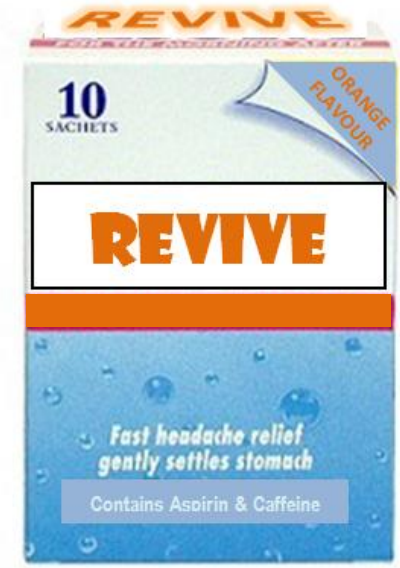
SCHOOLS' ANALYST COMPETITION 2012

MIDLANDS REGIONAL HEAT



INSTRUCTION BOOKLET

ROYAL SOCIETY OF CHEMISTRY
ANALYTICAL DIVISION

LENNARD-JONES LABORATORIES
KEELE UNIVERSITY

Disgruntled Employee: Tablet Contamination?

Welcome:

Welcome to the RSC Schools' Analyst competition at Keele University. Today's competition is based around three analytical experiments which aim to give answers to a number of questions. You will need to carry out the experiments and make decisions about the data you obtain in order to answer the questions.

To be able to complete the tasks you will need to work as a team and distribute the workload in the most effective way. Make sure you read through the safety information and the instructions before you start on any experimental work.

We hope that you enjoy your day with us at Keele University and wish you good luck in the competition!

The Scenario:

On 26th April 2012 the police were alerted to several status updates on a social networking site. Mr X works at the PharmaHax pharmaceutical manufacturing plant, and had recently posted a number of status updates indicating he had applied for a promotion to the role of production supervisor, and later that he had been overlooked in favour of a younger member of staff for the post. A few days later Mr X updated his status, bragging that he had taken his revenge by contaminating the production line of 'Revive' – an orange flavoured soluble aspirin tablet also containing caffeine. The police have thoroughly questioned members of the 'Revive' production team, and have concluded that Mr X can only have contaminated the tablets with the components already present (i.e. aspirin, caffeine and sunset yellow). However, increased quantities of active ingredients may still have serious health consequences for people taking 'Revive'. As a precaution, 'Revive' has been withdrawn from all shops whilst a full investigation is carried out.

As analytical chemists, it is your job to determine whether Mr X has altered the quantities of ingredients in 'Revive'. **ONE** Revive tablet taken from the suspected contaminated batch has been dissolved in **200 mL** of water. (It is important to consider this when drawing your conclusions). For each experiment, you have been provided with a sample of this solution that has had all active ingredients removed apart from the one you are analysing. The table below shows the quantity of aspirin, caffeine, and sunset yellow that one Revive tablet should contain.

Substance	Quantity per soluble tablet (mg/200 mL)
Aspirin	250
Caffeine	44
Sunset Yellow	2

N.B. You will need to refer to this table when drawing your conclusions.

The Tasks:

Your tasks today are to:

1. Determine the quantity of sunset yellow in the questioned tablets by UV-vis spectrophotometry.
2. Determine the quantity of aspirin in the questioned tablets by pH titration.
3. Determine the quantity of caffeine in the questioned tablets using High Performance Liquid Chromatography.

Planning:

To be successful you will need to plan how the tasks can be best distributed amongst team members to achieve the objectives in the allocated time. Our estimate of the time required for the experiments is:

UV-vis spectroscopy	1.5 hours
HPLC	1.5 hours
Titration	1 hour
Write-up / Questions	1 hour

Note: For the HPLC experiment your team will need to book a 20 minute slot on the HPLC sign-up sheet. We recommend that you book this slot at the start of the day.

Results:

This instruction booklet contains space for your observations as you conduct the experiments. You will have a copy of this booklet each and you should fill this in as you are conducting the experiments.

