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Royal Society of Chemistry

Analytical Division North West Region

Schools Analyst Competition

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Experimental Handbook

ROYAL SOCIETY OF CHEMISTRY

SCHOOLS ANALYST COMPETITION 2017

The Scenario

An unnamed man, 75, who lived alone has been found dead in an armchair late one afternoon at his home. He was visited by his GP early that morning and found to be in excellent health so foul play cannot be ruled out.

A half-empty bottle of potassium chloride solution lay on the floor. It could have accidentally fallen from the bag of his GP that morning. It is of concern because potassium ions which will affect the sodium / potassium balance can cause the heart to stop beating by interfering with nerve impulses. Indeed, cardiac surgeons have used potassium chloride to temporarily stop the heart during operations. Also in the room are a couple of empty whisky glasses, thought to be left from a late morning visit by a friend of the deceased.

The detective inspector who is in charge of the investigation, has arranged for a sample of urine to be taken and you as forensic analysts, are asked to determine the ethanol and potassium concentrations in the urine*; You have access to data on normal and abnormal levels for potassium and alcohol below so that you can advise on the likely cause of death.

You will be provided with the necessary laboratory equipment, and a method which you must use to measure the alcohol and potassium content.

At the end of the experiment your results will be assessed by the judges and they will select the winner on the basis of Accuracy, Precision and also the standard of your laboratory technique and data presentation. The 'true' value for the alcohol and potassium ion the sample has been worked out using the same method by department's staff, and your aim is to get as close as possible to this value.

Ethanol in urine

Urine should be zero if no alcohol has been ingested.
The threshold for drink driving in England, Wales and Northern Ireland is 1.07mg/ml. Levels greater than 3-4mg/ml can be fatal due to respiratory depression.

Potassium in urine

The standard reference range of potassium in an adult human should be between 136.5mg/l and 198.9mg/l

**due to hygiene and safety the sample given is not urine but a spiked coloured water sample made up by the department's staff to match the original urine sample*

THEORY

The ethanol analysis is based upon the oxidation of ethanol by potassium dichromate (orange) under acidic conditions to produce acetaldehyde, (see reaction 1 below). The dichromate then oxidizes the acetaldehyde to acetic acid, (see reaction 2). As the dichromate oxidizes the various reactants, it is in turn reduced to Cr^{3+} which is green. The green colour is the result of a characteristic absorbance of light at a wavelength in the region of 600nm.

Reaction 1

$$3CH_3CH_2OH + K_2Cr_2O_7 + 4H_2SO_4 \rightarrow 3CH_3CHO + Cr_2(SO_4)_3 + K_2SO_4 + 7H_2O$$

Reaction 2

$$3CH_3CHO + K_2Cr_2O_7 + 4H_2SO_4 \rightarrow 3CH_3CO_2H + Cr_2(SO_4)_3 + K_2SO_4 + 4H_2O$$

The intensity of the green colour is measured using a spectrophotometer. The absorbance is directly proportional to the concentration of Cr^{3+} in the solution (The Beer-Lambert law). The concentration of Cr^{3+} is also proportional to the concentration of ethanol in the original sample. Measurements are made for a series of standard solutions containing a known concentration of ethanol and a graph is plotted of absorption against concentration. This graph, known as a calibration curve, can then be used to calculate the concentration of ethanol in an unknown sample.

Because the urine sample will also contain other organic material, such as sugars, it is necessary to separate the ethanol by distillation prior to carrying out the oxidation. If this were not done the result would be higher than it should due to oxidation of these ‘interfering’ compounds.

Dr Tom Parry Jones OBE, an eminent Welsh entrepreneur and inventor, developed the world's first Electronic Breathalyser based on dichromate in 1974 and established Lion Laboratories to manufacture and market the products worldwide. It has since been replaced by more sophisticated electrochemical devices.

For determining the potassium concentration you will make up a series of standards and then run these along with the unknown sample on the Atomic Absorption Spectrometer (AAS).
Atomic absorption uses a high temperature flame to vaporize the sample and measures the amount of light absorbed at a wavelength unique to the element of interest. In comparison to spectrophotometry which measures the absorbance of light by an Iron containing molecule, Atomic Absorbance spectroscopy measures the absorbance of light by Iron atoms. The greater the amount of light absorbed, the higher the concentration.

