

# **DRINK, DIABETES OR DEADLY DEED?**



**A group experiment in Forensic Analysis for the  
Royal Society of Chemistry Analytical Division  
North East Regional Heat of the  
Schools' Analyst Competition 2006**

**Analytical Chemistry** is all about solving problems. The context could be forensic analysis such as the experiment that you are doing today, industrial analysis ensuring that an industrial process works efficiently and that the products are of the correct composition, clinical analysis analysing patient samples or environmental monitoring. In all these, analysts have to design experiments, carry them out and interpret the data.

Today's exercise is designed to give you a taste the type of work that an Analytical Chemist has to do. We hope that you find it interesting and challenging and perhaps consider Analytical Chemistry as a career.

First you will have to decide how to tackle your problem, so that you can distribute the workload among your team of three so that each of you is always busy. Then you will need to carry out the experiments, perform the calculations and make some decisions based on the data obtained. A few questions complete the exercise.

You should already have enough background knowledge (but feel free to ask a demonstrator if there is anything that you do not understand), but you will need to show common sense and good organisational skills.

It is part of the RSC Schools' Analyst Competition being carried out in several centres and so we have to operate under the constraints of the competition and keep to time but primarily we hope that you enjoy doing the exercise.

**Please read and understand the instructions before commencing and note that there is a strict time limit of three hours for the exercise.**

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### **Health and Safety**

This is a practical exercise, so normal rules for safety in the laboratory apply.

Wear laboratory coats and safety spectacles at all times.

Do NOT eat or drink in the laboratory.

Always use the pipette fillers provided, and handle glassware carefully to avoid breakage and cuts.

Keep long hair under control.

**IF IN DOUBT ABOUT ANYTHING THEN ASK A DEMONSTRATOR.**

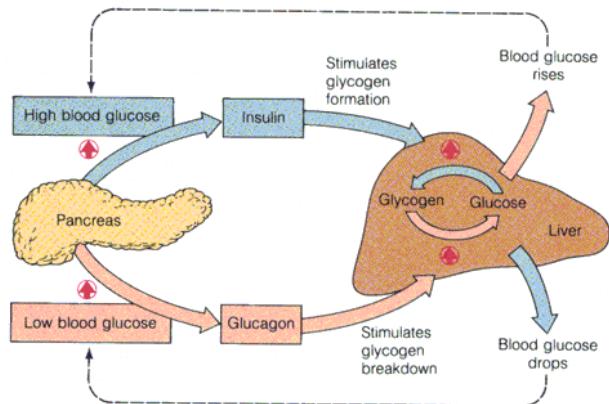
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***THE ORGANISERS THANK YOU FOR PARTICIPATING AND WISH YOU ALL THE BEST FOR YOUR FUTURE.***

## The Scenario

Alan Appleton, 75, who lived alone since his wife died a year or so ago, has been found dead in an armchair late one afternoon at his home. He suffered from diabetes but was otherwise in excellent health so foul play cannot be ruled out.

Mr Appleton suffered from insulin-dependent diabetes mellitus. In this disease, the pancreas does not produce enough insulin to control the level of glucose in the blood. The high levels cause serious problems and so the sufferer requires regular injections of insulin to reduce the blood glucose. On the other hand, too much insulin can reduce blood glucose to dangerously low levels (hypoglycaemia) and possibly death.



A half-empty bottle of potassium chloride solution lay on the floor. It could have accidentally fallen from the bag of his GP, Dr Betty Bolland, who had made a routine visit early 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 from Alan Appleton's friend Charlie Campbell, a retired biochemist. Drinking alcohol is more risky for diabetics than others, since alcohol interferes with the release of stored glucose from the liver increasing the chance of hypoglycaemia.

D.I. Doorstop, who is in charge of the investigation, has arranged for samples of blood and urine to be taken. Serum (a cell-free preparation) has been obtained from the blood and you as forensic analysts, are asked to determine

- i) the ethanol concentration in urine;
- ii) the glucose concentration in serum; and
- iii) the potassium concentrations in serum.

You have access to data on normal and abnormal levels for all of these so that you can advise D.I. Doorstop on the likely cause of death.

