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<tr>
<th>Author</th>
<th>Hywel Evans</th>
</tr>
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CONTENTS

Contents ........................................................................................................................................... 5
Learning Outcomes & Assessment .................................................................................................. 7
Pre-Laboratory Exercises ................................................................................................................ 9
Gel Electrophoresis of DNA .............................................................................................................. 40
Identification of Accelerants for Fire Investigation ........................................................................ 44
Fibre Analysis 1 - Optical Microscopy for Forensic Science ............................................................. 50
Fibre Analysis 2 – TLC and FTIR ....................................................................................................... 54
Drugs of Abuse ................................................................................................................................. 58
Acknowledgements ......................................................................................................................... 62
LEARNING OUTCOMES & ASSESSMENT

Assessment of your Laboratory Notebook

Your laboratory notebook will form the basis for assessment. It should contain the following elements:

- **Pre-laboratory exercises (10%)** - these must be completed and written up in your laboratory notebook prior to completing the laboratory reports.
- **Laboratory reports (75%)** – completed in full with appropriate sections, figure and table captions.
- **Summary (15%)** - a 400 word summary giving an objective account of the findings for each experiment and an evidential matrix showing the matches between control and retrieved evidence for all suspects. Do not include any figures or tables other than the evidential matrix, however, you may refer to results in your laboratory reports if correctly referenced.

### Learning Outcomes

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Learning Outcome</th>
<th>Part</th>
<th>Weight</th>
<th>Mark</th>
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</thead>
<tbody>
<tr>
<td>Laboratory Notebook</td>
<td>Practical Skills</td>
<td>the student has recorded data and observations accurately and clearly in their notebook</td>
<td>Overall assessment of the notebook</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Knowledge and understanding</td>
<td>the student has applied the appropriate theory, graphical and mathematical procedures for data treatment</td>
<td>Pre-labs</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Fibre 1 (microscopy)</td>
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<td>Fibre 2 (TLC)</td>
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<td></td>
<td>DNA profiling</td>
<td>2</td>
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<td></td>
<td></td>
<td></td>
<td>Drugs analysis</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fire analysis</td>
<td>2</td>
</tr>
<tr>
<td>Summary</td>
<td>Key transferable skills (written communication)</td>
<td>the student has presented the experiment clearly, with correct presentation of tables, figures and graphs with appropriate captions</td>
<td>Overall assessment of the notebook</td>
<td>3</td>
</tr>
<tr>
<td>Summary</td>
<td>Key transferable skills (written communication)</td>
<td>the student has structured their report in a clear and concise fashion with appropriate referencing of figures, tables and graphs in their your laboratory notebook</td>
<td>Overall assessment of the report</td>
<td>1</td>
</tr>
<tr>
<td>Summary</td>
<td>Key transferable skills (problem solving)</td>
<td>the student has interpreted the evidence objectively and formulated logical conclusions based on the forensic evidence alone</td>
<td>Overall assessment of conclusions</td>
<td>2</td>
</tr>
</tbody>
</table>
PRE-LABORATORY EXERCISES

A crime has been committed: This pre-lab is designed to prepare you to undertake a series of practicals in which you will apply forensic analysis techniques to the evidence collected from the scene of the crime and from the suspects. The methods used are as close as possible to those in a real forensic lab.

Despite what you have seen on the TV, the methods used in forensic analysis are often rather mundane and unexciting. It is the context in which they are used which makes it interesting. In this, more than any other branch of analytical science, care and attention to detail is paramount. When performing the practical work you should place the emphasis on attention to detail, careful technique, observation, comprehensive note-taking and unambiguous presentation of the results.

At the end of the investigation you will be assessed on your laboratory notebook containing: the completed pre-lab exercises and completed laboratory reports for each experiment.

In addition, at the end, you should include a summary, of no more than 400 words, giving the conclusions you have drawn from the evidence.

The Crime

The Police Report

At 3.00am on Friday January 9, 2009, a 999 call was received from Lesley Jones who said that she had found the body of her fiancé, Richard Ernesettle, dead on his living room floor. At 3.20am Police Officer George Cornwall (721029) arrived at a flat 23 St Ivel Close, Plymouth and found that the door to the flat was closed but not locked and that there was no sign of forced entry. He discovered the body of a white male, aged approximately 20 years old, sprawled on the living room floor apparently dead. An attempt had been made to set fire to the flat, but the sprinkler system had activated leaving only the sofa and curtains partially burned. At 4.25am the Police surgeon confirmed that death had occurred around 2.00am. Crime Scene Investigators arrived at 4.45am.

The Coroner’s Verdict

The Coroner’s report stated that Richard Ernesettle had died from smoke asphyxiation at around 2.00am, on Friday morning.
A high concentration of the barbiturate drug barbitone was found in a sample of the victim's blood, though it was not possible to say whether this level would have proved fatal.

Police consider the evidence is sufficient to treat the case as murder / manslaughter.

The Evidence
Forensic evidence gathered from the crime scene:

EV1: Crime Scene DNA analyses of victim’s blood
EV1A: DNA analysis of Gareth’s blood
EV1B: DNA analysis of Dean’s blood
EV1C: DNA analysis of dried blood sample taken from Dean’s clothing
EV1D: DNA analysis of Paul’s blood
EV2: Carpet, soaked in a solvent, retrieved from the crime scene
EV3: Colourless liquid in green metal can, smelling of solvent, retrieved from Sofie’s apartment
EV4: Colourless liquid in a white plastic can, smelling of solvent, retrieved from Gareth’s car.
EV5: Fibre samples from the carpet at the crime scene
EV6A: Fibre samples from Paul’s shoes
EV6B: Fibre samples from Niamh’s shoes
EV6C: Fibre samples from Sofie’s shoes
EV6D: Fibre samples from Gareth’s shoes
EV6E: Fibre samples from Dean’s shoes
EV6F: Fibre samples from Lesley’s shoes
EV7: Capsule taken from a packet of painkillers in Sofie’s handbag.

The police returned to Richard’s flat and made an extensive search of the premises. They found nothing new inside but a bottle with some white wine was discovered in an adjacent garden.

