Royal Society of Chemistry
Analytical Division

North West Regional Heat

Schools' Analyst Competition

Wednesday 26th April 2017

University of Central Lancashire

LABORATORY HANDBOOK

School Name:.....

Student Name:.....

WELCOME!

Welcome to the University of Central Lancashire and RSC Schools' Analyst Competition. We hope that your will enjoy participating in today's event and that your visit to the university is a warm and memorable one.

Chemistry @ UClan

Chemistry provides an important understanding of our world and how it works. Through an understanding of the chemistry of materials we can design and manufacture drugs to fight disease; computer chips to enhance communication; pesticides to protect our health and crops; fertilizers to grow abundant food; fuels for transportation; fibres to provide comfort and variety in clothes; plastics to package food and replace worn-out body parts; and much, much more. However, it is becoming increasingly more apparent if we are to continue to enjoy the lifestyle which is so heavily reliant upon chemicals, then we will have to give more consideration to the impact that this has on our environment.

In addition to the chemistry degree courses we provide here at the university, chemistry also forms a large part of the Forensic Science degree programmes. As a result of this we are constantly striving for excellence in both our teaching and research, providing our students with an outstanding educational experience. Our approach to the research and teaching of chemistry is based on the interdisciplinary relationship of physical, organic, inorganic and analytical chemistry, with the focus of this approach being the student.

Competition Entries 2017

Bacup and Rawtenstall Grammar School

Bolton School

Bolton School Girls' Division

Bolton UTC

Bury Grammar School

Hutton Grammar School

Lancaster Girls' Grammar School

Moor Park High School

Preston's College

Queen Elizabeth's Grammar School (Blackburn)

Range High School

Rossall School

Runshaw College

St Christophers C of E High School

St John Rigby College

St. Mary's College Blackburn

Stockport Grammar

Tauheedul Islam Girls' High School and Sixth Form College

Westholme School

Winstanley College

Brief History and Outline

The first competition was organised in London by the then Polytechnic of North London (now London Metropolitan University) in 1982. In 1985, a similar competition was held in the Manchester area and then, when these proved to be so popular, other regional events were also held. These were brought together for the first time in 1990 in a National Final for the winners of all the regional competitions.

Since then, the Schools' Analyst Competition has become a nationally recognised competition run by the RSC's Analytical Division for first year sixth form/college students studying AS level Chemistry or equivalent. Regional heats are held at various universities around the country and the winners of each heat progress to the National Final.

The Final of the Competition sees up to 20 teams competing for a Challenge Shield. First, Second and Third placed teams in the National Final receive prizes of £1000, £600 and £300 respectively, with the other finalists receiving £100 each. There are also prizes for the individual team members of the first and runner-up teams.

Teams are required to undertake various analytical practical's based on problems relevant to industrial or social needs. These are judged on skill, understanding and accuracy and are intended to promote team work and safety in the laboratory. Each competition aims to provide some tasks which are relatively familiar to the students such as titrations and others which are likely to be unfamiliar, such as chromatographic separations or atomic and molecular spectroscopy. In this way, it is hoped that the competitors will learn new skills, as well as demonstrate their existing knowledge, abilities and aptitude for analytical science.

Analytical Chemistry

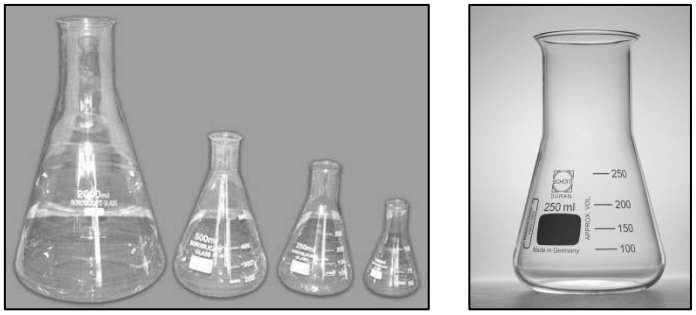
Analytical Chemistry is an essential aspect of many scientific disciplines and it requires good problem solving skills. It is used in identifying unknown compounds, determining purity of a newly synthesised substance, monitoring how a reaction proceeds, analysing evidence collected at a crime scene as well as making sure the quality of food you buy is of a particular standard. These are just a few examples of its uses.