## **Planning**

To be successful you will need to plan how each member of the group will use their time. Our estimate of the time required for the experiments is

Experimental	2.0 - 2.5 h each
Calculation	0.5 - 1.0 h each

Decide how you will organise the work within your group; then write out a plan in the form of a flow chart (this is a simple diagram showing the key steps to be taken, in a series of boxes linked by arrows to show the sequence of events). Each box should explain, in brief, the action to be taken at that point. Individual responsibilities should be indicated for each step. The flow chart will be handed in with the results, so do it neatly.

Record the results neatly on the sheets provided, plot your graphs (remember to include titles), perform the calculations then, as a group, draw conclusions from your data. Finally, as a group, answer the questions in the spaces provided.

When you have finished hand in your flow sheet, the result sheets and your graph to the organiser.

## **Experimental work**

### **Dilution**

When making up dilutions, always do so accurately by using a pipette and by making up to a fixed volume in a standardised flask. For example, if you start with a  $200 \text{ gL}^{-1}$  solution and wish to make up a  $10 \text{ gL}^{-1}$  solution, you need a 20 fold dilution so you could pipette 5.0 mL of the standard into a 100 mL volumetric flask and then dilute to the mark.

### **Measurements**

Except where you need to run a high concentration first to check that the concentrations will be on scale, you should run your standards in order of increasing concentration to reduce the risk of cross contamination.

Your unknown test samples should have a reading within the calibration range. If this is not the case, then you should normally dilute the sample quantitatively until it gives a signal within the range.

### **Assessment**

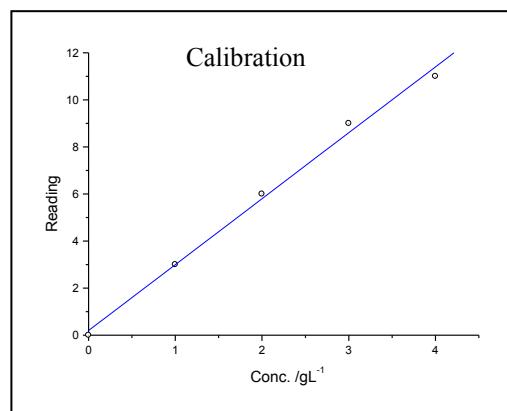
For the purpose of the competition, you will be assessed on your analytical results, your presentation of the results and your deductions.

## **Treatment of results**

### **Calibration**

Draw the points onto clearly labelled graphs. The methods here should give straight lines and unless the points are clearly better fitted with a curve, you should estimate by eye the best fit straight line which will be one which minimises the distances from the line to the points rather than the one which passes through the most points.

### **Calculation**



Ensure that your unknown sample gives a reading within the range of your calibration. Dilute the unknown if necessary. Read the concentration off the graph and correct for any dilution before quoting the value in the original sample supplied.

For each result reported try to estimate the uncertainty in the answer by quoting the range within which you are reasonably confident that the answers lies. (Suggestions for how to do this are given for each individual experiment.)

### **Expected levels**

The reference ranges (concentrations expected for normal individuals) for your analytes are:

Potassium in serum  $3.5 - 5.3 \times 10^{-3} \text{ mol L}^{-1}$

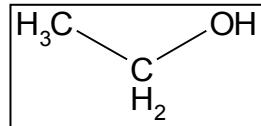
Glucose in serum  $65 - 130 \text{ mg dL}^{-1}$  (RMM for glucose is 180.16.)

Ethanol in urine Nominally zero in the absence of ingestion.

The threshold for drink-driving prosecution is 106 mg per 100 mL urine (said to be achieved with about one and a half pints of ordinary bitter or a double measure of spirits).

## 1. Determination of ethanol in urine using gas liquid chromatography.

There are many methods for the determination of ethanol in various sample matrices. Gas liquid chromatography is usually the method of choice for analysis of volatile compounds and works well for ethanol. An internal standard, here propan-1-ol, is often used to compensate for variations in the volume of sample injected. The same amount of propan-1-ol is added to each solution measured. Chromatograms of each of the standards and of the unknown sample are then obtained by injection of a small volume of each solution into the chromatograph.



