulfate to it and gently swirl the flask.
7. After 5-10 minutes filter off the drying agent into a round bottomed flask which has previously been weighed.
8. Evaporate the solution to dryness on a rotary evaporator (see a demonstrator for how to operate it) and weigh the flask again.
9. Record the weight of your solid (material X) on the report sheet.
10. At the end of the experiment wash the aqueous extract down the sink with plenty of tap water.



Results:
Weight of four tablets =

Weight of empty round bottomed flask =

Weight of round bottomed flask with material X =

Experiment 2: Record the Infrared (IR) spectrum of material X

To get any meaningful information out of the IR spectrum, it needs to be a decent spectrum with sharp peaks. If a high intensity is observed, all the impurities will seem to be more significant than they really are, and the peaks with high intensity will be 'cut off'. A **nujol mull** is obtained by grinding up the solid and mixing it with mineral oil to form a suspension, which is placed in between KBr discs.

Safety:

- Nujol (liquid paraffin) is a slight irritant. Take care when handling.

Equipment:

- FTIR spectrometer
- Mortar and pestle
- Nujol
- Microspatula
- KBr or NaCl discs
- Sample holder for spectrometer
- Tissues
- Acetone

Procedure:

1. Use the microspatula to put a small amount of material X into the mortar; some solid on the tip of the spatula is enough. Add 1 drop of nujol and using the pestle, grind the nujol and material X together to form a paste; grind the paste for at least 10 minutes in order to make the particle size as small as possible.
2. Wear gloves to prevent fogging of the KBr discs; DO NOT touch the KBr discs with bare hands. Take the discs out of the jar and check that they are clean; if they need cleaning use a tissue and acetone to wipe them. BE CAREFUL: DISCS ARE FRAGILE.
3. It is essential to obtain a background spectrum before collecting the sample spectrum. To do this the background must be taken of just the nujol. Put a tiny dab of nujol onto the centre of one of the discs. Place the other KBr disc on top of the mixture to form a sandwich, and twist the disc so that the nujol becomes a thinly smeared circle in the middle of the discs.
4. Place the sandwiched discs into the sample holder and go to the IR spectrometer.
5. ASK A DEMONSTRATOR TO HELP WITH USE OF THE SPECTROMETER. Obtain a background spectrum.
6. The KBr discs must be thoroughly cleaned after this procedure to prevent contamination of future samples. Wipe the windows with a tissue, then rinse several times with acetone and wipe them with tissue. The cleaned surface should be clear and free from scratches.
7. Using the pestle, dot the material X/ nujol mixture onto the centre of one of the KBr discs. Place the other KBr disc on top of the mixture to form a sandwich, and twist the disc so that the mixture becomes a thinly smeared circle in the middle of the discs.
8. Repeat steps 4-6 with your sample. Obtain a print out of the spectrum.
9. Once the discs have been cleaned immediately place the discs in their jar and close the lid.
10. Refer to the table of IR absorption bands on page 13 of this handbook. Identify the major peaks in your spectrum.

Experiment 3: Thin Layer Chromatography for the detection of organic components

Manufacturers add many substances to medicines in addition to the active ingredient. You are going to be using Thin Layer Chromatography to see how many substances are contained in material X.

Safety:

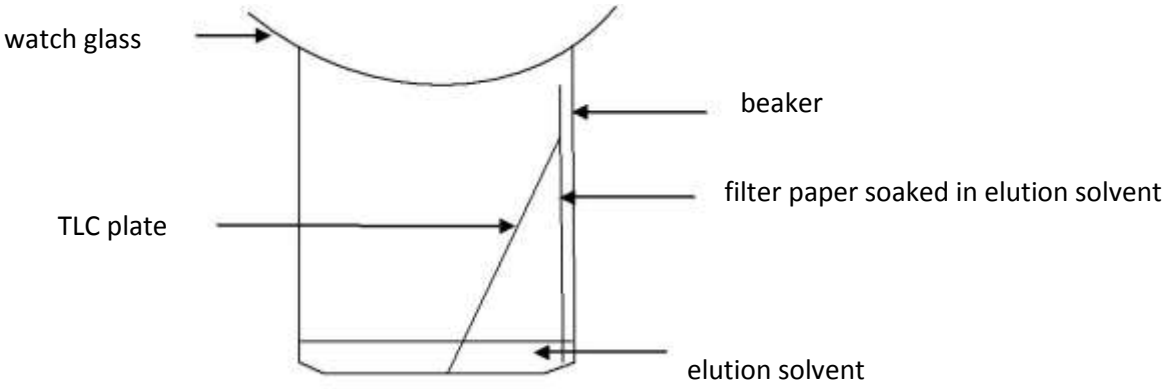
- Ethyl ethanoate is flammable and **must** be handled in the fumecupboard ONLY.
- Wear gloves when using ethyl ethanoate; if you get any on your skin wash off with water.
- Short wave UV - may cause skin cancer and eye damage. Do not observe directly. The viewer should be screened from direct radiation.