EV8: Sample of white wine from discarded bottle
The Victim

Richard Ernesettle: Born in Plympton, educated locally. Currently in the final year of a Biotechnology degree course at Plymouth University. Engaged to Lesley Jones.

The Suspects

Dean Thompson: Dean was Richard’s oldest friend – from their school days. Dean was completed a diploma in graphic art and in 2008 set up his own specialist graphic art sign-writing business. He arranged Richard’s stag night celebration. Dean is currently going out with Richard’s ex-girlfriend, Sofie.

Gareth Jones: Gareth is originally from Cardiff. He graduated in Business & Language from Plymouth in 2007 and then joined Dean as a business partner. Gareth is Lesley’s brother and shares a flat with her.

Paul Efford: Paul was born and bred in Plymouth. He met, and became friendly with Richard in the first year of their Biotechnology course. He is currently working as a laboratory technician in a drug development company.

Niamh Smith: Niamh is from Ireland. She is in the third year of Behavioural Studies at Plymouth University. She shares a flat with Sofie.

Lesley Jones: Lesley is sister to Gareth and was brought up with him in Cardiff. She joined the second year of the Biotechnology course and shared a flat with Sofie. She became engaged to Richard in December 2007.

Sofie Liu: Sofie was born in Plymouth and met Richard during Induction week. She is also in the final year of her Biotechnology course. She shares a flat with Niamh.

The Statements

Statements from the bar and restaurant managers:
The four men met at ‘The Ship Inn’ on Thursday night between 8.00 and 8.15pm, had a few drinks, and then went on to the “Drake’s Drum” restaurant. The booking had been made the previous week by Mr Thompson. The party had the ‘Golden Hind Special’. Towards the end of the meal, they were joined by two girls. The party left together around 11.00pm.

Lesley’s statement:
“Richard and I were due to be married on the following Friday. Dean and a few of the boys were taking Richard out on Thursday evening on his Stag night. I of course wasn’t invited. Richard had promised to phone me afterwards. When I he didn’t phone and I didn’t hear Gareth come in I was so concerned that I went round to Richard’s flat and found him”. She also said, “Sofie would do anything to stop us getting married”.

Sofie’s statement:
“Niamh and I had decided to eat out and, by coincidence, we chose the same restaurant at which Richard and his friends were celebrating. I did not know it was his Stag night. We joined the party and everyone left at the same time. Niamh and
Paul had ‘gone off together’ and the rest of us walked home with Richard. Everybody went into Richard’s for a final drink and then we all left together about back of 1.00 am and went our separate ways. I couldn’t be sure as everybody was pissed”.

Niamh’s statement:
Niamh said, “Sofie asked me out for a meal and she chose the restaurant”. She knew Dean but hadn’t met the other men before that evening. Dean and Richard had had “a bit of a punch-up” in the street after they left the ‘Dake’s Drum’ and Sofie had intervened. After the meal, she and Paul took a cab back to the flat and he stayed there overnight. She heard someone, presumably Sofie, go into the bathroom about 1.30am.

Dean’s statement:
Dean said he arranged the Stag night and called for Richard about 7.30pm. They had started the evening with a drink or two before going on to the bar. He admitted he had too much to drink. He said they drank white wine at the “Drake’s Drum”. He had a fight with Richard for ‘dumping Sofie and said that Sofie had intervened before it got too out of hand. They had then gone to Richard’s flat, had more to drink and parted on good terms. He and Sofie walked back to her apartment, where he left her some time after midnight, and he then went home. No one heard him come in.

Gareth’s statement:
Gareth said that Richard, Paul and Dean were already in the bar when he met them at about 8.10pm. He thought they had drunk white wine during the meal. He remembered Dean and Richard having a bit of a barny outside the restaurant. At some point Paul left with Niamh. The others walked back to Richard’s flat and had a final drink together. Everyone left about 1.00am and got a taxi but he decided to walk home. He thought it was about 4.00am when he eventually got back to the flat.

Paul’s statement:
Paul met Dean and Richard at ‘The Ship Inn’ about five minutes before Gareth arrived. He said Sofie intervened when Dean and Richard had a go at each other outside the restaurant. He had spent most of the night chatting Niamh up and they left around 11.00pm as they did not want to drink too much. “There was a bit of a disagreement between Dean and Richard but it soon blew over”.

Analyzing the Evidence

Now work through the following exercises. These will introduce you to the various techniques used to analyse the evidence.

- DNA profiling
- Gas chromatography for fire analysis
- Fibre analysis 1: optical microscopy
- Fibre analysis 2: thin layer chromatography
- Drugs of abuse
You will need to work through them so that you complete each exercise before the relevant practical.
DNA Profiling Pre-Lab
(Adapted from ref. 1)

**Aim**
This exercise is designed to prepare you for the ‘Gel Electrophoresis of DNA’ practical by allowing you to:

1. Develop an understanding of gel electrophoresis
2. Manipulate data obtained from the gel electrophoresis of the TH01 locus

In the practical itself you will be using the BXP007 locus

**Background**
DNA profiling distinguishes one human being from another by determining the exact genotype of a DNA sample that is unique to every individual. This powerful tool assists in investigations of crime scenes, missing persons, mass disasters, immigration disputes, and paternity testing. Crime scenes often contain small biological specimens (such as blood, semen, hairs, bones, pieces of skin) from which DNA can be extracted. One of the reasons that DNA profiling is so powerful is that a profile can be obtained from even very tiny samples by using the polymerase chain reaction (PCR) to amplify the DNA.

The DNA sequences used in forensic DNA profiling contain segments of short tandem repeats, or STRs. STRs are very short DNA sequences that are repeated one after another. The example below shows the TH01 locus (actually used in forensic DNA profiling). Its specific DNA sequence contains five repeats of [TCAT].

...CCC[TCAT][TCAT][TCAT][TCAT][TCAT]AAA...

For the TH01 STR locus, there are many alternate forms (alleles) that differ from each other by the number of [TCAT] repeats present in the sequence. More than 20 different alleles of TH01 have been discovered in people worldwide. Each of us still has only two of these, one inherited from our mother and one inherited from our father.