THERE IS A STRICT TIME LIMIT OF THREE HOURS TO COMPLETE AND HAND IN YOUR EXPERIMENTS. IT IS UP TO YOU TO DECIDE HOW TO TACKLE AND DISTRIBUTE THE WORKLOAD AMONGST YOUR TEAM.

List of Equipment

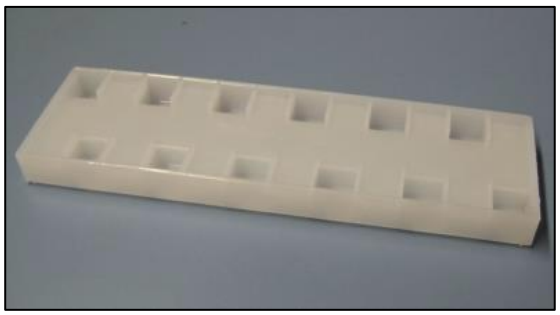
Burette

Pipette | Measuring Cylinder | Volumetric Flask



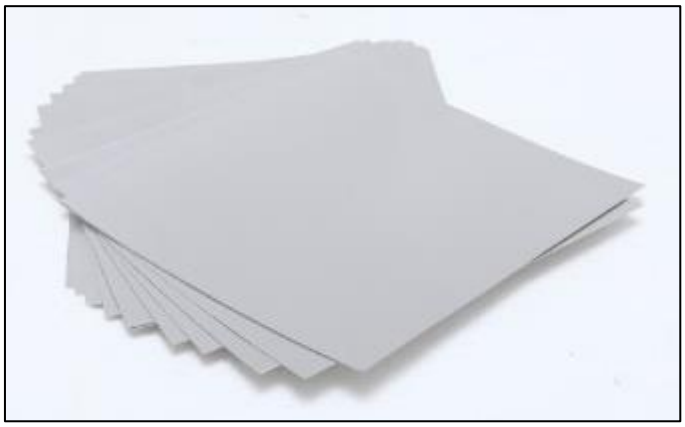
Narrow and Wide neck conical Flasks

Cuvette



Cuvette Holder

Analytical Balance



TLC Plates

UV Spectrophotometer:

Scenario

Mr Conrad Clarke was found dead in the playing field of his local gym at 10.45 am Saturday 16th February. His death is being treated as suspicious and police have a list of prime suspects.
You are to analyse various pieces of evidence that have been collected relating to the crime scene.

1. **Post Mortem Analysis:** Toxicological reports showed unusually high levels aspirin and caffeine in his bloodstream, levels high enough to have induced a heart attack. Mr Clarke was seen leaving the changing room drinking from a bottle of soft drink.
    a. Three bottles of soft drink from the crime scene have been recovered. It is suspected that Mr Clarkes drink had been purposely tampered with and spiked.
    b. A sachet of white powder was collected from Mr Clarkes sport bag. It is suspected to be a contaminated aspirin sample.
2. **Crime Scene Evidence:** Pieces of grass of been collected from both Mr Clarkes and a suspect's footwear. These have been sent for analysis along with a standard sample taken from the field Mr Clarke was found in.

You are to analyse these evidence samples using the following techniques:

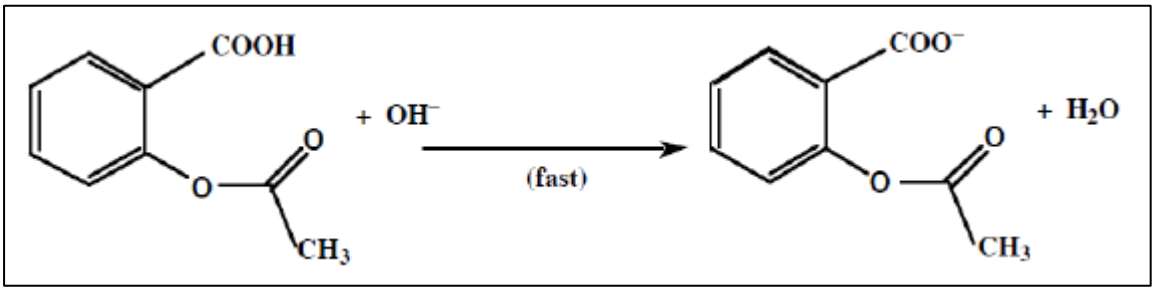
1. **Volumetric Analysis of Aspirin.**
    - This will determine the unknown mass of aspirin present in the sachet found at the scene of the crime.