Materials and apparatus:

- Ethyl ethanoate – elution solvent
- Vial of 'Active ingredient'
- 2 x 2 dram glass vials
- Felt pen to write on glass
- 250 ml beaker plus large watch glass or petri dish to act as lid (chromatography tank)
- Filter paper
- 2 pieces of TLC plate
- Pencil
- 25 ml measuring cylinder
- 2 x micro-spotters
- UV viewer – short wavelength

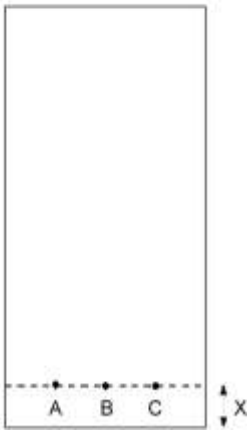
Procedure:

1. Work inside the fumecupboard. To make the chromatography tank (diagram below) fold the filter paper in half then half again, then put it inside the beaker so it rests against the side.

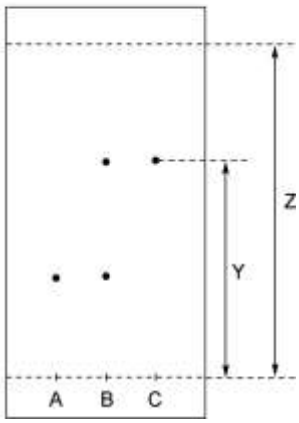


2. Measure 15 ml ethyl ethanoate in a measuring cylinder and pour into the chromatography tank soaking the filter paper as you do it. Replace the lid. Allow the tank to stand to saturate the air with vapour.
3. Place a small amount (about the tip of a spatula measure) of 'Active ingredient' into the glass vial. Add 1 ml ethyl ethanoate to the vial to dissolve 'Active ingredient'. Repeat the same procedure to prepare a TLC sample of material X. Make sure each vial is properly labelled.

TLC plate before elution



TLC plate after elution



4. Make sure that you do not touch the surface of the TLC plate with your fingers during this activity. Handle the plate only by the edges. Take a TLC plate and using a pencil lightly draw a line across the plate about 0.5 cm from the bottom.
5. Mark 3 points on the baseline so that they are roughly equidistant from each other and the sides of the plate, and underneath in pencil label them A, B, and C.
6. Dip a micro-spotter into your 'active ingredient' solution. Capillary action will draw up some of the solution into the micro-spotter. Lightly touch the spotter onto points A and B on the TLC plate (the smaller the applied spots the better). Allow the spots to dry, and then repeat 3 more times. The spot should be about 1-2 mm in diameter.
7. Repeat the same process with your material X solution *but* on points C and B only; apply the spotter to C first then spot B.
8. After the spots are dry, place the TLC plate in the chromatography tank so that it stands near vertical and rests against the filter paper, making sure that the original pencil line is above the level of the developing solvent. Put the watch glass on the tank and allow to stand in the fume cupboard until the solvent front has risen to within a few millimetres of the top of the plate.
9. When the solvent has reached approximately 0.5cm from the top of the plate, remove the plate from the tank and carefully mark that position of the solvent front by drawing a pencil line along where the solvent has risen to.
10. Wait until the plate is dry and then place the TLC plate under the UV lamp. The compound spots should fluoresce under UV light. Mark the position of each spot with a pencil by drawing an outline around the spot.
11. To calculate the Retention factors (R_F) of each spot, measure the distance from the baseline to the solvent front and the distance each spot has travelled.

The R_F value for each spot is calculated by the equation $R_F = Y/Z$

R_F value for 'active ingredient' (A) = _____ (quote to 2 decimal places)

R_F value for material X (C) = _____ (quote to 2 decimal places)

Experiment 4: Melting point of material X

Safety:

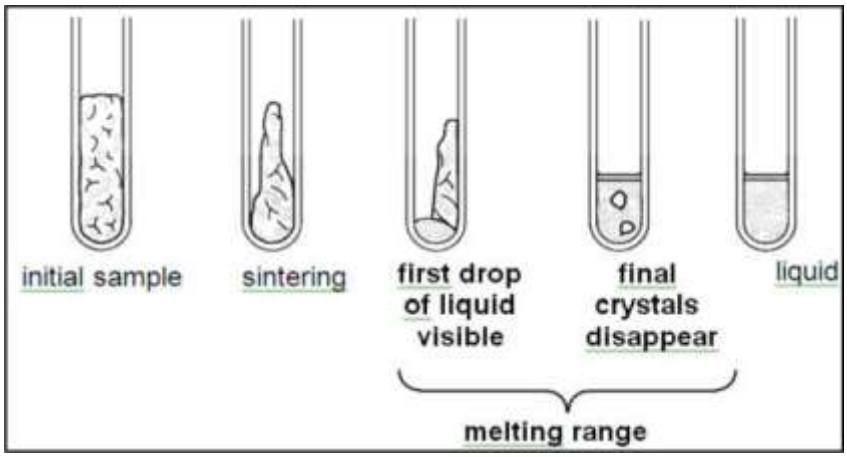
- Take care when using the capillary tubes – alert a demonstrator if any tubes break and they will help clear away any broken glass.

Materials and apparatus:

- Watch glass
- 2 capillary tubes
- Melting point apparatus

Procedure:

1. Fill a capillary tube with material X about 3 mm high; to do this put the capillary tube (open end down) into the material and tap it on the bottom of the watch glass to get the solid into the tube. Force the solid to slide to the bottom of the tube by tapping the tube (open end up) on the lab bench.
2. Place the capillary tube into the melting point apparatus. Set the apparatus to rapidly heat your sample to a 'plateau temperature' of 160°C. Above the plateau, the sample will continue to be heated but at a slower rate of 2-3 degrees per minute which will allow you to record an accurate melting range of your compound. Observe the melting process though the magnifying lens. The first temperature is recorded when the first drop of liquid is apparent. The second temperature is recorded when the entire mass of crystals has been converted into a liquid.



Record your first temperature here _____°C
Record your second temperature here _____°C
Give the melting range of your temperature _____°C

3. Once a melting point range is determined, prepare another capillary tube (tubes should only be used once and then discarded) and set the apparatus to the appropriate power level. This time, make sure that the increase in temperature is no more than 1°C per minute. Again, observe through the lens, and record the melting range/ point of X.
   Note: A sharp melting point (actually, a melting range of less than about 1°C) is often taken as evidence that the sample is fairly pure, and a wide melting range is evidence that it is not pure.
4. Dispose of used capillary tubes in the glass bin.

Melting point/ range of material X = _____ °C

Experiment 5: Solubility of material X

Investigate the solubility of X by adding small samples to different solvents.

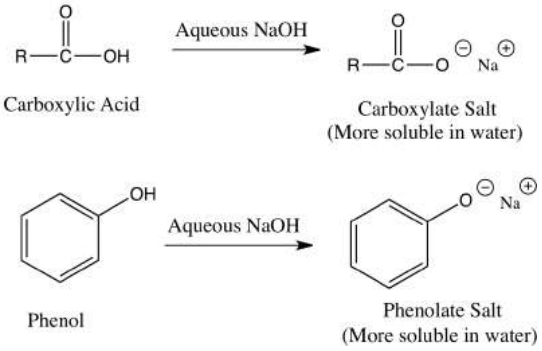
Information:

Solubility in H_2O

If your unknown is soluble in water, it suggests that you have at least 1 functional group capable of hydrogen bonding with the water per 4-5 carbon atoms. For example, simple alcohols containing 1-3 carbons (methanol, ethanol, propanol) are completely soluble in water. Butanol and pentanol (containing 4 and 5 carbon atoms respectively) are slightly soluble in water. While hexanol (6-carbons), and larger homologues, are essentially insoluble in water.

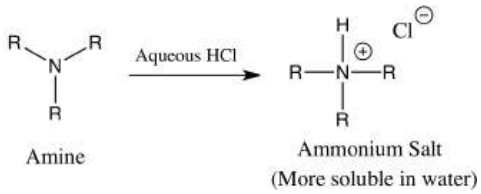
Solubility in Aqueous NaOH

If your unknown is insoluble in water, but does dissolve in 5% sodium hydroxide (NaOH) solution, then your unknown probably contains an acidic functional group (pKa < 15) that is deprotonated by the sodium hydroxide producing an ionic compound. Two common functional groups with this property are carboxylic acids (pKa ~5) and phenols (pKa ~10).



Solubility in Aqueous HCl

If your unknown is not soluble in water, but does dissolve in 5% HCl, then your unknown probably contains a basic functional group that is protonated by the hydrochloric acid producing an ionic compound. The most common organic functional group with this property is an amine.



Safety:

- Dilute HCl and NaOH are irritants; if you get any on your skin wash off with water.