Suspect A’s DNA type for the TH01 locus is (5-3)

\[
\begin{array}{cccccccc}
C & C & C & \square & \square & \square & \square & A & A & 5^* \\
C & C & C & \square & \square & A & A & 3^*
\end{array}
\]

Suspect B’s DNA type for the TH01 locus is (6-10)

\[
\begin{array}{ccccccccccccccc}
C & C & C & \square & \square & \square & \square & \square & \square & A & A & 6^* \\
C & C & C & \square & \square & \square & \square & \square & \square & \square & \square & A & A & 10^*
\end{array}
\]

*Number of [TCAT] repeats

Each STR allele has a different length depending on the number of tandem repeats it contains. When the alleles are amplified by PCR, alleles of different lengths can be distinguished by gel electrophoresis. The number of tandem repeats contained in each allele can be determined by comparing the locations of the DNA bands with an allelic ladder (DNA fragment size standard) that corresponds to the known sizes of TH01 alleles.
Exercise 1 – Running a Gel
It is not possible to run your own gel in this prelab but you can view an animation in the LABplus virtual box at the University of Plymouth that explains the process.

1. Access the virtual box by visiting www.ssb.plymouth.ac.uk/labplus/ and click on projects, and then Forensic science prelab - Dr Hywel Evans or by clicking on this link:
2. Click on ‘DNA Profiling’ and then ‘gel electrophoresis animation’.
3. When promoted, click ‘Run’ twice
4. Watch the animation (it does not require sound).

When you have finished, continue with Exercise 2 – manipulating data obtained from the gel electrophoresis.

Exercise 2 – Manipulating Data obtained from the Gel Electrophoresis
In the virtual box, you should find Figure 1.1. Gel electrophoretogram of crime scene and suspect DNA. The allele ladder in the electrophoretogram represents all the possible alleles at the TH01 locus, and is used as a reference to calculate the relative sizes of the fragments. There are 8 possible alleles, with the largest near the well and the smallest furthest away.

1. Measure the distances the bands have moved, in mm, from the leading edge of the wells at the top of the plate and complete the data in Table 1 (a copy of the table that you can write on is also available in the virtual box).

<table>
<thead>
<tr>
<th>Allele</th>
<th>Size (b.p.)</th>
<th>$\log_{10}(\text{size})$</th>
<th>Distance moved</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>11</td>
<td>203</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>199</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.3</td>
<td>197</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>195</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>191</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>183</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>179</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Plot $\log_{10}(\text{size})$ versus distance which should give a straight line. Using this as a calibration, determine the allele sizes for the crime scene and each of the
suspects and complete Table 2 (a copy of the table that you can write on is also available in the virtual box).

Table 2. Data table for crime scene and suspects

<table>
<thead>
<tr>
<th>Allele</th>
<th>Log_{10} size (b.p.)</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS</td>
<td>A</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Does the crime scene DNA sample have a genotype that matches any of the suspects?

4. Refer to Table 1 in reference 2 (Butler et al., 2003. Allele frequencies for 15 Autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations). Copies of this can be found in the box and virtual box.

This gives the frequencies of each of the alleles at the TH01 locus. An example of how to calculate the overall genotype frequency is given in Box 1.

Box.1. Example of how to calculate the overall genotype frequency for the crime scene sample

Frequency in Caucasian population
Allele 9.3 = p = 0.368
Allele 7 = q = 0.190

Chance of 9.3 from Dad and 7 from Mum = pq
Chance of 7 from Dad and 9.3 from Mum = pq

Overall frequency = 2 pq
= 0.1398

14% of Caucasians have the 9.3,7 genotype at the TH01 locus

Using the frequency data in Table 1, calculate the overall frequencies of the genotypes for the crime scene and each of the suspects and complete Table 3 (a copy of the table that you can write on is available in the virtual box).
Table 3. Overall frequencies for the crime scene and suspect genotypes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Identified allele</th>
<th>Allele frequency</th>
<th>Genotype frequency</th>
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<tr>
<td>CS</td>
<td>9.3</td>
<td>p = 0.368</td>
<td>2pq = 0.1398</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>q = 0.190</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. If the crime scene genotype matches one of the suspects, how good a match do you think it is?

References

1. Crime Scene Investigator PCR Basics™ Kit, Bio-Rad, UK
Gas Chromatography for Fire Analysis Pre-Lab

**Aims**
This exercise is designed to prepare you for the ‘Identification of Accelerants for Fire Investigation’ practical by allowing you to:

1. Measure retention times and peak heights
2. Identify a possible fire accelerant from a gas chromatograph

**Background**
Liquid and semi-liquid distillate products (e.g. petrol, paraffin, and diesel) can be analysed and compared in order to establish a common source (i.e. it can be determined whether or not two or more products are indistinguishable). Such analyses are invaluable for cases involving theft of fuel, or arson cases where a liquid accelerant was left at the scene and one is subsequently found in the suspect’s possession.

Paraffin is the second most popular accelerant after petrol. It is found in a number of common household products, from charcoal lighters to paint thinner, so is the most common incidental accelerant. It is more difficult to ignite than petrol, due to a lower volatility, but it will burn longer and hotter. It is similar to diesel but contains a greater proportion of lighter hydrocarbon components making it more volatile but, due to its relatively high boiling range, it is more likely to leave a detectable residue after a fire than petrol.

**Exercise 1**
A chromatogram of unevaporated paraffin is shown in Fig.1. It displays seven evenly spaced characteristic peaks due to n-alkane fractions.

![Fig. 1. GC chromatogram of paraffin. Peak identities: (1) C₉, (2) C₁₀, (3) C₁₁, (4) C₁₂, (5) C₁₃, (6) C₁₄, (8) C₁₅](image)

1. Measure the peak height and retention time of the seven n-alkane. These are the most intense peaks which have been numbered for you in Fig. 1. Use a ruler to measure the peak height, in mm, and read the retention time from the
x-axis, in minutes. Record the data in Table 1 – a copy you can write on can be found in the virtual box.

Table 1. Data table for GC of paraffin

<table>
<thead>
<tr>
<th>Peak id</th>
<th>Carbon no.</th>
<th>Retention time/min</th>
<th>Peak height/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C₉</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C₁₀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C₁₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C₁₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>C₁₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C₁₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>C₁₅</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2 shows a GC trace of the headspace from a partially burned carpet retrieved from a crime scene.