2. **Spectrophotometric Analysis to Determine the Concentration of Caffeine.**
    - This will determine whether or not Mr Clarkes drink had been spiked.

3. **Thin Layer Chromatography Analysis of Grass Samples.**
    - This will determine whether or not the samples of grass obtained from the crime scene, Mr Clarkes shoes and the suspects shoes are from the same area.

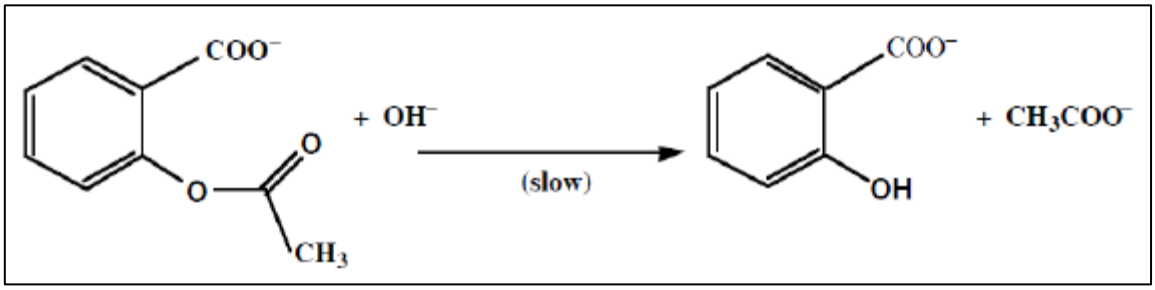
Back Ground Theory

Many reactions are slow or present unfavourable equilibria for direct titration. Aspirin is a weak acid, therefore it can react with a base, such as sodium hydroxide, NaOH. This is an example of an acid/base titration. (See Reaction 1).



Reaction 1: Acid/base reaction of aspirin with sodium hydroxide, NaOH.

Additionally, aspirin can also undergo a slow base hydrolysis reaction. This is where the ester functional group is hydrolysed to an alcohol and a carboxylate salt (See Reaction 2). This means that each aspirin molecule reacts with two hydroxide ions (OH^-).



Reaction 2: Base hydrolysis reaction of aspirin with sodium hydroxide, NaOH.

In the back titration of aspirin, a known excess amount of NaOH is added to the sample solution to ensure that both reactions have gone to completion. Then, an additional titration is performed using HCl to determine the amount of unreacted NaOH. This is subtracted from the initial amount of NaOH to find the actual amount of NaOH that reacted with the aspirin and hence the quantity of aspirin in the unknown sample.

Analytical Method:

Part A: Aspirin Sample preparation.

1. Accurately record the weight of your impure aspirin sample.
2. Transfer your impure aspirin sample to a labelled 100 mL conical flask and add 20 ml of ethanol and 30 ml of distilled water. Gently swirl to ensure all the aspirin dissolves.
3. To remove any undissolved solid particulates, filter your solution in to a 250 ml volumetric flask. Rinse the conical flask with 10 ml of distilled water and poor over the filtrate.
4. Make the volumetric flask up to the mark with distilled water and mix thoroughly.