Each group will have one set of answer sheets. They ask you to record your results and conclusions from the three experiments and also to answer a number of questions relating to the experiments. You will need to complete one set of answer sheets per team. Show all of your working out for calculations. The answer sheets will be collected in and marked at the end of the day, however, to speed up the marking process please hand in answer sheets as soon as you have completed them.

Units:

In this booklet you will encounter two ways of describing concentration. The first is 'molar concentration', M or mol L^{-1}, which describes the number of moles per litre of each chemical. The second is 'mass concentration', mg L^{-1}, which describes the number of mg of a particular analyte per litre of aqueous solution.

Glassware:

During the day you may need to reuse some of your glassware, if you do please ensure that you rinse the glassware thoroughly with distilled water, in order to avoid contamination.

Important Safety Information

Lab coats and safety spectacles must be worn at all times in the laboratory.

Do not eat or drink in the laboratory.

Long hair should be tied back.

No shorts, skirts, leggings or tights should be worn.

No open-toed shoes - only enclosed shoes may be worn in the laboratories i.e. no sandals, ballet-type shoes or flip flops.

Some of the chemicals you will be using are hazardous. The hazards and precautions associated with each chemical are listed below:

Chemical substance	**Hazards**	**Precautions**
Phenolphthalein indicator in ethanol solution	Irritant	Avoid skin contact, wear gloves.
0.02 M NaOH solution (aqueous)	Harmful, irritant, causes burns. Toxic.	Avoid skin contact, wear gloves. In case of contact with eyes, wash with plenty of water.
Acetonitrile	Irritant. Flammable.	This will be in sealed vials.
Sunset Yellow	Irritant.	Avoid skin contact, wear gloves.
Aspirin	Harmful if swallowed. Irritant.	Do not ingest. Avoid skin contact, wear gloves.
Caffeine	Harmful if swallowed.	Do not ingest.

Experiment 1 - Background Information

UV-Vis Spectroscopy

Many compounds absorb light in the near-UV or visible region of the electromagnetic spectrum. UV-Vis spectrophotometry can be used to measure how much light is absorbed by a compound, and this information can be used to measure the concentration of the compound.

A spectrophotometer measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I_0). The proportion of light that exits from the solution is called the transmittance (T) and is given by:

$$T = \frac{I}{I_o}$$

The transmittance depends on the concentration of a sample (c), and the length of the sample through which the light travels (the pathlength, l). This relationship is given by:

$$-\log T = \varepsilon cl$$

The quantity $-\log_{10}T$ is known as the absorbance (A), and the spectrophotometers you will be using today display this quantity automatically. The quantity ε is a constant called the absorption coefficient, which is specific to a particular compound at a particular wavelength. Hence, we arrive at the Beer-Lambert law which shows that the absorbance (A) of a sample is directly proportional to its concentration (c):

$$A = \varepsilon cl$$

This relationship suggests that a plot of absorbance (A) versus concentration (c) will be a straight line going through zero. The absorbance of a solution is normally recorded at $\boldsymbol{\lambda_{max}}$ (the wavelength corresponding to the peak absorbance of the solution).

Dilutions

For this experiment you will be required to make up dilutions from a stock solution of known concentration in order to obtain a range of solutions of differing concentration. To work out the volume of stock solution required to do this, the following equation will be useful:

$$\mathbf{c_1V_1 = c_2V_2}$$

(Where c_1 is the concentration of the stock solution, V_1 is the volume of stock solution you need to calculate, c_2 is the concentration you want to achieve, V_2 is the final volume of the solution).

1. Determination of the sunset yellow concentration using UV-Vis spectrophotometry

In this experiment you will use UV-Vis spectrophotometry to construct a calibration graph by measuring the absorbance of standards of known sunset yellow concentration. You will then use this graph to determine the concentration of sunset yellow in the questioned tablet.

Equipment:

• 5 × 50 mL volumetric flasks • Graduated pipette & pipette filler • Plastic pipettes • 7 × Plastic cuvettes & 1 × cuvette holder • UV-vis spectrophotometer • Graph paper	• Standard sunset yellow solution (50 mg L^{-1}) • Distilled water • Questioned tablet sample

Experimental procedure:

1. From the sunset yellow stock solution provided, prepare 5 calibration standards in the range of: 5 mg L^{-1} - 35 mg L^{-1}. Do this by accurately using a graduated pipette and pipette filler to pipette an appropriate volume of stock solution (you will need to calculate this) into a 50 mL volumetric flask and making up to the mark with distilled water.