Materials and apparatus:

- Dilute HCl – about 1 ml
- Dilute NaOH – about 1 ml
- Deionised water
- 1 x 2 dram glass vial
- Microspatula

Procedure:

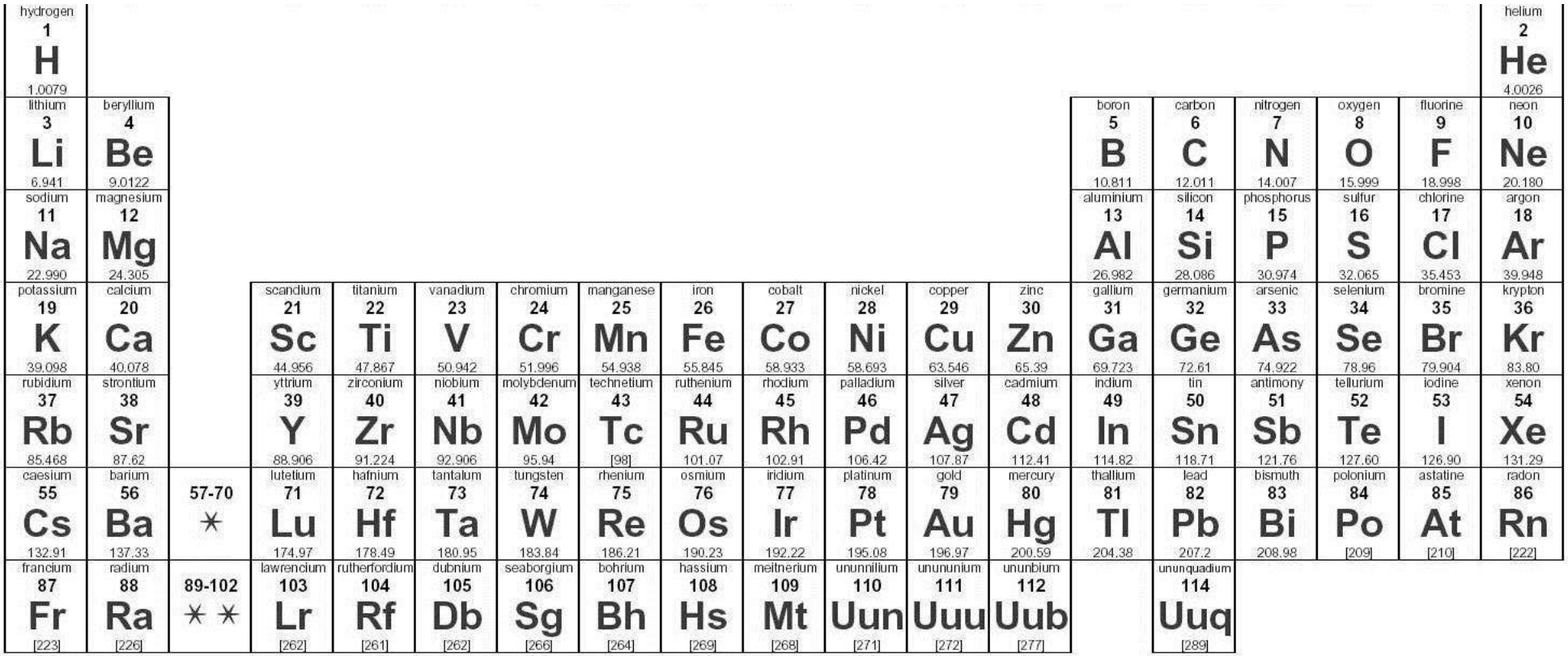
1. Into a glass vial add approx. 1ml of deionised water.
2. To each vial add a microspatula tip measure of material X. Give each test about 2-3 minutes for something to happen.
3. Record your observations in the table:
    - Cold water – is X very, slightly, or not soluble?
    - Dilute HCl – is X soluble or not?
    - Dilute NaOH – is X soluble or not?
4. From the information provided on page 9, make inferences as to the structure and functional groups of material X.

Substance	Solubility in water	Solubility in HCl	Solubility in NaOH
Material X			

Additional analytical information

Refer to the Answer Booklet for additional information and questions about material X.

NOTES:



Characteristic infrared absorptions in organic molecules

Bond	Location	Wavenumber / cm^{-1}
C–C	Alkanes, alkyl chains	750–1100
C–X	Haloalkanes (X = Cl, Br, I)	500–800
C–F	Fluoroalkanes	1000–1350
C–O	Alcohols, esters, carboxylic acids	1000–1300
C=C	Alkenes	1620–1680
C=O	Aldehydes, ketones, carboxylic acids, esters, amides, acyl chlorides and acid anhydrides	1630–1820
aromatic C=C	Arenes	Several peaks in range 1450–1650 (variable)
C≡N	Nitriles	2220–2260
C–H	Alkyl groups, alkenes, arenes	2850–3100
O–H	Carboxylic acids	2500–3300 (broad)
N–H	Amines, amides	3300–3500
O–H	Alcohols, phenols	3200–3600