Part B: Aspirin Titration with Base (Reaction 1)

1. Pipette 25 ml of your prepared aspirin sample in to a 100 ml conical flask. Add three drops of phenolphthalein indicator and swirl gently to mix.
2. Titrate the aspirin sample with the 0.1 M NaOH solution until the solution turns a permanent pink.
3. This is the completion of reaction 1 between aspirin and NaOH

Part C: Heating the Reaction to Completion (Reaction 2)

1. The aspirin/NaOH acid-base reaction consumes one mole of sodium hydroxide per mole of aspirin. The slow aspirin/NaOH hydrolysis reaction also consumes one mole of sodium hydroxide per mole of aspirin, and so, for a complete titration we will need to use a total of twice the amount of NaOH that you have already used. Additionally, to make sure the reaction has gone to completion you will need to add an excess amount of NaOH.
   (For example: if you used 15 mL of NaOH in Part B: Step 2, you will need another 15 ml of NaOH for the hydrolysis step. Then you will need to add an additional 10 ml of NaOH to ensure that it is in excess. Thus, you would have added a total of 15 + 15 + 10 = 40 mL of NaOH.) **It is essential that you record all this information.**
2. Gently heat the flask contents in a water bath. To make a water bath, fill the 400 ml beaker with approximately 100 ml of water and place it on the hot plate. Carefully put your 100 ml conical flask into the water. **Avoid boiling, because the sample may decompose.** While heating, swirl the flask occasionally. After 15 minutes, remove samples from the water bath and allow to cool for 5 minutes.
3. If the solution is colourless, add a few more drops of phenolphthalein. If it remains colourless, add 5 mL more of the NaOH and gently reheat. (Don't forget to add this additional volume of base to the previously recorded total volume.) Repeat this process until the solution remains pink and no longer goes back to colourless.

Part D: Neutralising the Excess NaOH

1. The NaOH remaining in each flask will be excess base that has not reacted with the aspirin. Using your burette with your ~0.1 M HCl solution, titrate the excess base in each flask with HCl until the pink colour just disappears. The endpoint is best described as clear/white.

2. Record all the volumes of bases and acids used in the data table.

Data Table:

Part B:	**Titre 1**	**Titre 2**	**Titre 3**	**Titre 4**
Initial Burette Reading				
End Burette Reading				
Volume of NaOH (**V1**)				
Part C:	**Titre 1**	**Titre 2**	**Titre 3**	**Titre 4**
Step 1: Excess NaOH (**V2**)				
Step 3: Excess NaOH				
Step 3: Excess NaOH				
Step 3: Excess NaOH				
Step 3: Total (**V3**)				
Part D:	**Titre 1**	**Titre 2**	**Titre 3**	**Titre 4**
Initial Burette Reading				
End Burette Reading				
Volume of HCl (**V4**)				

Calculations:

Volume of the base (NaOH) used in the first titration (V1) =

Volume of the extra base (NaOH) added in the flask (V2) =

Total volume of the base (NaOH) used in the reaction (V3) =

Volume of the acid (HCl) reacted with the mixture in the second titration (V4) =

Actual volume of the base (NaOH) reacted (V5) = V3 – V4

Number of Moles of NaOH reacted = Volume of the base in liters X Molarity of the base

Number of Moles of aspirin reacted (Asp_{mol}) =

Mass of Aspirin reacted = M_r X Asp_{mol}

Mass of Aspirin in Impure Sample =

NOTES:

- M_r is the Relative Molecular Mass
- Remember the molar ratio of NaOH to aspirin. You will need to divide the number of moles of NaOH by 2 to find the moles of aspirin.
- Remember that the mass of aspirin reacted is only a fraction of the total mass of aspirin in the impure sample.

Use this space for notes and calculations.

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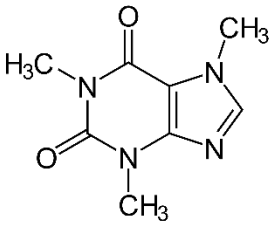
Background Theory

Many compounds can absorb radiation in the UV or visible regions of the electromagnetic spectrum. Caffeine absorbs UV radiation in the spectral range of 240 – 300 nm



Structure 3: Caffeine

The amount of UV radiation absorbed is influenced by three parameters:

- **Concentration, c,** of the species absorbing the UV radiation
  - This is the parameter you will be changing so you can plot a calibration curve to determine the concentration of caffeine present in the soft drink.
  -
- **Molar Absorptivity Coefficient, ε**, of the molecules at the wavelength used.
  - This is determined by the calibration graph of absorbance versus concentration where you will measure the absorbance of a series of solutions of known concentration.
  -
- **Path length, l,** through the solution.
  - This is 1 cm and is achieved using a glass or plastic cuvette

These three parameters fit in to the following equation:

$$A = \varepsilon \times c \times l \quad \text{(eq. 1)}$$

Once you have produced a calibration graph it is relatively straight forward to determine the unknown concentration of your sample by measuring it's absorbance and comparing it to your graph.