   IF YOU ARE UNSURE OF HOW TO USE A GRADUATED PIPETTE PLEASE ASK A DEMONSTRATOR.

2. Use separate plastic pipettes to transfer a small amount of these prepared solutions, the questioned tablet sample, and distilled water (to use as a blank) to separate cuvettes (fill about ⅔ full).

   CONSULT A DEMONSTRATOR ABOUT THE OPERATION OF THE SPECTROPHOTOMETER.

3. Using water as a blank, measure the spectrum of each solution from 600 to 300 nm, using a UV-vis spectrophotometer. Record the absorbance of each solution at λ_{max} (~480 nm).

Results:

Sunset Yellow concentration (mg L^{-1})	**Absorbance**

Questioned Tablet Solution	

Data treatment:

Plot a Beer-Lambert calibration graph by hand of absorbance (at λ_{max}) versus concentration for the standard sunset yellow solutions, and use the graph to determine the concentration of sunset yellow in the questioned tablet sample. Record your results on the answer sheet.

Attach calibration graph

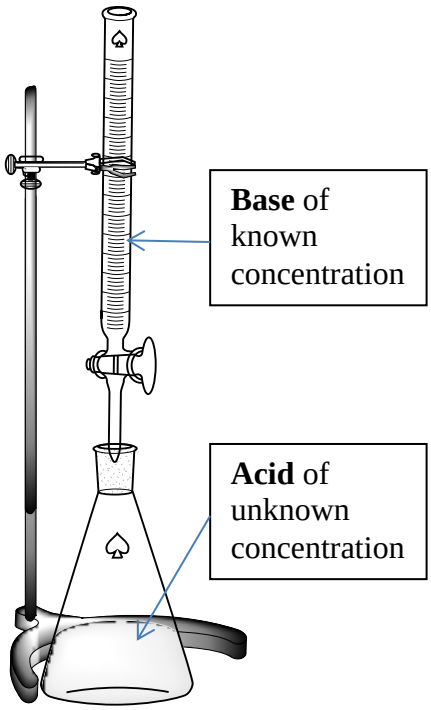
Concentration of Sunset Yellow in tablet sample: _____mg L^{-1}
(Show your working out on the graph)

This analysis should answer the question as to whether the amount of sunset yellow in the contaminated sample is different to what it should be.

What is your conclusion?

Experiment 2 - Background Information

The concentration of an acid can be determined by titration with a base of known concentration. The base is delivered from a burette in small increments to a conical flask containing a known volume of the acid. A point will be reached during the titration where the number of moles of acid equals the number of moles of base (neutralisation); this is the end point of the titration. From the volume of base used to reach this point, you can calculate the concentration of the acid.



The end point of a titration can be detected visually using an appropriate acid-base indicator. Acid-base indicators tend to be organic dyes that display different colours in acid and alkaline solutions. In this practical we will use phenolphthalein indicator, which is colourless in acid solution, but pink in basic (alkaline) solution. When you are close to the end point a pink colour will form and then disappear after mixing; at this point you should reduce the amount of base you add to 0.1 ml increments. As the persistence of the pink colour increases, you should add the base dropwise. Once the pink colour is permanent, you should record the volume of base that has been added.

In this experiment the acid is acetyl salicylic acid (Aspirin) and the base is sodium hydroxide (NaOH).

2. Determination of aspirin quantity by titrimetric analysis

In this experiment you will determine the concentration of aspirin in the questioned tablet by pH titration.

