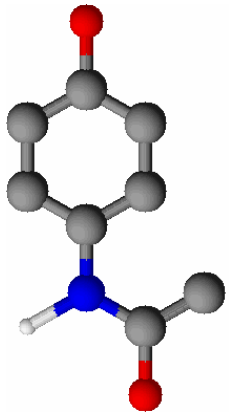
PAIN RELIEF
Parallel synthesis of paracetamol analogues



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Languages available
English, Hungarian

Summary
Pharmaceutical companies synthesise vast numbers of organic compounds in their search for one that will be active towards a particular disease or illness. Carrying out these syntheses efficiently and effectively is crucial. Combinatorial chemistry is used - in particular, a form of combinatorial chemistry called parallel synthesis.

Paracetamol is one of a number of over-the-counter (OTC) pain-relievers. In this activity, students work in groups to prepare a sample of paracetamol and to investigate the formation of a range of organic compounds that are structurally related to paracetamol. Reaction mixtures are examined using thin layer chromatography.

Activity type **F**
Use of scientific knowledge and understanding to solve problems
Working in teams to solve problems
Resource/ budget management
Time and workload management

Techniques
Organic synthesis
Thin layer chromatography

Field
Pharmaceuticals

Time
Practical lessons: 360 minutes
Theory lessons: 240 minutes
Out of class time: 120 minutes

StandardBase procedures
None

StandardBase techniques
None

Other resources
GlaxoSmithKline, R&D, Stevenage; www.gsk.com
Other resources are in the activity.

Student's document

PAIN RELIEF: Parallel synthesis of paracetamol analogues

Pharmaceutical companies synthesise and test tens of thousands of compounds in the search for one that shows significant therapeutic biological activity. Carrying out these syntheses efficiently and effectively is crucial. Combinatorial chemistry is used - in particular, a form of combinatorial chemistry called parallel synthesis. This is often done using robotic systems, but a few manual parallel syntheses are also carried out. Only small quantities are needed. For example, using plastic well plates, ten tests for biological activity can be carried out with only 1 mg of compound.

Your brief

Paracetamol is one of a number of over-the-counter (OTC) pain relievers. In this activity, you will work in a small group. Each of you will prepare a sample of paracetamol. Then, between you, members of the group will investigate the microscale preparation of paracetamol and the formation of a range of organic compounds structurally related to paracetamol. You will examine the reaction mixtures using thin layer chromatography.

From your results, the team needs to provide advice to the research and development (R&D) management team about which reactions appear to give products and, therefore, might be worked up to provide samples that can be tested for biological activity. This advice must also come with a costing exercise, saying how much it would cost to make these compounds.

Your investigation

Work in a group of four. Your group will be provided with three **sheets**:

- *Preparation of paracetamol*
- *Microscale investigation of paracetamol synthesis*
- *Parallel synthesis of compounds structurally similar to paracetamol*

Planning

Each member of the group should make a sample of paracetamol on a semi-micro scale (*Preparation of paracetamo*l). Compare your results - percentage yield and melting point of recrystallised paracetamol.

Next you need to work as a group to carry out the microscale synthesis of paracetamol and the parallel synthesis of some organic compounds that are structurally similar to paracetamol.

Each member of the group should read the **sheet** *Microscale investigation of paracetamol synthesis*. Make sure you are all clear about the instructions for the microscale preparation and the thin layer chromatography investigation of the reaction mixture.

Now read *Parallel synthesis of compounds structurally similar to paracetamol*. Check with your teacher what chemicals are available for you to use.

Plan what you are going to do and share out the work between group members. The group will now carry out the type of manual parallel synthesis usually done

by a single chemist. In parallel synthesis as little as 5 mg of a compound is made. High yield is not important. The desired compound is separated and purified by chromatography.

Your group will share the task of making an array of compounds (sometimes called a library) that are structurally related to paracetamol. How many there are in the array depends on the chemicals available and your ability to manage time and workload.

Practical work
Once you have decided what each member of the group will be doing, set up your work area and collect the equipment and materials you need.

You must be sure a risk assessment has been carried out before starting work.