Figure 2. GC chromatogram of burnt carpet

2. Identify the seven peaks in the n-alkane series from the retention times your recorded in Table 1, and record their peak heights and retention times, in Table 2 – a copy you can write on can be found in the virtual box.
### Table 2. Data table for GC of burnt carpet

<table>
<thead>
<tr>
<th>Peak id</th>
<th>Carbon no.</th>
<th>Retention time/min</th>
<th>Peak height/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C_9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C_{10}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C_{11}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C_{12}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>C_{13}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C_{14}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>C_{15}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Plot peak height data from Table 1 against the peak height data in Table 2 and fit a regression line. What does the $R^2$ value tell you about any relationship in the data and what does this tell you about the possible accelerant that was used to set fire to the carpet? Is there a statistical test that you could use to test if the relationship is significant?
Fibre Analysis 1: Optical Microscopy Pre-Lab

**Aims**

This exercise is designed to prepare you for the ‘Fibre Analysis 1 - Optical Microscopy for Forensic Science’ practical by allowing you to:

1. Set up a light microscope for Kohler illumination
2. Use optical microscopy to observe the type, shape and colour, and measure the size of fibre samples

**Background**

When fibres are analysed for forensic purposes a hierarchy of operations are performed in the following order:

1. The initial examination is done under a microscope to determine the fibre type, for example cotton or wool.
2. Colour is determined.
3. Physical features such as cross-sectional shape are distinguished.
4. The width of the fibre is measured.
5. The fibre is examined to see if it contains any delustrant – this is used by manufacturers to determine how bright and shiny a finished garment is.
6. The fibre is examined under ultraviolet and blue light.

If the fibres can be said to match after these examinations the following two procedures are performed:

1. UV/Visible microspectrophotometry is used to more accurately determine the colour.
2a. If the fibre is large enough the colour can be extracted from it and a thin-layer chromatography test can be done to identify the dyes mixed by the manufacturer to give the fibre its finished colour and hue.
2b. For man-made fibres infra red spectrophotometry can be performed to tell whether the fibre is made of polyester, nylon or acrylic.

**Light microscopy**

Small objects may be observed with various kinds of microscopes. The most common are optical (light) both low power stereo and higher power compound. For more resolution electron microscopes both scanning and transmission are used. There are also many other forms of microscopy such as confocal, scanning, tunnelling and atomic force microscopy.

The compound light microscope normally has a magnification range of x100, x400 & x1000 and has a condenser to help illuminate the object. It is most commonly used for looking at prepared slides. The depth of field (the amount that is in focus in the z direction) and the working distance (distance between lens and object) are both very limited. Illumination is commonly transmitted but in forensic work incident illumination (EPI) is common. The illumination system needs to be set up for Kohler illumination for optimal viewing and resolution. The microscopes used in this exercise will be for normal microscopy termed ‘bright field’ but there are many others.
The stereo is two microscopes side by side built into one body. This allows 3D vision so is often used for dissection/sorting. Instead of set magnifications as with the compound microscopes most stereo have a zoom control for variable magnification between x7 and x45. The depth of field and working distance is much better than the compound microscope. Illumination is possible from below or above (incident).

**Magnification and resolution**

A *compound light microscope* is limited to a maximum magnification of 1500 times and has a maximum resolving power of approximately 0.2\(\mu\)m (micrometers). The *transmission electron microscope* can magnify objects 500,000 times and has a resolving power of approximately 0.2nm (nanometres). Compare this to the naked eye whose resolving power is about 0.1mm (=100\(\mu\)m). The *scanning electron microscope* is used for studying surface structures. Different illumination (light or electrons) is used, as the much smaller wavelength of an electron beam allows much greater resolution to be achieved.

**Specimens**

Slide 1: Acrylic (man-made)

Slide 2: Cotton (plant)

Slide 3: Wool (animal)
**Exercise 1 – Setting up a Compound Microscope for Kohler Illumination**

**Bold type** indicates the object is labelled on the microscope diagram above.

Please follow this schedule in the order written

1. Make sure the microscope is on a firm ‘footing’ away from chemicals. Sit directly in front of the microscope. Adjust your chair so that you are level with the eyepieces.

2. With the **rheostat** at its logarithm setting and the **switch** on the microscope off, plug in the microscope.

3. Switch on the microscope using the **switch**.

4. Place the x10 or x4 **objective** above the **stage**. Swing the objectives around using the **nosepiece** not the objectives.

5. Place a slide on the stage. Orientate the specimen using the **stage controls** so that the specimen is below the objective. Raise the stage using the **coarse focus** until the coverslip/slide is almost touching the objective or until the stage reaches its stop. Adjust the rheostat for a suitable light level. Look down the **eyepieces** and rack the stage away from the objective again using the coarse focus until the specimen is in focus. You can finish off the focusing by using the **fine focus**, which you will also need if you scan the slide as the slide thickness varies.

6. Adjust the distance between the **eyepieces** (DO NOT HOLD THE EYEPIECE TUBES). The eyepieces should be the same distance apart as the interpupillary distance (the gap between your pupils). This distance is displayed on the scale between the tubes. Now adjust the **diopters** (the tubes the
eyepieces sit in). Do this by closing the left eye and focusing the image for the right eye. Now open the left eye and close the right eye, and focus the image for the left eye using the focus knob.

7. Close down the **field iris** and open up the **condenser iris**. Focus the **condenser** to give an image of the field iris (NB. IT HAS ITS OWN FOCUS KNOB SO DON’T USE THE COARSE OR FINE FOCUS KNOBS). The image will be polygon in shape (see Figure 1). The image of the field iris can also be centred using the centring screws. Finally, open the field iris enough to just illuminate the whole field.

![Figure 1. Field iris closed](image)

8. Adjust the condenser iris for contrast/resolution.

9. When you wish to go to a higher magnification, if you have adjusted the dioplers correctly, then hardly any focusing adjustment will be required (parfocal). When you change from x4 to x10 to x40 all that is required is to swing the new objective over the stage then fine focus. On changing magnification the field iris will need adjusting to only illuminate the field. The condenser iris will also need adjusting for contrast/resolution. When you lower magnification again, you will need to open the field iris and close down the condenser iris to their optimal positions.