### Equipment

Gas chromatograph with suitable column set for isothermal operation at 70 C. Integrator. Injection syringe. Standard ethanol solution (20.0 g L <sup>-1</sup> ) Standard propan-1-ol solution (1.00 g L <sup>-1</sup> ) Deionised water in wash bottle	10 mL graduated pipette 1 mL and 5 mL bulb pipettes Pipette filler 5 x 100 mL volumetric flasks 6 x 10 mL volumetric flasks "Corpse urine" sample
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### Procedure

- 1 From the ethanol stock solution, prepare four calibration standards to cover the range 1 to 4 g L<sup>-1</sup> by pipetting an appropriate volume of stock solution into a 100 mL volumetric flask and making up to the mark with deionised water. Stopper each flask. (A fifth calibration standard of zero ethanol concentration will be made from deionised water alone.)
- 2 Add the internal standard to the five calibration solutions and the unknown test sample as follows. To a 10 mL volumetric flask, add by pipette, 1 mL of each calibration standard or test sample and 5 mL of propan-1-ol solution and make up to the mark with deionised water.

*ASK FOR A DEMONSTRATION OF THE USE OF THE GAS CHROMATOGRAPH.*

- 3 Take the highest concentration standard and inject 1 µL into the gas chromatograph. Start the integrator immediately. Rinse out the syringe with deionised water.
- 4 Check that the two peaks are clearly resolved and that the larger one gives between 50% and 90% of full scale on the paper. Ask for assistance in changing sensitivity if it is not.
- 5 Now, starting with the lowest ethanol calibration standard, for each of the calibration standards and then the unknown test sample, flush the syringe with the solution and then inject 1 mL of solution.

### Calculation

Calculate the ratio of the peak areas (ethanol/propan-1-ol) for each standard and for the unknown test sample. Construct a calibration graph of these peak area ratios against the standard concentrations and then read off the unknown concentration from it.

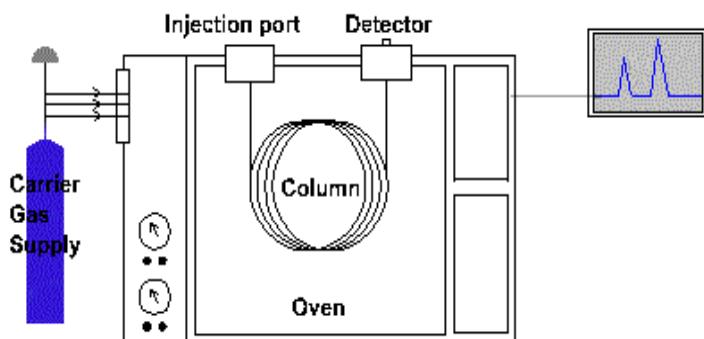
## Background

Chromatography is a separation technique that involves two phases, a mobile phase which contains the sample being analysed flows over a stationary phase which retards the components of the sample by different amounts (e.g., water and paper in the separation of dyes in ink).

In gas liquid chromatography the mobile phase is a gas (in this experiment it is nitrogen) and the stationary phase is a liquid. To achieve a large surface area of liquid (and therefore allow free interaction of the components of the mixture with the stationary phase) it is coated onto tiny particles of an inert material and packed into a column. These columns are about two metres long and so have to be coiled up to fit in the instrument.

Gas liquid chromatography is normally performed at elevated temperatures in order to maintain a high vapour pressure of all the components of the mixture. The column is therefore contained in a thermostatted oven.

Samples are injected with a microsyringe through a rubber septum into the gas stream, the components of the mixture instantly volatilise and are carried onto the column by the gas stream. The mixture is separated during passage down the column, at the outlet of which is a detector which can measure the amount of eluting analytes. The injector and detector are maintained at higher temperatures than the column so that all the mixture components are in the gas phase when in the injector or the detector.



The flame ionisation detector is the most widely used type. In this, partial combustion in a small hydrogen flame causes fragmentation and ionisation of organic molecules eluting from the column. A charged electrode attracts these fragment ions and so an electrical current, proportional to the number of ions, can be measured when a compound elutes from the column.

The resulting chromatogram is displayed as a series of peaks on a time-based recorder. There are two key measurements that we make for each peak displayed.

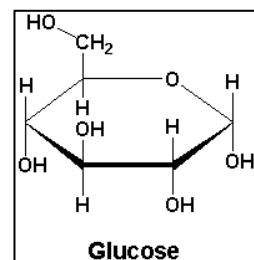
The first is the **retention time** for each peak (the time between injection of the sample and appearance of the top of the peak on the recorder), which permits qualitative identification by comparison with known components.

The second is the **peak area**, which is directly proportional to the amount of the component present (a calibration curve is produced by injection of known amounts and the quantity of unknown determined by comparison of peak areas).

Modern gas chromatographs, such as you will use here, have automatic integrators attached which will calculate the retention times and peak areas for each component detected.