Method 1: Calibration Standards

You have been provided with a 200 mg L^{-1} standard caffeine solution.
Prepare caffeine standard solutions containing 4.0, 8.0, 12.0, 16.0 and 20 mg/L in distilled water.

1. To prepare an 8 mg/L solution, pipette 4.0 ml of caffeine standard solution into a 100 ml volumetric flask and dilute with distilled water to the mark.
2. Stopper the flask and mix thoroughly. Prepare other solutions in a similar manner.

NOTE: It is crucial that you take every care to be as accurate as possible as these solutions will be used to make you ***calibration graph***.

Method 2: Calibration Graph

The UV-Spectrophotometer wavelength has been at 270 nm.

Calibrating the UV Spectrophotometer

1. Fill a cuvette with distilled water up to the fill line (figure 1). This cuvette is known as ***The Blank***.
2. Place the cuvette in the spectrophotometer so that the clear smooth sides are in line with the beam path.
3. Ask a member of staff to show you how to use the spectrophotometer.

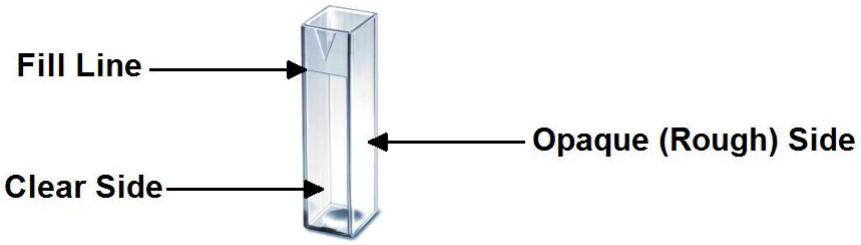


Figure 1: A cuvette has two opaque and two smooth clear sides. For a successful absorbance reading to be taken it is essential that the smooth clear side is in line with the path of the UV-beam.

Running Your Standards

1. Fill a clean cuvette with your 4 mg L-1 solution.
2. Place the cuvette in the spectrophotometer and run your sample.
3. Record the absorbance.
4. Repeat steps 1-3 for the remainder of your standard solutions.
5. Record the absorbance data you have collected in the table provided and then plot a graph of concentration versus absorbance.

NOTE: You can always ask a demonstrator to go through how to use the UV Spectrophotometer, even if they have already shown you before.

Absorbance Data:

Caffeine	
Concentration mg/L	**Absorbance**
4.0	
8.0	
12.0	
16.0	
20.0	

Method 3: Contaminated Drink Analysis

1. Warm approximately 20 ml of the standard soft drink provided in a beaker on a hot plate to expel any CO_2 present (be very careful not to boil the liquid.)
2. Then filter the warm liquid through filter paper to remove any particulates.
3. After cooling to room temperature, pipette 4.00 ml into a 100 ml volumetric flask and dilute with distilled water to the mark.
4. Measure the UV absorption spectrum for the 4:100 dilution of the standard soft drink. Record your result in the table provided and then determine the amount of caffeine in a Standard 500 ml bottle.
5. Repeat steps 1-4 for the three suspect samples. (***NOTE:*** *You will need to rinse and re-use your 100 ml volumetric flask step 3)*
6. Using the calibration graph you produced earlier, determine the concentration of caffeine in the standard drink. **Remember to take in to account the dilution factor, 4 ml in 100 ml and that the units in the answer are mg L^{-1}**

Results:

	Concentration	**Absorbance for Caffeine**
Standard Soft Drink	4: 100	

Concentration of Caffeine in Standard Drink	mg/L
Amount of Caffeine in Standard 500 ml Bottle	mg/L

	Absorbance for Caffeine	**Concentration of Caffeine**	**Concentration of Caffeine (500 ml)**
Suspect Drink A		4:100	
Suspect Drink B		4:100	
Suspect Drink C		4:100	

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Introduction

Chromatography is a physical technique used for the separation of a mixture of compounds and to purify a substance. Chromatography takes advantage of the fact that different substances possess different affinities for two phases, one a stationary phase and the other a mobile phase. The mobile phase can be liquid (liquid chromatography) or gaseous (gas chromatography).