**Exercise 2 – Fibre Observation**

You are now ready to study the fibres in detail. You have been given three different fibres; one man-made (acrylic) and two natural (cotton and wool).

Observe each of these in turn using the x40 objective. Make a sketch and record any distinctive differences. **Copies of recording tables that you can write on are available in the virtual box.** Examples of these three fibre types are also given so see if you can spot the same features.

<table>
<thead>
<tr>
<th>Slide 1: Acrylic Example</th>
<th>Man-made fibres have very even features and look smooth. They can also be shaped and have a variety of cross-sections. Some of the fibres in the example are shaped this way.</th>
</tr>
</thead>
</table>

Forensic Analysis Laboratory_Student Manual.docx 26
Slide 2: Cotton Key features

<table>
<thead>
<tr>
<th>Key features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton is a plant fibre which can become twisted during drying but dyeing can make them more even.</td>
</tr>
</tbody>
</table>

Slide 3: Wool

<table>
<thead>
<tr>
<th>Key features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wool is animal fibre from sheep or breeds. It has a cortex of spindle-shaped cells surrounded by a cuticle of overlapping scales. The scales erode over time so the fibre looks smooth. The cortex can have a hollow core (medulla) which can be broken or uninterrupted.</td>
</tr>
</tbody>
</table>

**Exercise 3: Fibre Measurement**

1. Remove the right eyepiece from the microscope and unscrew the lens at the top.

2. Place the eyepiece graticule (transparent disc with accurately spaced marks) in the eyepiece, screw the top lens back in place and replace the eyepiece. **If the graticule or any other surface is dirty, please see the technician for lens cleaning tissues. Never clean them with any other material, including your clothes!**

3. Place the graduated slide (also called a stage micrometer) on the stage and with the x10 objective in place. Centre the marks of the slide in the field of view and focus.
Graduated slide

Accurate scale markings in central ring of graduated slide

4. Use the graduated slide to calibrate the graticule. The slide is marked in parts of a millimetre. By aligning the two scales you can work out how the divisions of the graticule correspond to micrometres at this magnification. An example is given in box 1.

Box 1: Calibrating the eyepiece graticule

Align the eyepiece graticule with the scale on the graduated slide as shown below (the eyepiece graticule is the smaller scale graduated from 0 to 100)

100 eyepiece units = 26.5 stage units
Each stage unit = 0.01 mm so:
100 eyepiece units = 26.5 × 0.01
  = 0.265 mm
  = 265 µm
So: 1 eyepiece unit = 2.65 µm

N.B. If the eyepiece graticule scale is longer than the graduated slide scale then rearrange the method. For example:
40 eyepiece units  = 100 stage units
So: 1 eyepiece unit = 2.5 stage units (100 ÷ 40)

As 1 stage unit  = 0.01 mm
2.5 stage units = 0.025 mm (or 25 μm)
Therefore: 1 eyepiece unit = 25 μm

5. Do the calibration for the x40 objective. If you change objective lens you will need to repeat the calibration.

6. Replace the graduated slide with one of the specimen slides and rotate the eyepiece graticule so you can measure the diameter of one of the fibres.

7. Make 3 separate measurements of diameter at different points for each fibre type and record the results in Table 1. A copy of this table you can write on is available in the virtual box.

Table 1. Measurements of fibre diameter

<table>
<thead>
<tr>
<th>Fibre diameter</th>
<th>Graticule units</th>
<th>Microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slide 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When Finished

1. Turn down the rheostat, turn the microscope switch off and then unplug from the mains socket.

2. Remove the graduated slide and eyepiece graticule and return them to the box. Return all the other materials to the box.

3. Centre the stage under the x4 magnification and return the condenser and field iris’s to an open position.

4. Place the cover on the microscope and wrap the lead around it. Return the microscope to the cupboard.
Fibre Analysis 2: Thin Layer Chromatography Pre-Lab

**Aims**

This exercise is designed to prepare you for the ‘Fibre Analysis 2 – TLC and FTIR’ practical by allowing you to;

1. Spot a TLC plate with a dye extract
2. Perform an efficient TLC separation of the mixture

**Background**

When fibres are analysed for forensic purposes a hierarchy of operations are performed in the following order:

1. The initial examination is done under a microscope to determine the fibre type, for example cotton or wool.
2. Colour is determined.
3. Physical features such as cross-sectional shape are distinguished.
4. The width of the fibre is measured.
5. The fibre is examined to see if it contains any delustrant – this is used by manufacturers to determine how bright and shiny a finished garment is.
6. The fibre is examined under ultraviolet and blue light.

If the fibres can be said to match after these examinations the following procedures are performed:

7. UV/Visible microspectrophotometry is used to more accurately determine the colour.
8. If the fibre is large enough the colour can be extracted from it and a thin-layer chromatography test can be done to identify the dyes mixed by the manufacturer to give the fibre its finished colour and hue.
9. For manmade fibres an infra red spectrophotometry can be done – a chemical analysis to tell whether the fibre is made of polyester, nylon or acrylic.

**Exercise 1 – Spotting a TLC plate**

In the box you should find the following: (if these items are not in the box, see the technician)

1. A strip of aluminium-backed TLC plate (Fig. 1).
Figure 1. Aluminium-backed TLC plate

2. A small vial which contains a dye extract.

3. A drawn out capillary tube to use as a ‘spotter’.

1. Prepare the strip of TLC plate by **LIGHTLY** drawing a pencil line parallel to the short edge about 1 cm from the bottom.

2. Dip the end of the drawn-out capillary tube into the dye extract – the end of the capillary should fill by capillary action.

3. The next step is to CAREFULLY spot the dye onto the TLC plate WITHOUT damaging the plate. Hold the tip of the capillary as close as possible to the pencil line (Fig. 2) until a SMALL amount of the dye extract is drawn out onto the plate. With practise you should be able to do this without actually touching the plate.

4. When the spot has dried repeat the operation by spotting onto the same place until a reasonable concentration of dye has been built up (3 or 4 repeats). Try and keep the spot as small as possible.
5. Repeat this process by making a second spot on the same plate to ensure you have mastered the technique (Fig. 3)!