## 2. Determination of glucose in serum, using an enzyme assay with colorimetric detection.

**Glucose is not easy to analyse. It is not coloured and it is difficult to separate from the other molecules found in serum. For this reason, an indirect method of analysis has been developed which uses the specificity of an enzyme reaction to generate a coloured product molecule which is then measured by its spectroscopic absorption.**



### Equipment

Visible spectrophotometer (colorimeter)	Stock D-glucose solution ( $0.100 \text{ mol L}^{-1}$ )
Plastic 1 cm pathlength cells	Reagent solution 1
10 mL graduated pipette	Reagent solution 2
Pipette filler	"Deproteinised corpse serum" sample
50 $\mu\text{L}$ pipette	Deionised water in wash bottle
8 x 100 mL volumetric flasks	Pasteur pipettes.
	12 test tubes

### Procedure

- From the glucose stock solution, prepare four calibration standards to cover the range  $2$  to  $8 \times 10^{-3} \text{ mol L}^{-1}$  by pipetting an appropriate volume of stock solution into a volumetric flask and making up to the mark with deionised water. (A standard of zero glucose concentration will be made from deionised water alone.)
- Add 50  $\mu\text{L}$  of each of the standard solutions, deionised water and the serum sample to separate test tubes. Mix 2 mL Reagent solution 1 with 2mL Reagent solution 2 and add into the test tube. Leave for  $18 \pm 1$  mins at room temperature. Make duplicates of each.

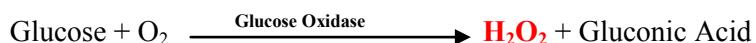
*ASK FOR A DEMONSTRATION OF THE USE OF THE SPECTROPHOTOMETER.*

- Set the spectrophotometer to a wavelength of 520 nm and set the zero absorbance with deionised water in the cell. Make sure that you hold the cell by the frosted faces and that the transparent optical faces are clean.
- For each of the mixtures from step 2, transfer some solution from the test tube, using a Pasteur pipette, into the cell, place in the instrument and record the absorbance.

Now plot a calibration graph using the absorbances read from each of your standard solutions, and read off the concentration of glucose in the serum from the graph.

### Background

*Glucose oxidase produces hydrogen peroxide as the co-product of the coupled enzyme assay. The hydrogen peroxide then reacts with the dye precursor(s) and horseradish peroxidase (HRP) yielding a coloured dye, shown below.*





*Once the dye has been formed, its absorbance is measured. This is proportional to its concentration which is in turn proportional to the original concentration of the glucose.*

### **UV/visible spectrophotometry**

*Light can be split into the spectrum of colours that we see in a rainbow; different colours signify different wavelengths and, therefore, different energies. We call a beam of light of one colour monochromatic. Light will be absorbed by an atom, ion or molecule when the energy of one quantum of a particular wavelength of light matches the energy required to cause an electron in an outer orbital to jump to a higher energy level.*

*Each absorption band is caused by the transition between a given pair of energy levels; because the energy level differences vary with different electronic structures, absorption spectra can often be used to help identify the analyte atom, ion or molecule.*

*The technique of spectrophotometry relies on the absorption of light by the analyte; the intensity of a beam of light is measured in the absence then presence of analyte and the decrease in transmitted intensity is used to determine the analyte concentration.*

*The Beer-Lambert law expresses the relationship between absorption and concentration:*

$$A = \varepsilon cd$$

*where  $A$  = absorbance,  $\varepsilon$  = molar absorptivity ( $\text{L mol}^{-1} \text{cm}^{-1}$ ),  $c$  = concentration ( $\text{mol L}^{-1}$ ) and  $d$  = optical path-length (the distance that light travels through the sample, in cm). If this relationship is valid, then a graph of absorbance against concentration for a solution will be a straight line, which passes through the origin.*

*This is the basic equation of spectrophotometry. The spectrophotometer can only measure the intensity of light, however, so we need an additional relationship linking absorbance to  $I$  and  $I_0$ .*

*This is  $A = \log(I_0/I)$ .*

### 3. Determination of potassium in serum using flame atomic emission spectrometry.

**Flame atomic emission spectrometry is an instrumental version of the flame tests for sodium and potassium that you will have seen. Potassium ions in solution are sucked into a flame, where they are converted to atoms. Some of the atoms are excited by the flame so that an outer shell electron is moved to a higher energy orbital. This configuration is unstable, and the electron can drop back down with the emission of a photon of light. We can measure the intensity of light emitted (i.e. the number of photons per second) and because one atom emits one photon we have a quantitative method for potassium measurement.**