HEALTH AND SAFETY

It is essential that you read the following risk assessment, and are fully aware of the hazards associated with the materials you are using.

Hazard	Risk	Control Measures	First aid in case of accident
Acidified Potassium Dichromate solution	Highly corrosive, Oxidizing agent, Causes burns to skin and eyes. Toxic by ingestion.	Very small quantities (1ml) issued in cuvettes. Substance must not be removed from the cuvette. Gloves, apron, and Safety glasses to be worn at all times	In case of contact with skin or eyes Rinse the affected area with cold water immediately for at least 5 minutes. Report the incident to a member of staff. Obtain medical advice
0.25% Ethanol solution	Harmful by ingestion, may cause dizziness and disorientation in excessive quantities. Serious long term chronic effects target organs Liver, Kidneys, Heart and Brain.	Small quantities issued (250ml) to each group.	In case of contact with skin or eyes Rinse the affected area with cold water Report the incident to a member of staff.
Unknown urine samples	Harmful by ingestion, Serious long term chronic effects. Target organs Liver, Kidneys, Heart and Brain	Issue of small quantities closely supervised by staff.	In case of contact with skin or eyes Rinse the affected area with cold water Report the incident to a member of staff
Boiling water	Burns caused by spillage, splashes or contact with liquid or steam	Eye protection worn and tongs used to handle hot items. supervision and assistance of trained staff at all times. Solutions should be allowed to cool before handling.	Wash affected area under cold water and seek first aid assistance immediately
Glassware	Glassware breakage causing cuts	All glassware is pre-checked by technical staff and any damaged equipment should be returned. Care to be exercised especially when pushing rubber pipette fillers on to glass.	Contact a member of staff and obtain first aid assistance immediately in the case of an accident

GENERAL LABORATORY SAFETY

Pay careful attention to the safety advice that will be given to you prior to working in the lab. Familiarize yourself with the location of emergency exits. Eating, drinking or smoking is not permitted in the lab and mobile telephones must be switched off. Whilst working in the lab you must only touch chemicals and equipment that are used in your experiment.
Do not run or fool around. You must report any accidents or incidents, however small, immediately to a member of staff.

Please ask one of the demonstrators or technicians if you have any questions.

CHEMICALS AND EQUIPMENT

Each team will be provided with;

EQUIPMENT

bulb pipettes (10ml, 20ml, 25ml, 50ml)
pipette fillers
Beakers (100ml x 2, 600ml x 1)
Graduated pipettes (25ml, 10ml, 5ml)
Measuring cylinder (25ml)
100ml round bottom flask and distillation apparatus (still head, condenser etc)
Heating mantle or hotplate
Small cork ring to support round bottom flask
Stopper for 100ml round bottom flask
100ml volumetric flask (x7) with stopper
Test tubes (x 9) and rack
Small funnel
Pasteur Pipettes and teat
Graph paper, ruler, pencil
Tongs
Plastic 1cm cuvettes (x 9) in a rack
Marker pen (medium)
Boiling chips

CHEMICALS

Urine sample (safe to handle)
Ethanol solution (2.5 mg/ml)
Potassium stock solution (1000 µg/ml)
Acidified potassium dichromate solution **CARE!**
Deionized water (in wash bottle)

EXPERIMENTAL PROCEDURE A – Ethanol determination

1 – Distillation

Transfer using a bulb pipette 25 mL of the unknown sample into a 100 mL round bottom flask and add 25 mL water and a boiling chip. Place the flask in a heating mantle and assemble a distillation apparatus (Please ask the technical staff for help – you will not lose any points). Ensure that the water is flowing within the condenser and heat the flask. Collect approximately 25 mL of distillate in a graduated beaker. Do not allow the heated flask to dry out. *(**Tip**: Whilst waiting for the distillation to finish you may wish to prepare the standards as detailed in section 2, the water bath (see section 3) can be prepared to save time later also the potassium determination can be started.)*

Transfer the distillate to a 100 mL volumetric flask and rinse the beaker several times with small amounts of deionised water, pouring the rinses into the volumetric flask. Make the volume up to the 100 mL mark with deionised water. Stopper the flask and invert it several times to mix the contents

2 - Preparation of the standards and sample

Label nine test tubes as described in the table below. You are provided with a standard 2.5 mg/ml solution of ethanol. In each test tube prepare the following solutions using a graduated pipette.