![Figure 3. Repeat spotting of the plate](image)

**Exercise 2: Developing the TLC plate**

In the box you should find a TLC development tank containing ethanol to a level of 0.5cm from the bottom. If there is not sufficient ethanol in the tank to conduct the following, then see the technician.

1. Take the TLC plate which you have just spotted and place it in the development tank with the pencil line parallel to the base (Fig. 4). **Make sure the solvent level is below the pencil line.**

![Figure 4. Development tank with plate](image)

2. Replace the lid of the development tank.

3. Allow the solvent to move up the plate until it is approximately 1cm from the top.

4. You should see the dyes in the mixture separate out (Fig. 5).
Figure 5. Separated dyes

When you have finished…

Dispose of your TLC plate in the bin.

Put the lid back on the development tank to save the ethanol for further use.

Dispose of the capillary tube in the sharps bin by the sink.
Drugs of Abuse Pre-Lab

Aims
This exercise is designed to prepare you for the ‘Drugs of Abuse’ practical by allowing you to;

1. Predict the effect of pH on the chemical form of drugs
2. Predict the effect of pH on absorption and extraction

Background
Many drugs can be classified as acidic or basic depending on the functional groups present.

Drug absorption depends on the lipid solubility of the drug, its formulation and the route of administration. A drug needs to be lipid soluble to penetrate membranes unless there is an active transport system or it is so small that it can pass through the aqueous channels in the membrane.

For weakly acidic and weakly basic drugs, absorption and distribution are pH dependent since only the unionized drug is lipid soluble. Strongly ionized drugs cannot cross membranes so they are not absorbed orally and cannot cross the blood-brain barrier.

Basic Drugs
The class of drugs known as the alkaloids are basic due to the presence of a tertiary amine group. Cocaine (benzoylmethyl eegonine) is an example, and can be intraconverted between the free base and the salt by changing the pH with ammonia solution (Scheme 1).

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\text{HCl} & \quad \Leftrightarrow \\
\text{NH}_3 & \quad \text{Cl}^- \quad \text{HN}^+ \\
\text{(1a) Cocaine free base} & \quad \text{(1b) Cocaine hydrochloride} \\
\end{align*}
\]

Scheme 1

Cocaine hydrochloride is a water soluble salt which is commonly administered by insufflations or ‘snorting’ where it dissolves in and is absorbed through the mucus membranes lining the sinuses. It has a high melting point (195 °C) so cannot be easily smoked. On the other hand, the free base (‘crack’) is more volatile so can be smoked and absorbed directly into the bloodstream through the lung membranes.
**Acidic Drugs**

Aspirin (acetylsalicylic acid) is an acidic drug with a $pK_a$ of 3.5, so is converted into the free acid form in the acidic conditions of the stomach where it becomes insoluble. For this reason most absorption occurs in the small intestine which has a higher pH (Scheme 2)

\[
\begin{align*}
(2a) & \quad \text{Aspirin free acid} \\
(2b) & \quad \text{Aspirin ammonium salt}
\end{align*}
\]

**Exercise 1 – Ecstasy or MDMA (3,4-methylenedioxy- N-methylamphetamine)**

MDMA is an amphetamine drug which has psychoactive effects. Commonly known as ecstasy, it gives a sense of well being.

\[
\begin{align*}
(3a) & \quad \text{MDMA free base} \\
(3b) & \quad \text{MDMA ammonium salt}
\end{align*}
\]

1. What type of drug is MDMA (acidic or basic)?
2. If you change the pH by adding HCl to a solution of MDMA in water the structure of the drug will change. Make a note of the structure of the drug (3b in Scheme 3). You can write on the downloadable template available in the virtual box.
3. What happens to MDMA in the stomach?
4. What effect does this have on absorption of the drug?

**Exercise 2 – Barbital**

Barbital is a barbiturate drug which has hypnotic effects and was prescribed as a sedative.

2. What class of drug is barbital?

3. If you change the pH by adding ammonia to a solution of barbital in water the structure of the drug will change. Make a note of the structure of the drug (4c).

4. If you wanted to solvent extract the drug from an aqueous solution, using the non-polar solvent chloroform, what would you have to do first to make it soluble in chloroform? Why?
SCHOOL OF Geography, Earth and Environmental Sciences  
COSHH and RISK ASSESSMENT

GEL ELECTROPHORESIS OF DNA

Assessment Date: 08/10/09  
Assessment no: 1

ASSESSMENT OF RISK:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Hazards</th>
<th>Probability</th>
<th>Severity</th>
<th>Risk Factor</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel electrophoresis</td>
<td>Electrical</td>
<td></td>
<td></td>
<td></td>
<td>Do not use above 100 V.</td>
</tr>
<tr>
<td></td>
<td>shock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

COSHH ASSESSMENT:

<table>
<thead>
<tr>
<th>DNA and restriction enzymes</th>
<th>Spillages of enzymes or DNA solution should be wiped up promptly. Used plastic (polypropylene) tubes and microsyringe tips can be disposed of in the normal waste or recycled with no special precautions.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose gel</td>
<td>WARNING! Hot, molten agarose can scald and it must be handled with care.</td>
</tr>
<tr>
<td>Tris-Borate-EDTA buffer</td>
<td>Presents no serious safety hazards. Spent buffer can be washed down the drain.</td>
</tr>
<tr>
<td>Electrode tissue</td>
<td>Carbon fibre electrode tissue may release small fibres, which can cause minor skin irritation. Wear protective gloves.</td>
</tr>
<tr>
<td>Azure A in ethanol</td>
<td>Flammable - keep away from naked flames. Azure A as a 0.04% solution in 20% ethanol presents no serious safety hazard, although care should be taken to prevents splashes on the skin or eyes e.g., wear protective gloves and safety glasses. Used stain can be diluted with water and washed down the drain.</td>
</tr>
<tr>
<td>Bromophenol blue/Sucrose</td>
<td>Presents no serious hazards. Used loading dye can be washed down the drain.</td>
</tr>
</tbody>
</table>

FIRST AID FOR ANY OF THE ABOVE CHEMICALS

<table>
<thead>
<tr>
<th>Mouth</th>
<th>If swallowed DO NOT induce vomiting, seek medical advice immediately.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Wash immediately with plenty of soap and water</td>
</tr>
<tr>
<td>Eyes</td>
<td>Wash with plenty of water and seek medical advice</td>
</tr>
<tr>
<td>Lungs</td>
<td>Remove from exposure, rest and keep warm. In severe cases seek medical advice.</td>
</tr>
</tbody>
</table>
GEL ELECTROPHORESIS OF DNA

(Adapted from the Crime Scene Investigator PCR Basics™ Kit, Bio-Rad, UK)

Objectives
- Submarine gel electrophoresis of crime scene and suspect DNA
- DNA typing of one "STR locus"
- Statistical analysis.