Information
- You will need to find the prices of chemicals from the catalogues of chemical suppliers. Examples include:
  http://uk.vwr.com/app/Home
  http://www.sigmaaldrich.com/Area_of_Interest/Europe_Home/UK.html

Your findings
Write a brief report that includes the group's findings. While writing your report, imagine you are writing it to the R&D management team of a pharmaceutical company. You should say which reactions appear to give products and, therefore, might be worked up to provide samples that can be tested for biological activity. You must also include calculations showing an estimation of how much it would cost to make these compounds.

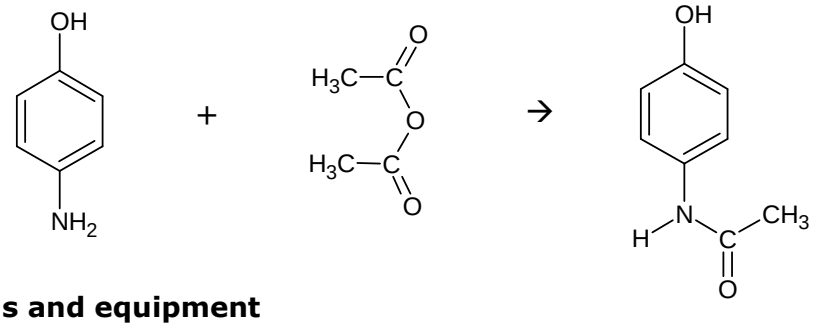
Attach to your report:
- the results of the semi-micro scale preparation of paracetamol
- the summary table showing the outcomes from the parallel syntheses of compounds structurally related to paracetamol.

Student self assessment
This activity was about using scientific knowledge and understanding to solve problems, working in teams to solve problems, managing time and workload, resources and budgets. For each case, write about 50-100 words explaining:
- what scientific skills and knowledge you used and how effectively you used them
- how you worked as a team and give examples of how you supported others in your group or were supported by them
- how you managed your time and workload
- how you managed resources and budgets.

Student sheet: Preparation of paracetamol

Paracetamol is a commonly used analgesic. Aspirin and ibuprofen are the two other most common analgesics. The chemical name for paracetamol is N-(4-hydroxyphenyl)ethanamide.

It may be prepared in the laboratory by reacting 4-aminophenol with ethanoic anhydride.



Materials and equipment

- 4-aminophenol
- ethanoic anhydride
- ethyl ethanoate
- balance weighing to the nearest 0.01 g
- 50 cm^3 conical flask
- Buchner flask, funnel and filter paper
- clamp and stand (to hold the Buchner flask)
- vacuum pump (water pump)
- apparatus for determining melting point
- distilled water wash-bottle
- Bunsen burner, tripod and gauze
- boiling tube
- 400 cm^3 beaker (for a water bath)
- equipment for thin layer chromatography (see *Microscale investigation of paracetamol synthesis*)
- access to a fume cupboard

Procedure

Eye protection must be worn. A risk assessment must have been made before starting this practical work.

1. Weigh 1.0 g of 4-aminophenol into a 50 cm^3 conical flask. Add 9 cm^3 of distilled water and shake the mixture briskly to suspend the solid in water.

2. In a fume cupboard, add 1.1 cm^3 (1.17 g) of ethanoic anhydride to the stirred suspension and gently shake to mix. The solid will dissolve after about 30 seconds. Continue shaking and a precipitate will form after 2 minutes.

3. After 10 minutes, filter off the solid under suction filtration, wash with a little cold water and dry.

Note: Use a Buchner flask and funnel connected to a vacuum pump (water pump). Clamp the flask to keep it stable. Put a filter paper in the funnel, attach the flask to the pump and pour through about 10 cm^3 water as a practice run

and to wet the filter paper. Remove the vacuum tube, pour out the water and then do the real filtration. If you do not manage to transfer all of the solid from the reaction mixture into the funnel at the first attempt, just pour the filtrate back into the conical flask and repeat the process.

Remove a small sample (A), dissolve in minimum quantity of ethyl ethanoate and keep in a labelled sample tube.

4. To recrystallise the sample, put it into a small conical flask. Dissolve the crude product in the minimum volume (probably about 15 cm^3) of distilled water. Heat it in a hot water bath at 80 °C until the solid dissolves. Add a few extra drops of hot water, if necessary, to dissolve the solid.