Thin Layer Chromatography (TLC) is a widely used technique for qualitative analysis of substances. It is a very sensitive technique enabling very small amounts of compounds to be separated. The technique involves the passage of a solution of the substance under study through a solid stationary phase; therefore, it is a form of *liquid-solid chromatography*. The mobile phase is a solvent system chosen for the substance under investigation and the stationary phase is a support substance (usually silica) coated on to a solid backing (glass, plastic or metal).

Principle of TLC

A 'spot' of a substance under investigation is placed on to the TLC plate, and the plate placed vertically into the prepared TLC tank. The solvent in the base of the TLC tank slowly rises up the TLC plate, and the test substance is carried (eluted) up the TLC plate by the solvent.
The distance travelled by the substance is determined by how strongly the molecules of the substance are bonded to the stationary phase, and how strongly they are bonded to the solvent molecules. Consequently, a substance that is not adsorbed by the stationary phase will move with the solvent front. On the other hand, a substance that is strongly absorbed by the stationary phase will stick at the baseline of the plate, where the original spot was placed. In this way mixtures of substances can be separated by polarity since they will elute(move) through different distances on the TLC plate.

Use of TLC

TLC can be used for the separation of coloured compounds such as inks, dyes and in the case of this practical plant pigments as well as many other coloured compounds. Separation of coloured compounds can be observed on the TLC plate making determination of R_f values relatively simple.

R_f Values

The R_f for a given substance, under a given set of TLC conditions, is the ratio of the distance travelled by the substance (analyte) from the baseline, to the distance travelled by the solvent front (**R**elative to the **f**ront) (See figure 2).

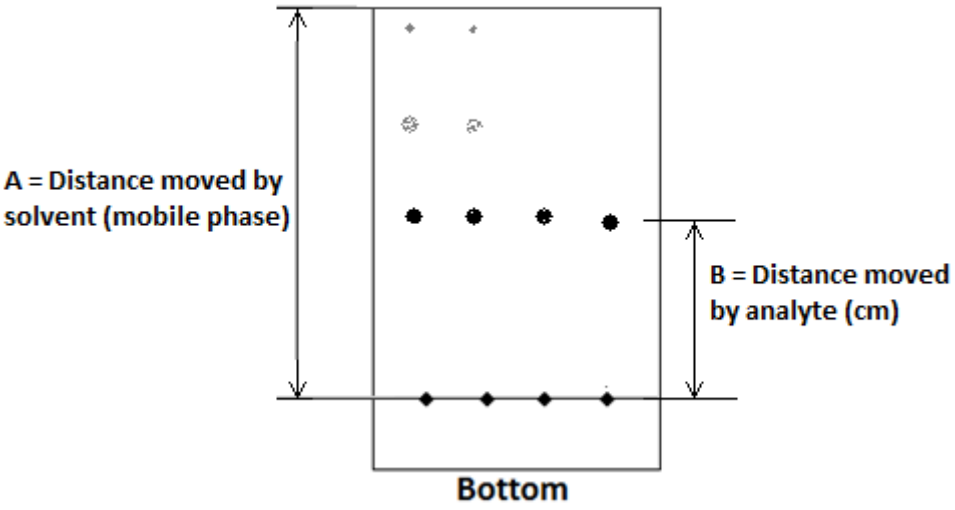

Figure 2: Calculating the R_f value of a substance (analyte)

R_f values will vary with different solvent systems and can also show some degree of variation from plate to plate for the same solvent system. For this reason, comparison of R_f values of two samples or a sample and a known standard will usually be carried out on the same plates. Rf values can be calculated using the following formula:

$$R_f = \frac{\text{Distance travelled by solvent}}{\text{Distance travelled by substance}}$$

Plant Pigments

There are a large number of coloured pigments in vegetative material that can be used for identification. Predominantly observed are chlorophylls and carotenoids. In reference table 1 are some of the common pigments and R_f values found in grass samples.

