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Analytical Division North West Region

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Experimental Handbook

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Counterfeit Medicines

Theory

The multi-billion-pound global trade in bogus medicines is responsible for thousands of deaths a year. All kinds of medicines have been counterfeited from drugs to treat malaria to generic painkillers. Research has suggested that a third of malaria drugs are counterfeit.

Although counterfeit medicines are a serious problem in the UK with counterfeit Viagra smuggled into the UK last year they are more prevalent in several African countries, parts of Asia and Latin America. The World Health Organisation says that one in every ten drug products in poorer nations is fake. Drugs purchased over the internet from illegal sites have also been found to be fake in over 50 % of cases.

Bogus pharmaceuticals come in all combinations - some with no active ingredient, some with too little active ingredient, others with too much and some with toxic constituents. Even cheap 'over the counter' vitamin and diet supplement pills are the target of counterfeiters.

Scenario

You are a forensic scientist. You are investigating suspect multi-vitamin pills seized by custom's officers that are believed to be counterfeit medicines. You will be using a spectroscopic technique to measure the concentration of iron in two suspect brands of pill. The concentration you measure can then be compared with that on the bottle to determine whether they may be fake.

There are two suspects believed to be involved in distributing the counterfeit medicines, Mr Jones and Mr Williams. However, only one of them is guilty. A smear of sunscreen was found on each tablet bottle. You will use UV-Vis spectroscopy to determine who is responsible for distributing the counterfeit medicines by comparing samples of sunscreen found on the suspects' skin to those found on the medicine bottles.

Due to the nature of the investigation (your results may be used in court) and the implications for human health, it is vital that you work to a professional standard and produce high quality data.

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
6M Hydrochloric acid – handling, heating and filtering	Damage to skin, eyes or lungs caused by corrosive liquid or fumes	Gloves, safety glasses and protective apron to be worn at all times. Heating of hydrochloric acid to be done in the fume hood. Close supervision of students by qualified and/or experienced staff and demonstrators. First aid trained persons on hand. Emergency showers and eye wash stations in the lab.	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.
Hot glassware etc	Burns caused by contact with hot surfaces	Instructions specify that tongs should be used to handle hot flasks, and that glassware should be allowed to cool before being moved. Close supervision of students by university staff.	In case of contact with skin cool the affected area with cold water. Report the incident to a member of staff. Obtain medical advice
Harmful and toxic chemicals	Adverse health effects caused by ingestion, inhalation, or skin/eye contact with chemicals	Chemicals are used in very small quantities and are dilute. Chemicals chosen to be of low toxicity unless ingested. Gloves and eye protection worn at all times. Constant supervision by trained staff. Pipetting by mouth prohibited.	In case of contact with skin or eyes rinse the affected area with cold water. Report the incident to a member of staff
Glassware	Glassware breakage	All glassware is pre-	Contact a member of

	causing cuts	checked by technical staff and any damaged equipment should be returned. Care to be exercised especially when pushing rubber pipette fillers on to glass.	staff and obtain first aid assistance immediately in the case of an accident
Allergenic chemicals or materials	Adverse, possibly serious health effects caused by allergic reaction.	Students asked to declare any known allergy prior to the event. Students asked to report any unusual symptoms during the event. Trained first aid staff on hand. COSHH data available for all reagents	If the risk cannot be contained the student will be prohibited from performing the experiment. Rinse the affected area with cold water. Report the incident to a member of staff
Fire	Death or serious injury caused by fire	Other laboratory operations in the laboratory prohibited during event. Two flammable reagents are used during the event. Instructions specify that they should be handled away from any sources of ignition. Fire Alarms, smoke detectors and other building safety systems tested and operational at all times. Students instructed on the evacuation procedure prior to the event.	Evacuate the building.

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.

Method outline

Part 1

Your team will be provided with two brands of vitamin tablets, labelled A and B. You will use spectrophotometry to determine the amount of iron present in the tablets.