5. Cool the solution under a tap or preferably in ice. Collect the recrystallised product by suction filtration, washing with 5 cm^3 of ice-cold distilled water.

6. Dry the recrystallised product between filter papers and then in an oven (about 105 °C). Determine the yield. Calculate the percentage yield.

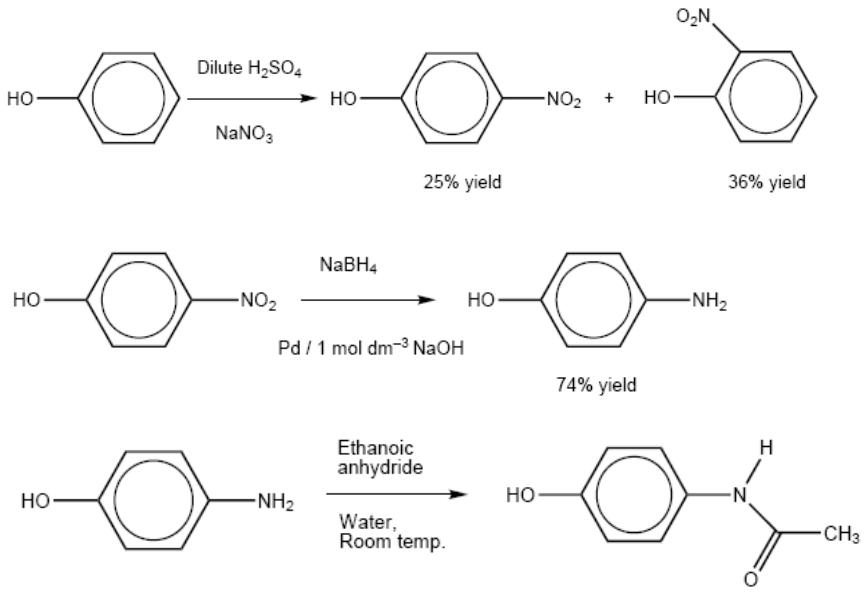
Remove a small sample (B), dissolve in minimum quantity of ethyl ethanoate and keep in a labelled sample tube.

7. Find the melting point of the dry, recrystallised product. The melting point of pure paracetamol is 169-171 °C.

8. Now run a thin layer chromatogram using the method in *Microscale investigation of paracetamol synthesis*. Run the crude paracetamol (**A**) and recrystallised paracetamol (**B**) against (a) 4-aminophenol, (b) pure paracetamol and (c) a mixture of 4-aminophenol and paracetamol (all dissolved in ethyl ethanoate).

Results

- Calculate the percentage yield of recrystallised paracetamol.
- Record the melting point of the recrystallised paracetamol.
- Sketch a diagram of the chromatogram obtained (or put the chromatogram in a transparent plastic bag and fix it in your notebook).
- Compare your results with other members of your group and explain any differences.
- Look at this scheme for making paracetamol from phenol:



Using the data shown together with the percentage yield you estimated for the conversion of 4-aminophenol to paracetamol, calculate the yield of paracetamol that might be obtained from 50 g of phenol.

Student sheet: Microscale investigation of paracetamol synthesis

Preparation

Materials and equipment
- 4-aminophenol
- ethanoic anhydride
- anhydrous magnesium sulfate
- ethyl ethanoate
- distilled water
- 2 x small sample tubes
- 3 x dropping pipettes
- balance weighing to the nearest 0.001 g
- access to a fume cupboard

Procedure
Eye protection must be worn. A risk assessment must have been made before starting this practical work.

1. Using a weighing boat measure out 0.1 g of 4-aminophenol into a small sample tube. Add 1 cm^3 of distilled water. Shake briskly at room temperature in order to suspend the solid in water.

2. In a fume cupboard, add 1 drop (about 0.12 g) ethanoic anhydride to the suspension and continue to shake the mixture gently for 2 minutes. (You could check the weight of 1 drop from your pipette before doing this).

3. Add 1 cm^3 ethyl ethanoate and shake gently for 10-15 seconds. Leave to settle. Two layers will form. The product is in the ethyl ethanoate upper layer (the lower layer is mainly water).

4. Using a clean dropping pipette, carefully transfer the upper layer to another sample tube containing a small amount of anhydrous magnesium sulfate. This will remove any traces of water.