Test tube	mL of deionized water	mL of 2.5 mg/ml ethanol	Resulting mg/ml ethanol
blank	2.50	0.00	0.00
Standard 1	2.00	0.50	0.5
Standard 2	1.50	1.00	1.0
Standard 3	1.00	1.50	1.5
Standard 4	0.50	2.00	2.0
Standard 5	0.00	2.50	2.5
Sample (reading 1)	Each of these three tubes will contain 2.5ml of your sample distillate only. Three replicate measurements can then be made		
Sample (reading 2)			
Sample (reading 3)			

3 – Oxidation of the sample and standards

Prepare a water bath by adding about 200ml of tap water to a 600ml beaker and place the beaker on a hotplate in one of the fume cupboards. Heat the water to a gentle boil.

Warning: Acid dichromate solution is highly corrosive and an oxidizing agent. <u>Gloves and eye protection must be worn</u> and the following operation must be performed in a fume cupboard using extreme caution.

Add 2.5 mL of acid dichromate solution to each of your nine test tubes. Place the test tubes into the water bath and once boiling re-commences heat the test tubes for 15 minutes. Remove the test tubes using tongs and allow them to cool to room temperature. Using a Pasteur Pipette carefully transfer enough solution from each tube to two thirds fill a disposable plastic cuvette. Note the position in the rack of each cuvette to avoid confusion. **DO NOT WRITE ON THE CUVETTES!**

4 – Measurement of absorbance

Take your cuvettes containing the standards and samples to the spectrophotometer and with the assistance of a demonstrator measure the absorbance of each cuvette at a wavelength of 590 nm. Record your results. The blank should be placed in the reference beam of the spectrophotometer. After recording your absorbances, pour the dichromate solutions into the labeled waste bottle. **Do NOT pour down the sink!**

5 – Treatment of results

You may now use the data that you have obtained to plot a graph of the absorbance against the concentration of each standard. You can then use the graph to read the concentration of ethanol in your sample. Remember that you initially took 25ml of the urine sample and diluted the ethanol obtained to a total volume of 100ml. You will need take this into account when calculating the ethanol concentration in the urine by multiplying the result by 2.

EXPERIMENTAL PROCEDURE B – Potassium determination

You are provided with a stock solution containing 1000mg/l of potassium.
Use the following method to make a range of standards to generate a calibration curve:

In a 100ml volumetric flask dilute the following up to the mark using deionised water:

Stock solution (ml)	Concentration (mg/l)
10	100
20	200
30	300
40	400
50	500

With what's left in the unknown sample bottle and your standards you will be taken down to the 1st floor research laboratory with a demonstrator and be shown how to run your samples on the AAS instrument, please take a book with you and note down the values so you can generate a calibration graph and work out the concentration of potassium in the sample.

RESULTS SHEET ETHANOL DETERMINATION

(to be handed in with your graph)

NAME OF SCHOOL _____

SAMPLE	ABSORBANCE	CONCENTRATION (mg/ml)
Standard 1		0.5
Standard 2		1.0
Standard 3		1.5
Standard 4		2.0
Standard 5		2.5
Sample (reading 1)		
Sample (reading 2)		
Sample (reading 3)		

Average absorbance for three replicates_____

Concentration of ethanol in unknown _____mg/ml

Concentration of ethanol in urine sample*_____mg/ml

** Remember to multiply your answer by two to account for the dilution factor*

RESULTS SHEET POTASSIUM DETERMINATION

(to be handed in with your graph)

SAMPLE	ABSORBANCE	CONCENTRATION (mg/l)
Standard 1		100
Standard 2		200
Standard 3		300
Standard 4		400
Standard 5		500
Sample (reading 1)		
Sample (reading 2)		
Sample (reading 3)		

Average absorbance for three replicates_____ mg/l

Concentration of Potassium in unknown _____

Given your findings what would you suggest contributed to the death?

_____

_____

_____

When you have completed your results sheet please hand it to the judges with your calibration graph

FOR JUDGES USE ONLY	
Accuracy (Actual value – Measured mean value) / Actual Value	
Precision (spread of replicate values)	
Graph presentation	
Laboratory technique (Observed during experiment)	