In spectrophotometry, the amount of light that a sample absorbs is measured. This absorbance is related to the concentration of the chemical compound being analysed, by a relationship called the Beer-Lambert law.

$A = \varepsilon cl$

Where:
A = absorbance
ε = molar extinction coefficient (Unique for an analyte at a given wavelength)
c = concentration
l = path length, this will be 1.0 cm for this experiment

This relationship may be represented graphically.

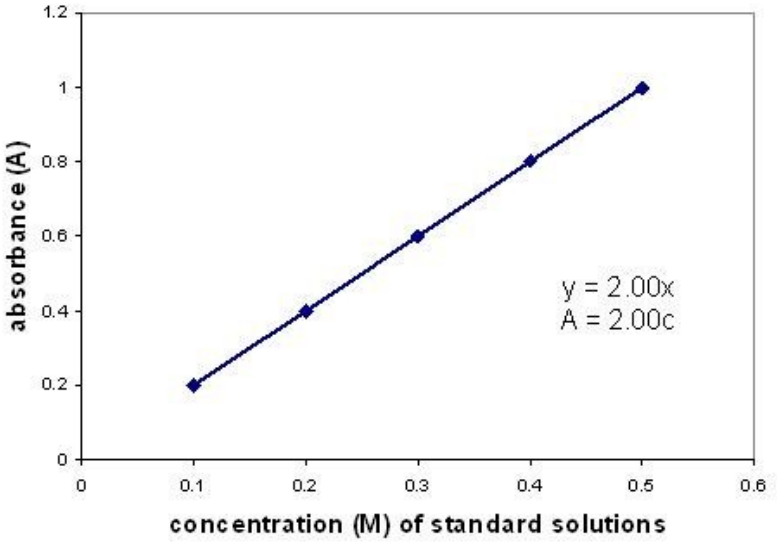

Figure 1: Beer-Lambert law "standard curve"

In order to determine the extinction coefficient ε, you must make a calibration curve using a series of standard solutions. A standard solution is a solution in which the concentration of the analyte is known. The absorbances of several standard solutions are measured and absorbance vs. concentration is plotted. The plot is called a "standard curve", and should be a straight line with y-intercept virtually zero, whose equation y = mx + c corresponds to the equation A = εcl. In these equations, y corresponds to A, x corresponds to c, and the slope corresponds to εl. Once the calibration curve is complete, the absorbance of the

unknown sample is measured, and the Beer Lambert's Law is used to determine the concentration of the unknown sample.

During this analysis we firstly digest the iron tablets. It is necessary to do this as it ensures that all of the iron present is in the Fe^{2+} state. This is done by the addition of hydroxylammonium chloride which reduces any Fe^{3+} present to Fe^{2+}. Spectrophotometry is only useful if the analyte absorbs light to a significant extent, but Fe^{2+} ions do not absorb light very strongly. Luckily, it is possible to modify certain analytes by reacting them with another substance and forming a strongly absorbing complex. In the case of Fe^{2+} this is necessary and consequently, we add 1,10-phenanthroline, a chelating agent, to produce the strongly coloured red complex by the following reaction:

$$Fe^{2+} + 3\ PhenH^{+} \rightarrow [Fe(Phen)_3]^{2+} + 3\ H^{+}$$

This complex absorbs light strongly at a wavelength of 508 nm

Part 2

In the second part of this experiment four different sunscreen samples will be analysed. You will firstly extract the active ingredients from the sunscreen samples by mixing a sunscreen sample with isopropanol. The UV-Vis spectrum of each sample will then be run from 200 nm to 400 nm as UVA (causes suntan) and UVB (causes skin to burn) absorbs in this region. Compare the spectra obtained for the sunscreen on the tablet bottles to the spectra obtained from Mr Jones and Mr Williams to determine who was distributing the counterfeit medicines.

Experimental

Tip: You may do part V at any point when you are waiting. Also, if you need to reuse some glassware rinse it with tap water and then deionised water. You do not need to dry the glassware.