5. Now use thin layer chromatography (see method below) and run this solution against (a) 4-aminophenol in ethyl ethanoate, (b) pure paracetamol in ethyl ethanoate, and (c) a mixture of 4-aminophenol and paracetamol in ethyl ethanoate.
In each case, make these solutions by dissolving half a small spatula of material (equal to about 1-2 grains of rice) in 1 cm^3 ethyl ethanoate.

Thin layer chromatography (TLC)

Materials and equipment

- solvent mixture – 2 parts by volume ethyl ethanoate and 1 part by volume cyclohexane
- iodine
- TLC plates (coated in silica gel and containing a fluorescing agent)
- screw-top jar large enough to take a 10 cm x 5 cm TLC plate
- finely drawn glass tubing (for spotting the plates)
- UV lamp

Procedure

Eye protection and gloves must be worn. The ultraviolet radiation may cause skin cancer and eye damage - do not observe directly. A risk assessment must have been made before starting this practical work.

1. Pour the solvent mixture into a screw-top jar to a depth of about 0.5 cm. Put the lid on and leave so that the atmosphere becomes saturated with the solvent vapour. This process could be accelerated by laying a piece of filter paper soaked in the solvent mixture on the inner wall of the screw-top jar. The atmosphere should be saturated with solvent vapour in 30 minutes.

2. Lay a 10 cm x 5 cm TLC plate on a piece on clean paper. Make sure you handle the plate by the edges only; do not touch the surface. Draw a pencil line across the plate about 1 cm from the bottom. On three small pencil crosses, spot first the product, then a mixture of your product and starting material and finally 4-aminophenol. Allow the spots to dry – they should be 1-2 mm in diameter. This process could be accelerated by using a hairdryer.

3. When the spots are dry, place the TLC plate in the screw-top jar. Do this quickly, but carefully. Try not to disturb the liquid. Immediately replace the lid on the jar.

Note: The original pencil line must be above the level of the solvent.

4. Run the chromatogram until the solvent front is within about 1 cm of the top of the plate. Remove the plate and mark the position of the solvent front.

5. Allow the plate to dry. Observe under UV light and mark any spots seen. Calculate the R_f values of the spots observed.

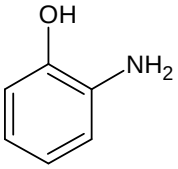
6. Finally, if time, place the chromatogram in a covered beaker containing a few crystals of iodine and wait for spots to appear.

Student sheet:
Parallel synthesis of compounds structurally similar to paracetamol

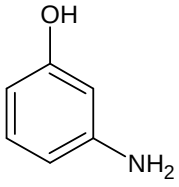
The microscale method for exploring the reaction between 4-aminophenol and ethanoic anhydride may be adapted to investigate the reaction of various compounds that are structurally similar to 4-aminophenol with various acid anhydrides.

You will need to check with your teacher what chemicals are available. However, here are some that may be:

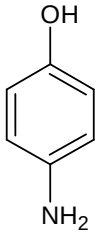
Possible aminophenols



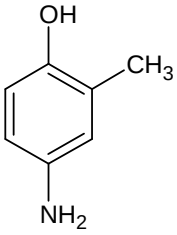
2-aminophenol



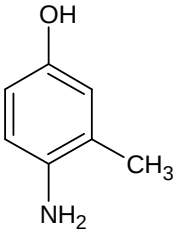
3- aminophenol



4- aminophenol

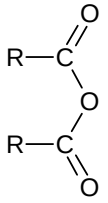


4-amino-2-methylphenol



4-amino-3-methylphenol

Possible acid anhydrides



R = CH_3 ethanoic anhydride (acetic anhydride)

R = C_2H_5 propanoic anhydride (propionic anhydride)

You can plan and record your results in a table similar to the one on the next page. Copy it and fill in the aminophenols used by members of your group. Indicated clearly which one(s) you investigated.

In each box, record:

- The initials of the group member who investigated
- The name and chemical formula of the aminophenol used
- The chemical structure of the compound you expected to be formed
- Evidence for new compound formation (or not) from the TLC studies
- The cost of making 1 g of the compound for further study

  Note: You will not have measured yields (in fact, the idea of parallel synthesis is simply to get evidence of product formation; optimising reaction conditions comes later). However, it is extremely unlikely that 100% yields would be obtained. Therefore, give costs based on 25%, 50%, 75%, and 100% yields.