#### Equipment

Flame photometer or emission spectrometer 10 mL graduated pipette 5 x 100 mL volumetric flasks Deionised water in wash bottle	Pipette filler Potassium chloride solution ( $0.010 \text{ mol L}^{-1}$ ) "Deproteinised corpse serum" sample
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#### Procedure

- From the potassium stock solution, prepare four calibration standards to cover the range 2 to  $8 \times 10^{-4} \text{ mol L}^{-1}$  by pipetting an appropriate volume of stock solution into a volumetric flask and making up to the mark with deionised water.
- Take the four concentration standards, the test solution and deionised water to the flame emission spectrometer.

*ASK A DEMONSTRATOR TO SHOW YOU THE FLAME EMISSION SPECTROMETER AND THEN FOLLOW THE INSTRUCTIONS PROVIDED.*

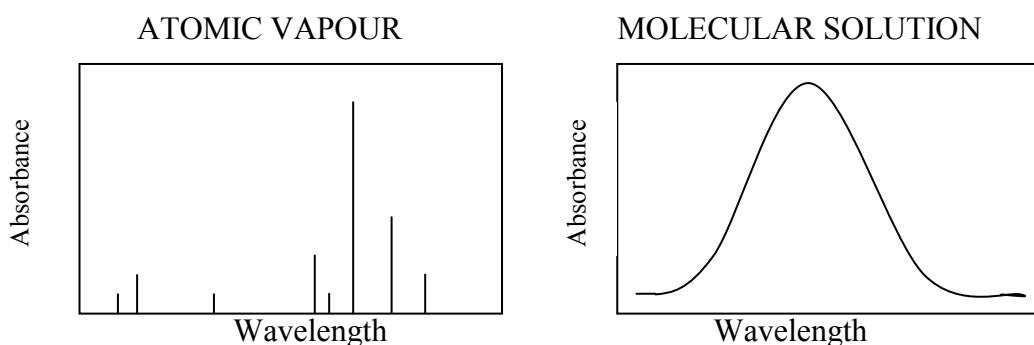
- Flush the spectrometer thoroughly by aspiration of deionised water for 5 minutes before starting the analyses.
- Zero the instrument with deionised water.
- Run each calibration standard, in order of increasing concentration, and record the results. Each measurement should be made in duplicate.
- Make an accurate dilution of the test sample so that the expected range of values will fall within the calibration range.
- Run the test sample and record the result. Again take a duplicate reading. Check that the actual reading is within the calibration range. If it is not, then it will be necessary to accurately dilute your test solution.

## Calculation

Draw a calibration graph with concentration as the x-axis and emission reading as y-axis. Draw the best fit line. Use the fitted line to convert the emission reading of the test sample to a concentration. Correct for any dilution of the test sample that you may have made.

## Background

In atomic spectrometry free metal atoms are generated in the gas phase and either absorption of light by the atoms or, as here, emission of light from excited atoms is used to measure the concentration of the metal ions in solution. Each element absorbs or emits radiation of particular wavelengths, and spectra of free atoms show very sharp lines, in contrast to the spectra of molecules in solution (the latter show broad absorption bands in the uv/visible region because molecules have vibrational energy levels as well as electronic energy levels and because of bonding to water molecules).



To produce free atoms from a solution of metal ions, a special gas burner is used. The solution is sucked up with the fuel and oxidant gases (e.g. ethyne and air respectively) and dispersed into tiny droplets. In the flame the solvent is evaporated and free atoms of the metal are produced. The flame itself is the 'sample cell'.

Atomic absorption spectroscopy is employed for most metals, but atomic emission spectrometry (AES, as used here) has advantages for some metals, particularly those of group 1. Greater sensitivity can be achieved for these elements with AES, and the instrumentation is simpler than for absorption measurements. When metal atoms are heated in a flame the energy of the flame causes excitation of outer shell electrons from their usual orbitals to levels of higher energy (e.g. 3s to 3p for sodium). Some of these excited atoms relax back by giving out light of a wavelength characteristic for the particular element and transition (e.g. the orange 589nm light from sodium 3p to 3s).