Part I - Digesting the vitamin tablet

1. Place each of the tablets provided into two separate 100 ml beakers. Label the beakers with the name of your school and also A or B to distinguish between the tablets. Working in a fume cupboard carefully add 25 ml of 6 M hydrochloric acid to each beaker and cover with watch glasses. Place the beakers on a hot plate. Boil the solutions for about 10 minutes. Remove the beakers carefully using tongs and allow them to cool until the beakers can be safely handled.

2. Whilst the solutions are cooling set up the Buchner flask and funnel. You may ask a demonstrator to help you set up the apparatus. Empty the contents of the beaker containing unknown A into the funnel and collect the filtrate (the liquid) in the flask. Use a wash bottle to rinse the beaker and filter paper to ensure quantitative transfer of the dissolved iron. Do not use more than about 20 ml of water to rinse at this stage. Discard the filter paper and residue and transfer the filtrate (the liquid) to a

100 ml volumetric flask. Again use a wash bottle to rinse the contents of the Buchner flask into the volumetric flask but do not use more than about 20 ml of water. Add deionised water up to the 100 ml mark and shake well.

3. Using a volumetric pipette, transfer 25 ml of the solution into a 250 ml volumetric flask. Add (using a measuring cylinder) 10 ml of dilute hydrochloric acid (2 M) to the 250 ml volumetric flask to maintain a low pH. Add deionised water up to the 250 ml mark and mix well. You can now discard the solution remaining in the 100 ml volumetric flask. Label the 250 ml flask as 'Unknown extract A'.

4. Repeat steps 2 and 3 using the unknown B solution.

Part II – Preparing standard solutions for the calibration curve

In this stage of the experiment you will need to dilute a stock solution of iron to provide four standard solutions which will cover a range of concentrations similar to that expected in the unknown solution.

5. You are provided with a bottle labelled 'IRON STOCK SOLUTION' This solution contains about 0.04 mg of iron per ml. Record the exact concentration that is noted on the bottle. Pipette 10 ml of this stock solution into a 100 ml beaker and record the pH using universal indicator. Using a burette, add sodium acetate solution drop wise until the pH is about 3.5 (use tweezers to periodically dip a small piece of pH paper into the solution). Note down the volume of sodium acetate required (around 4 to 6 ml will be required) for the pH to reach 3.5. Dispose of this solution.

6. Pipette a fresh 10 ml aliquot of the stock solution into a 100 ml volumetric flask and add the volume of sodium acetate solution determined in step 5. Also, add 5 ml of hydroxylammonium chloride solution and 4 ml of phenanthroline solution. Add deionised water up to the 100 ml mark and mix well. Transfer enough of this solution to ¾ fill a labelled clean glass sample tube for storage until you are ready to record the absorbance data. Discard the remainder.

7. You will now need to prepare three more standard solutions containing 5 ml, 2 ml and 1 ml of the iron stock solution. Repeat stage 6 three times using 5 ml, 2 ml and 1 ml of the iron stock solution. The amount of sodium acetate solution needed should be in proportion to the amount of iron solution used (e.g. if 10 ml of iron solution requires 5 ml of sodium acetate solution then 5 ml of iron solution requires 2.5 ml of sodium acetate solution etc.). The remaining instructions are the same.

8. Prepare a blank by adding 5 ml of hydroxylammonium chloride solution and 4 ml of phenanthroline solution to a 100 ml volumetric flask. Add deionised water up to the 100 ml mark. Mix well. Transfer enough of this solution to ¾ fill a labelled clean glass sample tube for storage until you are ready to record the absorbance data. Discard the remainder.

Part III – Preparing the digested vitamin solution for spectrophotometry

9. As in step 5 above, determine how much sodium acetate solution is required to bring 10 ml of the unknown solution A to a pH of around 3 to 4. Discard the solution after recording the volume.

10. Pipette a fresh 10 ml aliquot of the unknown solution into a 100 ml volumetric flask. Add the required amount of sodium acetate solution, 5 ml of hydroxylammonium chloride solution and 4 ml of phenanthroline solution to the flask. Make up to the mark with deionised water and mix well.