- A prediction about the drug potential of the product.

  Note: Use the *OSIRIS Property Explorer* http://www.organic-chemistry.org/prog/peo/ to estimate a 'Drug-Likeness Prediction' and 'Overall Drug-Likeness Score'. You need to have Java installed to use the molecular modelling programme.

You may need more or less boxes depending on what reactions the group was able to study.

initials	**aminophenol used**	**ethanoic anhydride**	**propanoic anhydride**

Teacher's document

Overview
Students work in a research and development group of a pharmaceutical company. The group has been asked to investigate the synthesis of paracetamol and an array of other structurally related compounds. The cost and drug potential of these other compounds must also be reported.

Developing students' skills
Learning objectives
Students:
- manage relationships to work effectively with others to solve problems
- manage resources and budgets effectively
- manage time and workload effectively
- use scientific knowledge and understanding to solve problems
- observe, measure, analyse and evaluate scientific data.

Guidance and help
It may be useful to use molecular modelling kits or computer drawing packages such as *ChemSketch* to illustrate the reactions that occur in the syntheses.

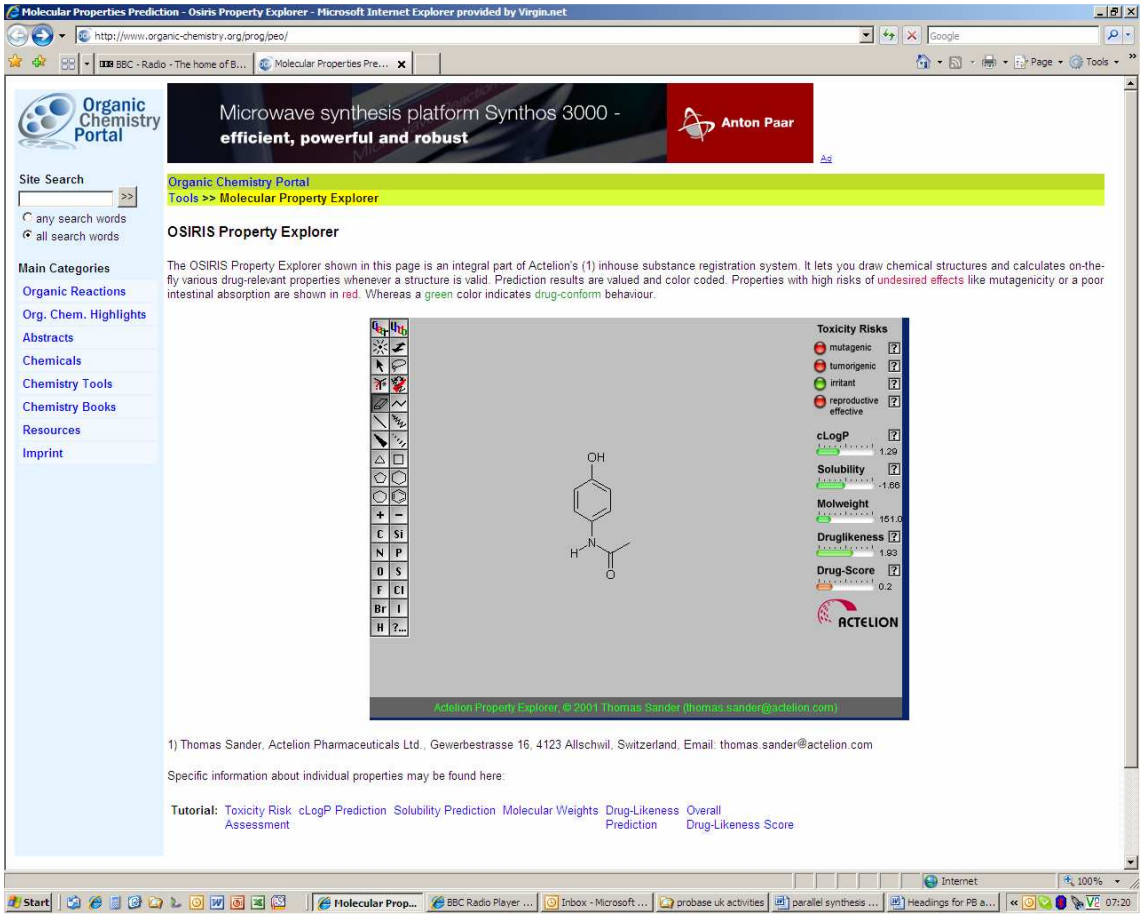
Students might be introduced to the idea of parallel synthesis and the advantages/disadvantages of robotic methods compared with manual syntheses.