Determination of plant material such as the drug cannabis will often include analysis by TLC.

Pigment Visible	Colour R_f	Pigment Visible	Colour R_f
Carotene Yellow	0.98	Violaxathin Yellow-brown	0.66
Alpha Carotene Yellow-orange	0.97	Xanthophyll Yellow	0.5
Xanthophyll Yellow	0.86	Neoxathin Yellow-brown	0.28
Beta Carotene Yellow-orange	0.94	Chlorophyll Light blue-green	0.48
Xanthophyll Red	0.8	Chlorophyll Dark blue-green	0.46
Lycopene Yellow-orange-red	0.81	Chlorophyll Light yellow-green	0.30
Phaeophytin Dark-gray	0.67	Chlorophyll Dark yellow-green	0.25
Leutein Yellow-brown	0.75	Xanthophyll Yellow	0.15
Phaeophytin Light-gray	0.6		

Reference Table 1: Various R_f values for pigments commonly found in grass.

Method 1: Analysing Your Known Sample.

TLC of Extracted Plant Material

A number of compounds dealt with in chemistry such as dyes, paints, and plant material will produce coloured components when separated using TLC. As these compounds will be visible on the chromatogram plate, the need for visualisation is eliminated. The correct solvent system has to be chosen however, to ensure the best possible separation of components.

Procedure 1: Sample Preparation

1. Crush 0.5g of the plant material provided from the suspect area using a pestle and mortar with 2 ml of ethanol and 4 ml of petroleum ether (a 1:2 ratio) added. Grind until a dark green coloured extract is observed.
2. Pour the solvent into a sample bottle and place astopper. If 2 layers of solvent are visible shake the bottle vigorously, if not add 1 ml of petroleum ether then shake vigorously.

Procedure 2: TLC Plate Preparation (Figure 3)

1. On a pre-cut TLC plate *lightly* draw a pencil line across each plate 1 cm from the bottom and 0.5 cm from the top. (NOTE: Do not press too hard with a pencil as this will damage the stationary layer)
2. Using a clean TLC spotter, carefully spot the extracted sample onto the TLC plate. Ensure the spot is as small as possible, and concentrated enough by applying the sample a number of times ensuring the spot dries between each application. Allow each spot to dry before re-applying.

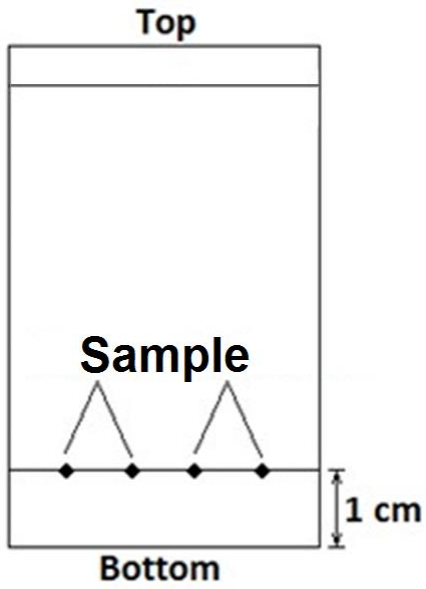

Figure 3: Schematic diagram of a prepared TLC plate.

Procedure 3: Running the TLC Plates (See figure 4)

1. Using petroleum ether as the solvent run a TLC plate of the plant extract following the steps 2-5.
2. To run the TLC plates, in a 100 ml beaker add less than 1 cm^3 of eluting solvent (i.e. solvent must be below spotting line on the TLC plate).
3. Carefully place the TLC plate into the beaker and cover with a watchglass. Make sure the TLC plate us level.
4. When the solvent front has reached the pencil line at the top of the plate, remove the plate and immediately place on a paper towel. Allow the plate to thoroughly dry before examining the plate.
5. When the best solvent system has been determined calculate the R_f values of the spots observed and record your results in the table provided.
6. Repeat step 2-5 using acetone as the solvent. Once you have completed that use the information to determine if a mixed solvent system would produce a better separation and what ratio of solvents would be needed.
7. Run a TLC plate with your chosen solvent system.