11. Repeat steps 9 and 10 using unknown solution B.

Part IV - Recording UV-VIS Spectroscopic data

12. Allow the unknown and standard solutions to stand for at least 20 minutes. Transfer an aliquot of each of the solutions to the plastic cuvettes using a Pasteur pipettes. Add enough liquid to fill the cuvette to about 0.5 cm from the top. Do not label or write on the cuvettes as they have to be optically transparent. To identify the samples make a note of their positions in the plastic rack.

13. Take your samples to the spectrophotometer where a demonstrator will show you how to take a reading for each of the samples. Please be patient if there is a queue. Record the absorbance for each of the samples on the results sheet. You will then be able to plot a calibration curve and determine the iron content of your sample. From this value you will then be able to calculate the amount of iron in the original tablet.

Part V - Analysing the sunscreen samples

14. You are provided with four different sunscreen samples, one from bottle A, one from bottle B, one from Mr Jones and one from Mr Williams. Transfer a small amount of the sunscreen sample (using a toothpick) and place it in a 100 ml beaker. Add 20 ml of propan-2-ol to this beaker (please make sure that there are no sources of ignition close to the propan-2-ol). Stir the solution vigorously for a few minutes. Allow the sample to settle (you may leave the solutions at this stage if you are waiting for the glass cuvettes). Transfer the solution to a glass cuvette (the glass cuvettes will need to be shared between all the groups). Record the spectrum from 200-400 nm. Rinse the glass cuvettes with acetone (please make sure that there are no sources of ignition close to the acetone) and repeat the procedure for each of the samples.

RESULTS SHEET

(Each team should hand a copy of this sheet attached to the graphs)

NAME OF SCHOOL_____

Exact concentration of Iron in the IRON STOCK SOLUTION [1] = _____M

	Volume of Stock solution used (ml)	Concentration of Iron in each standard (M) [2]	UV Absorbance measured at 508nm (A)
Blank	**0**		
1	**1**		
2	**2**		
3	**5**		
4	**10**		
		Molar extinction coefficient [3] (ε) =	

TABLET 'A'

UV Absorbance measured at 508nm (A)	
Concentration of Iron in extract	**mol l^{-1}**
Concentration of Iron in original 100 ml extract[4]	**mol l^{-1}**
Total number of moles of Iron in the tablet[5]	**moles**
Weight of Iron in the tablet[6]	**mg**

TABLET 'B'

UV Absorbance measured at 508nm (A)	
Concentration of Iron in extract	**mol l^{-1}**
Concentration of Iron in original 100 ml extract [4]	**mol l^{-1}**
Total number of moles of Iron in the tablet [5]	**moles**
Weight of Iron in the tablet [6]	**mg**

The counterfeit tablet was tablet_____

The person distributing the counterfeit medicine was_____

Notes

1 Record this from the bottle of iron stock solution

2 For each standard calculate the concentration of iron using this using the formula:

$$\frac{\text{(Vol. of stock solution used)}}{100} \times \text{(Conc. of Iron in Stock solution)}$$

3 Plot your graph using the two right hand columns in the table and use the graph to calculate the molar extinction coefficient (***ε***)

Use **A = εcl**

Where:

A = absorbance
ε = molar extinction coefficient
c = concentration
l = path length, this will be 1.0 cm for this experiment.

4 Remember the dilution stages! You will need to multiply the concentration measured by 100 to give the concentration in the original extract from the tablet.

5 The original extract was made up to 100 ml therefore all the iron in the tablet should be dissolved in this 100 ml. You have calculated the concentration in moles per litre (1000 ml). You therefore need to multiply your answer by 0.1 to give you the concentration per 100 ml and therefore the number of moles in the tablet.

6 The relative atomic weight of iron is 55.85. Multiplying the number of moles with this will give you the weight of iron in the tablet. Finally, multiply this by 1000 to express the value in milligrams (mg).