There is a substantial amount of practical work. Students should be given as much help as necessary to do this effectively (this activity is not about assessing practical skills).

Students need to show they can manage budgets. They need to recognise that the price of chemicals depends on quantities bought and purity. Their estimate of costs should reflect this. They need to recognise that yields are rarely 100% and so this must also be factored in to the calculation.

It may take some time for students to learn how to use the *OSIRIS Property Explorer* http://www.organic-chemistry.org/prog/peo/ to estimate a 'Drug-Likeness Prediction' and 'Overall Drug-Likeness Score'. They need to have an understanding of what these terms mean and be able to create different structures using the drawing package in the programme. Teachers may well want to try it out first!

Here is a screen print for paracetamol.



By this stage, students should have experienced working in groups on exercises to solve problems. Groups with successful experience of this can be left more to their own resources. Whether students are inexperienced or experienced, a sharp eye should be kept on the clock and it may be necessary to bring the whole class together at some point, to facilitate progress.

It is the nature of this task that it cannot be successfully completed in the time available without the need for team work and setting targets with deadlines. Nonetheless, students should be encouraged to take care and pride in their work. Troubleshooting vigilance may be needed to help with:

- technical aspects, including manipulative skills and correct handling of chemicals and equipment
- health and safety issues
- working relationships with others.

www.presentingscience.com/aflchem/key/groupwork.htm has ideas for setting up student groups.

Students will progress through the exercise by achieving higher levels of teamwork. In the beginning, it may be necessary for the teacher to set clear targets and deadlines and elicit team working strategies through appropriate

questioning. Short reviews at the end of sessions can be based on teacher observations of progress and help to set up the following sessions.

This exercise may allow a variety of assessment criteria to be met, according to the course being followed. Observations of practical work and the completed recording sheets and report should provide evidence that can be judged against published grading criteria.

Organising and managing the class
Students work in groups of four. One of the components of this activity is that students can manage their time and workload. They need to be given time and space to do this. Plans will need careful monitoring to ensure they are neither too ambitious/impracticable nor too trivial.

The smallest array investigated should consist of two aminophenols and two acid anhydrides. This would mean four microscale preparations, one for each member of the group. The activity can be extended easily, but substituted aminophenols are quite expensive.

Students should be told of times when they can access various facilities, e.g. laboratory, IT facilities, study centres. They will need to organise their work around these times.

Session plan for teachers
Exact timing depends on many factors, but the following is suggested:

- Introduction and checking of prior knowledge 60 minutes
- Arrange students in groups to discuss and plan the work 60 minutes
- Carry out the semi-micro preparation of paracetamol 210 minutes
- Carry out the parallel syntheses and thin layer chromatography 150 minutes
- Research costs and drug potential 120 minutes
- Complete table and write brief report 120 minutes

Technical notes

Preparation

A timetable of when facilities and equipment will be available should be prepared. Students will need this to organise their time and workload.

Amongst other things students need access to:

- laboratory with the appropriate equipment for the practical work
- computer or chemical suppliers' catalogues to find costs of chemicals (they may need help with the nomenclature)
- computer with broadband connection and Java installed to investigate drug potential.

Possible materials to use

Some are expensive, but the investigation requires only very small quantities.