Equipment:

• 25 mL bulb pipette & pipette filler • 3 x 100 mL conical flask • 50 mL burette • 50 mL beaker • White tile • Clamp stand & clamp • Stirrer plate & stirrer bead	• Questioned tablet sample • ~ 2×10^{-2} M NaOH solution (the exact concentration will be written on the bottle, you should note it down)

Experimental procedure:

1. Pipette 25 mL of the tablet solution into a 100 mL conical flask.
2. Place a magnetic stirrer bead into the conical flask and mount the flask on a magnetic stirrer plate.
3. Set the solution stirring at a moderate rate (ensure that the heat is not on!).
4. Using a beaker, transfer the ~ $2x10^{-2}$ M NaOH solution to the burette. (Do not transfer solutions above eye level).
5. Clamp the burette in place above the conical flask & place a white tile beneath the flask.
6. Add 5 drops of Phenolphthalein indicator to the conical flask.
7. Titrate the sample with ~ $2x10^{-2}$ M NaOH solution until the colour changes from colourless to pink.
8. You should repeat this procedure three times to obtain consistent results.

Results:

Titration	**Initial burette reading/mL**	**Final burette reading/mL**	**Volume of ~ $2x10^{-2}$ M NaOH used/mL**	**Average volume of ~ $2x10^{-2}$ M NaOH used/mL**
1				
2				
3				

Data treatment:

1. Calculate the number of moles of the NaOH solution that has reacted with the aspirin in the tablet solution.

$$moles\ \ mol\ = concentration\ mol\ L^{-1}\ \times volume\ (L)$$

2. Aspirin reacts with NaOH ions in a 1:1 ratio, so the number of moles of aspirin is equal to the number of moles of NaOH. From this and the volume of the contaminated tablet solution that you used, calculate the concentration of aspirin in the tablet sample.

$$concentration\ (mol\ L^{-1}) = \frac{moles\ (mol)}{volume\ (L)}$$

3. Convert the molar concentration (mol L^{-1}) to mass concentration (g L^{-1}) (molar mass aspirin = 180.16 g mol^{-1}).

Concentration of aspirin in questioned tablet sample: _____mg L^{-1}

This analysis should answer the question as to whether the amount of aspirin in the contaminated sample is different to what it should be.

What is your conclusion?

Experiment 3- Background Information

High Performance Liquid Chromatography (HPLC)

Chromatography involves the separation of components of a mixture. There are many different chromatographic methods of analysis, but they all work on the same principle; they all have a ***stationary phase*** (a solid, or a liquid supported on a solid) and a ***mobile phase*** (a liquid or a gas). The mobile phase, containing the components of the mixture, passes over (or through) the stationary phase. The different components travel at different rates according to how strongly each component is adsorbed onto the stationary phase, and are thus separated.

In HPLC, the mobile phase is a liquid (for this experiment it is 80% water:20% acetonitrile), and the stationary phase is made up of very small porous particles packed into a column. A small portion (20 μL) of the sample to be analysed is injected into the system using a special sampling loop. The components of the mixture are then carried through the column by the mobile phase solvent. Separation occurs in the column as a result of differential partitioning of the individual compounds between the mobile and stationary phases. Compounds that have a high affinity for the stationary phase, spend more time on the stationary phase, and hence take longer to move through the column, compared with those that have a lower affinity. The affinity depends on the chemical structure of the compound. As the compounds leave the column they pass through a detector, which results in a peak on the chromatogram.

The time taken for a compound to travel through the column is the called the ***retention time***. This can be used to identify the compound present by comparing with a standard. The area underneath the peak is proportional to the amount of compound that has passed the detector. This can be used to calculate the concentration of the compound in the sample. One way of doing this is to inject known concentrations of the compound, calculate the area under the peaks, and then produce a calibration plot of area vs. concentration.

Dilutions

For this experiment you will be required to make up dilutions from a stock solution of known concentration in order to obtain a range of solutions of differing concentration. To work out the volume of stock solution required to do this, the following equation will be useful:

$$\mathbf{c_1V_1 = c_2V_2}$$

(Where c_1 is the concentration of the stock solution, V_1 is the volume of stock solution you need to calculate, c_2 is the concentration you want to achieve, V_2 is the final volume of the solution).