Evidence you will examine
EV1: Crime Scene DNA analyses of victim’s blood
EV1A: DNA analysis of Gareth’s blood
EV1B: DNA analysis of Dean’s blood
EV1C: DNA analysis of dried blood sample taken from Dean’s clothing
EV1D: DNA analysis of Paul’s blood

Procedure

Setting up the PCR Reactions
1. Label 5 PCR tubes CS, A, B, C, or D, and include your group name or initials as well. Place each PCR tube into a capless micro centrifuge tube in the foam float on ice.
2. Using the chart below as a guide, transfer 20 µl of the appropriate template DNA into the correctly labelled tube. Important: use a fresh aerosol barrier pipet tip for each DNA sample.

<table>
<thead>
<tr>
<th>Evidence code</th>
<th>Label PCR tubes</th>
<th>Add DNA template</th>
<th>Add Master mix + primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV1</td>
<td>CS + your initials</td>
<td>20 µl Crime Scene DNA</td>
<td>20 µl MMP (blue)</td>
</tr>
<tr>
<td>EV1A</td>
<td>A + your initials</td>
<td>20 µl Suspect A DNA</td>
<td>20 µl MMP (blue)</td>
</tr>
<tr>
<td>EV1B</td>
<td>B + your initials</td>
<td>20 µl Suspect B DNA</td>
<td>20 µl MMP (blue)</td>
</tr>
<tr>
<td>EV1C</td>
<td>C + your initials</td>
<td>20 µl Suspect C DNA</td>
<td>20 µl MMP (blue)</td>
</tr>
<tr>
<td>EV1D</td>
<td>D + your initials</td>
<td>20 µl Suspect D DNA</td>
<td>20 µl MMP (blue)</td>
</tr>
</tbody>
</table>

1. Transfer 20 µl of the blue MMP (master mix + primers) into each of the 5 PCR tubes containing template DNA. Pipette up and down to mix. Cap each tube after adding blue MMP. Important: use a fresh aerosol barrier pipet tip each time. Immediately cap each tube after adding MMP.
2. Place your capped PCR tubes in their adaptors on ice.
3. When instructed to do so, place your tubes in the thermal cycler. Your instructor will program the thermal cycler for PCR.

Preparing the Agarose Gel
1. Completely dissolve 6 g of agarose powder in 200 mL of freshly prepared TAE buffer on a hotplate. Ensure that no lumps or fibres remain in the molten agarose.
2. Store in a water bath at 50 degrees for 5-10 minutes before use
3. Remove from waterbath and pre-stain agarose by adding 20 µL of SYBR Safe dye - do NOT add when agarose is above 50 degrees.
4. Place a 20 x 10 cm electrophoresis tank on a level surface, where you can leave it undisturbed for the next 20–30 minutes.

5. Slot a 16-toothed comb in place at one end of the tank insert and put the rubber retaining strips across each end to prevent the agarose gel running out before it is set.

6. Pour the molten agarose into the tank insert so that it fills the central cavity and flows under and between the teeth of the comb. Cover the tank with a piece of paper.

7. Place the insert in the tank, ensuring that the comb end is near the black (–) electrode.

8. Leave the tank undisturbed until the gel has set hard (agarose gel is opaque when set, about 30 min).

9. Remove the comb and tape and pour sufficient TAE buffer into the tank to cover the gel to a depth of 2 mm.

Electrophoresis of PCR Products

1. Obtain the 5 PCR tubes which have been prepared for you. Tap the tubes on the bench to collect all liquid to the bottom of the tube.

2. Transfer 20 µl of Orange G loading dye (from the tube labeled ‘LD’) into each of your PCR tubes. Pipet up and down to mix, and pulse-spin to collect liquid in the bottom of the tube.

3. Using a clean tip for each sample, load 15 µl of the samples into 6 wells of the gel by holding the tip above the well but under the buffer solution. Take great care not to puncture the bottom of the well with the microsyringe tip. Load the wells in the following order:

<table>
<thead>
<tr>
<th>Lane</th>
<th>Evidence code</th>
<th>Sample Load volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Allele Ladder</td>
<td>20 µl</td>
</tr>
<tr>
<td>2</td>
<td>EV1</td>
<td>Crime Scene 20 µl</td>
</tr>
<tr>
<td>3</td>
<td>EV1A</td>
<td>Suspect A 20 µl</td>
</tr>
<tr>
<td>4</td>
<td>EV1B</td>
<td>Suspect B 20 µl</td>
</tr>
<tr>
<td>5</td>
<td>EV1C</td>
<td>Suspect C 20 µl</td>
</tr>
<tr>
<td>6</td>
<td>EV1D</td>
<td>Suspect D 20 µl</td>
</tr>
</tbody>
</table>

4. Run the electrophoresis at 100 V for about 30 min or until the loading dye has moved at least 60 mm from the wells.

5. Disconnect the electrodes and pour off the buffer solution.

6. Remove the tank insert containing the gel and place into the plastic storage container provided.

7. Obtain a scanned fluorescence image of the gel.

Results

The allele ladder represents all the possible alleles at the BEXP007 locus, and is used as a reference to calculate the relative sizes of the bands. There are 8 possible alleles, with the largest near the well and the smallest furthest away. The sizes are 1500, 1000, 700, 500, 400, 300, 200, and 100 base pairs (bp).

Measure the distances, in mm, the bands in allele ladder have moved from the leading edge of the wells and plot \(\log_{10}(\text{fragment size})\) versus distance which should
give a straight line. Using this as a calibration, determine the allele sizes for each of the suspects for use in your final report.