Solvent system chosen (Ratio) _____

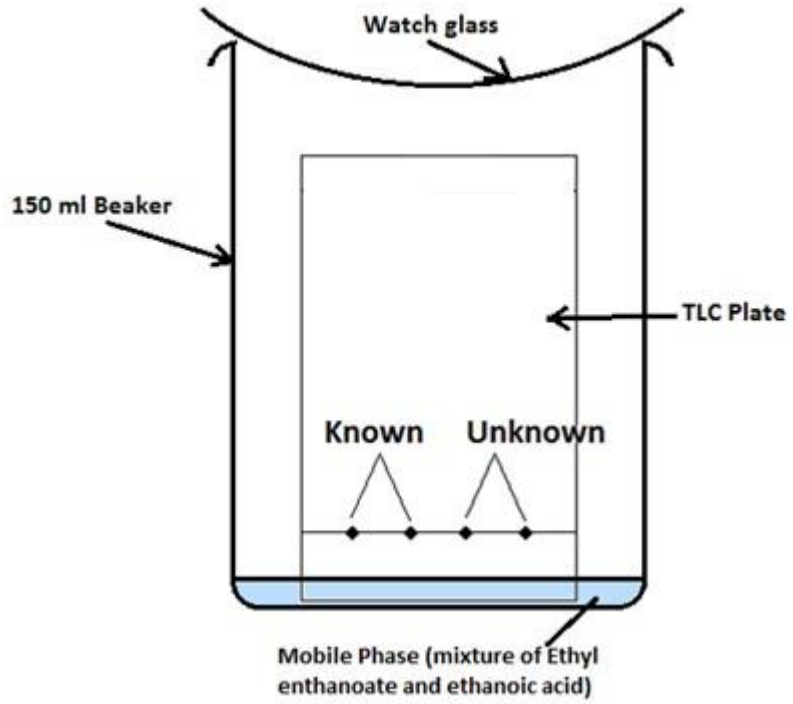

Figure 4: Simplified diagram showing the running of a TLC analysis.

Analysing Your TLC Plates

The components you ***may*** be able to see are (in order of decreasing R_f values):

- Carotenes (yellow-orange)
- Pheophytin a (gray, may be nearly as intense as chlorophyll b)
- Pheophytin b (gray, may not be visible)
- Chlorophyll a (blue-green, more intense that chlorophyll b)
- Chlorophyll b (green), Xanthophylls, (as many as three spots, yellow)

Using the chart given in reference table 1, identify any of the components by comparing similar R_f values, colour and order in which pigments separate.

Method 2: Analysing Unknown Sample

Following the identification of a suitable solvent system and pigmentation bands that can be separated in the grass sample, prepare an extraction sample from the two pieces of the plant material evidence recovered from the car, in the same manner outlined in Method 1 (page 26).

The two samples you have been provided with are:

- Sample A removed from the boot of the suspect's car
- Sample B removed from the wheel arches of the suspect's car

Compare the three extraction samples using TLC and your chosen solvent system and determine if the grass found in the car matches the grass from the crime scene.

Data Collection

Your results are to be recorded directly in the table below.

NOTE: Just because the table below has ten spots available for results does not mean that your samples will contain ten compounds. It is unknown how many compounds your samples contain.

Comparison of Determined R_f Values				
Order of Separation on TLC Plate (Start with the spot closest to the bottom)	**Known Sample R_f Values**	**Unknown Sample A R_f Values**	**Unknown Sample B R_f Values**	**Positive Match Y = Yes N = No**
Spot 1				
Spot 2				
Spot 3				
Spot 4				
Spot 5				
Spot 6				
Spot 7				
Spot 8				
Spot 9				
Spot 10				

Use this space for notes and calculations.

Use this space for notes and calculations.