3. Chromatographic analysis of caffeine

In this experiment you will use High Performance Liquid Chromatography (HPLC) to construct a calibration graph by measuring the area under the peaks of standards of known caffeine concentration. You will then use this graph to determine the concentration of caffeine in the questioned tablet

Equipment:

• 1 x 200 mL volumetric flask • Weighing boat & spatula • Glass funnel • 100 mL beaker • 5 × 25 mL volumetric flasks • 10 ml Graduated pipette & pipette filler • Plastic pipettes • HPLC instrument and computer • Injection syringe • Graph paper	• Caffeine • Distilled water • Questioned tablet sample • Acetonitrile (to clean syringe)

Experimental Procedure:

1. Prepare a 0.5 mg mL^{-1} caffeine stock solution by weighing out the correct amount of caffeine using an analytical balance (record the mass to 4 d.p.). Using a funnel transfer this to a 200 mL volumetric flask and add about 150 ml distilled water. When all the solid has dissolved dilute to the mark with distilled water.
2. From this caffeine stock solution, prepare 4 calibration standards of concentrations in the range of: 0.1 mg mL^{-1} - mg mL^{-1}. Do this by accurately using a graduated pipette and pipette filler to pipette an appropriate volume of stock solution (you will need to calculate this) into a 25 mL volumetric flask and making up to the mark with distilled water.

IF YOU ARE UNSURE OF HOW TO USE A GRADUATED PIPETTE PLEASE ASK A DEMONSTRATOR.

CONSULT A DEMONSTRATOR ABOUT THE OPERATION OF THE HPLC.

3. Inject the lowest concentration caffeine standard.
4. Once the run has finished (this will take about 3 minutes) determine the retention time of caffeine, and calculate the area under the peak
5. Repeat this procedure for the all of the standards and the questioned tablet sample.

Results:

Mass of caffeine used for stock solution =

Caffeine standard concentration	Retention time	Area under peak
Questioned tablet		

Plot a calibration graph by hand of area versus concentration for the standard caffeine solutions (express the area values in standard form to 3 decimal places). Use the graph to determine the concentration of caffeine in the questioned tablet sample. Record your results on the answer sheet.

Attach calibration graph

Concentration of caffeine in questioned tablet: _____mg L^{-1}
(Show your working out on the graph)

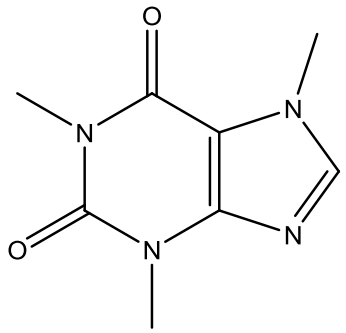
This analysis should answer the question as to whether the amount of caffeine in the questioned sample is different to what it should be.

What is your conclusion?

Questions

1. The acceptable daily intake (ADI) for Sunset Yellow is 1.0 mg/kg bodyweight per day. If a person weighing 65 kg consumed 4 'Revive' tablets (taken from the batch that you have tested today) in one day, would they exceed the ADI? Show your working.

2. The structure of caffeine is shown below. Using the periodic table provided, calculate the molecular weight of caffeine. Remember to include units.

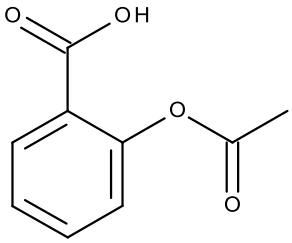


caffeine

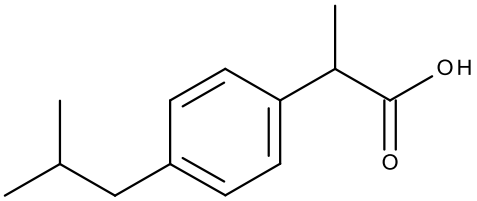
3. A 0.25 mol L^{-1} solution within a 1 cm path length placed within a UV-Vis spectrophotometer shows an absorbance of 0.85. Calculate the absorption coefficient ε for this compound from the relationship **A = εcl**. Show details of your calculation and work out the units of ε.

4. Name two other types of chromatography other than High Performance Liquid Chromatography.

5. The anti-inflammatory painkiller Ibuprofen has the same carboxylic acid functional group as aspirin. What volume of 2×10^{-4} mol L^{-1} aqueous NaOH solution would be required to neutralise 200 mL of 3.5×10^{-5} mol L^{-1} aqueous ibuprofen solution?



Aspirin



Ibuprofen