	CAS number	*indicative price (2008)*
aminophenols		
2-aminophenol	98-37-3	13€ / 100 g
3-aminophenol	591-27-5	12€ / 100 g
4-aminophenol	123-30-8	14€ / 250 g
4-amino-2-methylphenol	2635-95-2	48€ / 5 g
4-amino-3-methylphenol	2835-99-6	38€ / 5 g
acid anhydrides		
ethanoic anhydride (acetic anhydride)	108-24-7	12€ / 1 dm^3
propanoic anhydride (propionic anhydride)	123-62-6	16€ / 500 cm^3

Preparation of paracetamol

Equipment and materials

Materials and their CAS numbers

4-aminophenol	98-37-3
ethanoic anhydride	108-24-7
ethyl ethanoate	141-78-6

- balance weighing to the nearest 0.01 g
- 50 cm^3 conical flask
- Buchner flask, funnel and filter paper
- apparatus for determining melting point
- clamp and stand (to hold the Buchner flask steady)
- vacuum pump (water pump)
- distilled water wash-bottle
- Bunsen burner, tripod and gauze
- boiling tube
- 400 cm^3 beaker (to use for a water bath)
- access to a fume cupboard
- equipment for thin layer chromatography (see below)

Microscale preparations
Preparation
If you can not buy pure paracetamol, extract it from paracetamol tablets by dissolving a tablet in water and then extracting with ethyl ethanoate. The solution is then dried with sodium sulfate.

Equipment and materials
Materials and their CAS numbers

4-aminophenol or other aminophenol	98-37-3
ethanoic anhydride or	108-24-7
propanoic anhydride	123-62-6
anhydrous magnesium sulfate	7487-88-9
ethyl ethanoate	141-78-6

- pure paracetamol
- distilled water

Equipment
- 2 x small sample tubes
- 3 x dropping pipettes
- balance to weigh to the nearest 0.001 g
- access to a fume cupboard

Thin layer chromatography
Preparation
Solvent
- 2 parts by volume ethyl ethanoate and 1 part by volume cyclohexane

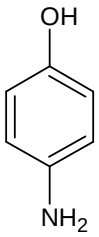
Equipment and materials
Materials and their CAS numbers

ethyl ethanoate	141-78-6
cyclohexane	110-82-7
iodine	7553-56-2

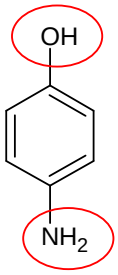
Equipment
- TLC plates (coated in silica gel and containing a fluorescing agent)
- screw-top jar large enough to take a 10 cm x 5 cm TLC plate
- finely drawn glass tubing (for spotting the plates)
- UV lamp
- gloves and eye protection

TESTING PRIOR KNOWLEDGE

1. Copy this structure and label the phenol group and the amine group:



Answer:

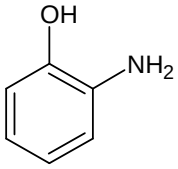


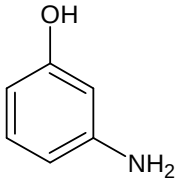
phenol group

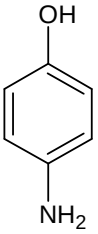
amine group

2. Explain the difference between and an alcohol and a phenol.
Answer:
Alcohol – hydroxyl group (-OH) is bonded to an aliphatic group
phenol – hydroxyl group (-OH) is bonded to an aromatic group

3. Explain why these three compounds are structural isomers.







Answer:
They have the same molecular formula and the same functional groups, but the functional groups are in different positions.

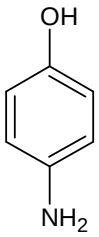
4. Name the isomers shown in question 3.
Answer:
left to right: 2-aminophenol, 3-aminophenol, 4-aminophenol

5. Write an equation to show how ethanoic anhydride might be formed from ethanoic acid.
Answer:
$2CH_3COOH \rightarrow (CH_3CO)_2O + H_2O$

Note: answers are given in red. These need to be removed before the test is used.

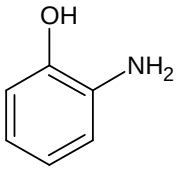
TESTING PRIOR KNOWLEDGE

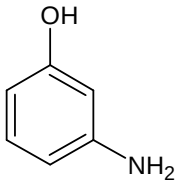
1. Copy this structure and label the phenol group and the amine group:

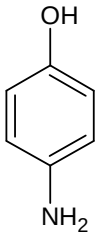


2. Explain the difference between and an alcohol and a phenol.

3. Explain why these three compounds are structural isomers.







4. Name the isomers shown in question 3.

5. Write an equation to show how ethanoic anhydride might be formed from ethanoic acid.