\*\*\*\*\* I hope you have all enjoyed the competition. Thanks should be given to Dr Dave Crowther and colleagues at the University of Sheffield for the original competition and Roger Jewsbury and later Dr Carl Hall and colleagues at the University of Huddersfield for modifications.

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## Results

Name of School or College: \_\_\_\_\_

Team members \_\_\_\_\_  
\_\_\_\_\_

Flow chart for experimental design and work allocation:

# 1. Determination of ethanol in urine using gas liquid chromatography.

Ethanol concentration /g L <sup>-1</sup>	Ethanol peak area	Propan-1-ol peak area	Ratio of peak areas Ethanol/Propanol

Test sample			
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(Attach calibration graph)

Concentration of ethanol in solution (from graph): \_\_\_\_\_ g L<sup>-1</sup>  
 (Keep to a realistic number of significant figures.)

Estimation of uncertainty

Obtain a very rough estimate of the uncertainty in the answer by looking at the distance between the points and the fitted line on your calibration graph. Assume for simplicity that all the error is in the reading. Use the data point which is furthest from the line and convert an error in the reading to an error in the concentration and assume that this will be the worst case uncertainty in the concentration of the answer.

Correction factor for dilution of urine sample (if appropriate) \_\_\_\_\_

Concentration of ethanol in urine solution is \_\_\_\_\_ ± \_\_\_\_\_ g L<sup>-1</sup>

Calculations:

**2. Determination of glucose in serum using an enzyme assay with colorimetric detection.**

Glucose concentration /mol L <sup>-1</sup> .	Absorbance reading 1	Absorbance reading 2	Mean absorbance reading
Test sample			

**(Attach calibration graph)**

Concentration of glucose in solution (from graph): \_\_\_\_\_ mol L<sup>-1</sup>  
 (Keep to a realistic number of significant figures.)

Estimation of uncertainty

Obtain a very rough estimate of the uncertainty in the answer by looking at the distance between the points and the fitted line on your calibration graph. Assume for simplicity that all the error is in the reading. Use the data point which is furthest from the line and convert an error in the reading to an error in the concentration and assume that this will be the worst case uncertainty in the concentration of the answer.

Correction factor for dilution of serum (if appropriate) \_\_\_\_\_

Concentration of glucose in serum is \_\_\_\_\_ ± \_\_\_\_\_ mol L<sup>-1</sup>

Calculations:

### 3. Determination of potassium in serum using flame atomic emission spectrometry.

Potassium concentration /mol L <sup>-1</sup>	Emission reading 1	Emission reading 2	Mean emission reading

Test sample			
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**(Attach calibration graph)**

Concentration of potassium in solution (from graph): \_\_\_\_\_ mol L<sup>-1</sup>  
 (Keep to a realistic number of significant figures.)

Estimation of uncertainty

Obtain a very rough estimate of the uncertainty in the answer by looking at the distance between the points and the fitted line on your calibration graph. Assume for simplicity that all the error is in the reading. Use the data point which is furthest from the line and convert an error in the reading to an error in the concentration and assume that this will be the worst case uncertainty in the concentration of the answer.

Correction factor for dilution of serum \_\_\_\_\_

Concentration of potassium in serum is \_\_\_\_\_ ± \_\_\_\_\_ mol L<sup>-1</sup>

Calculations:

## Deductions

The reference ranges (concentrations expected for normal individuals) for your analytes are:

Serum Potassium	$3.5 - 5.3 \times 10^{-3} \text{ mol L}^{-1}$
Serum Glucose	$65 - 130 \text{ mg dL}^{-1}$
Urine Ethanol	Nominally zero in the absence of ingestion. The threshold for drink-driving prosecution is 106 mg per 100 mL urine (said to be achieved with about one and a half pints of ordinary bitter or a double measure of spirits).

The results obtained for the deceased's samples were:

Serum potassium	
Serum glucose:	
Urine ethanol:	

Write your deductions about the possible cause(s) of death below.

You may also add "off the record" suggestions to D.I. Doorstop concerning the next steps in his enquiry. Explain the logic of your suggestions.

## Questions

1. What is the chemical symbol for potassium?

Potassium was not isolated as a metal until 1807, by which time P was already used for phosphorus. Why do you think that potassium was isolated much later than other metals such as copper and iron?

2. What is the name of the chemical process whereby sodium *ions* are converted to sodium *atoms* in the flame?
3. What is the formula for propan-1-ol and why do you think that it was chosen as an internal standard for the chromatography?
4. In what class of organic compound is glucose?