Further Reading
1. An introduction to forensic DNA analysis / Norah Rudin, Keith Inman. 2nd ed. CRC Press, 2002. 614.1 RUD
### SCHOOL OF Geography, Earth and Environmental Sciences
### COSHH and RISK ASSESSMENT

#### FIRE INVESTIGATION

Assessment Date: 08/10/09  
Assessment no:  1

#### ASSESSMENT OF RISK:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Hazards</th>
<th>Probability</th>
<th>Severity</th>
<th>Risk Factor</th>
<th>Controls</th>
</tr>
</thead>
</table>
| Pouring and moving solvents     | Spillage Evaporation    | 2           | 1        | 3           | Do not leave solvents in open containers  
                                  | Combustion               |             |           | Limit sample size  
                                  |                          |             |           | Do not take near to ignition source or naked flame  
                                  |                          |             |           | Work only in fume cupboards |
| Ignition tests                  | Uncontrolled fire        | 2           | 2        | 4           | Perform ignition tests in separate fume cupboard to solvents  
                                  |                          |             |           | Have cover ready to put over fire  
                                  |                          |             |           | Light fires only in crucibles |

#### COSHH ASSESSMENT:

- **Petrol**  
  All solvents are HIGHLY FLAMMABLE and VOLATILE and should be kept clear of naked flames.
- **Diesel**  
  All solvents are HARMFUL and may cause lung damage if swallowed.  
  Do not breathe in vapour and do not dispose of into drains.  
  Wear gloves.  
  Always work in fume cupboard.
- **White Spirit**
- **Turpentine Substitute**

#### FIRST AID FOR ANY OF THE ABOVE CHEMICALS

- **Mouth**  
  If swallowed DO NOT induce vomiting, seek medical advice immediately.
- **Skin**  
  Wash immediately with plenty of soap and water
- **Eyes**  
  Wash with plenty of water and seek medical advice
- **Lungs**  
  Remove from exposure, rest and keep warm.  
  In severe cases seek medical advice.
IDENTIFICATION OF ACCELERANTS FOR FIRE INVESTIGATION

Objectives
Upon conclusion of the experiment the student should be able to:
- prepare and sample fire debris for headspace gas chromatography
- run a headspace gas chromatograph
- interpret chromatograms and identify unknown accelerants

Evidence you will examine
EV2: Carpet, soaked in a solvent, retrieved from the crime scene
EV3: Colourless liquid in green metal can, smelling of solvent, retrieved from Sofie’s apartment
EV4: Colourless liquid in a white plastic can, smelling of solvent, retrieved from Gareth’s car.

Procedure
Identification of samples
You will use headspace gas chromatography to analyse the samples as follows:

EV2
1. Heat the sealed bottle containing the carpet in the oven at 100 °C for 15 minutes.
2. Remove the bottle from the oven, puncture with the gas-tight syringe and withdraw 50 μl of vapour.
3. Inject the sample into the injection port of the gas chromatograph (GC) and run the temperature program shown in Table 2. Allow an analysis time of 30 min.
4. Flush the gas-tight syringe by slowly pumping the plunger up and down for about 1 min.

EV3 & EV4
1. Take a 0.5 ml aliquot of the sample using the syringe provided, and place into a brown sample bottle. Seal the bottle with one of the rubber seals. Repeat for the remaining samples.
2. Place the bottles containing samples in the oven at 50 °C for 10 minutes
3. Remove a sample bottle and carefully pierce the rubber seal with the gas-tight syringe provided, and withdraw 50 μl of headspace.
4. Inject the sample into the injection port of the gas chromatograph (GC) and run the temperature program shown in Table 2. Allow an analysis time of 30 min.
5. Flush the gas-tight syringe by slowly pumping the plunger up and down for about 1 min.
Table 2 GC Operating Conditions

<table>
<thead>
<tr>
<th></th>
<th>Turpentine Substitute</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature program</strong></td>
<td>40-120 @ 5 °C min⁻¹</td>
</tr>
<tr>
<td></td>
<td>120-250 @ 20 °C min⁻¹</td>
</tr>
<tr>
<td><strong>Inject split</strong></td>
<td>30ml/min. @ 250°C</td>
</tr>
<tr>
<td><strong>Capillary column</strong></td>
<td>CP-Sil 5CB, 30m<em>0.32mm</em>0.25µm</td>
</tr>
<tr>
<td><strong>Carrier gas</strong></td>
<td>30 ml min⁻¹ nitrogen</td>
</tr>
<tr>
<td><strong>Detector</strong></td>
<td>Flame ionisation, 250 °C</td>
</tr>
<tr>
<td><strong>Headspace sample (with a gas-tight syringe)</strong></td>
<td>50 µl @ 100°C heating for 15 min.</td>
</tr>
</tbody>
</table>

**Results**

1. Study the GC chromatogram for turpentine substitute shown in Appendix 1.
2. Peaks 1 to 6 result from an alkane series and are easily identifiable in the chromatogram. Several other peaks interspersed between these alkane peaks have also been labelled (7 to 12).
3. For the turpentine sample shown you can measure the peak height for each of these 12 peaks and record the data as shown in Table 2. This has also been done for diesel and white spirit.

Table 2 Peak height data for selected peaks in turpentine, diesel and white spirit.

<table>
<thead>
<tr>
<th>Peak i.d.</th>
<th>Retention time / min</th>
<th>Turpentine</th>
<th>Diesel</th>
<th>White spirit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2</td>
<td>7756</td>
<td>27106</td>
<td>476753</td>
</tr>
<tr>
<td>7</td>
<td>5.9</td>
<td>5112</td>
<td>11108</td>
<td>473946</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>15603</td>
<td>21725</td>
<td>873383</td>
</tr>
<tr>
<td>8</td>
<td>7.0</td>
<td>7025</td>
<td>5677</td>
<td>200903</td>
</tr>
<tr>
<td>9</td>
<td>7.5</td>
<td>6382</td>
<td>2996</td>
<td>95574</td>
</tr>
<tr>
<td>10</td>
<td>8.2</td>
<td>6897</td>
<td>2995</td>
<td>71396</td>
</tr>
<tr>
<td>3</td>
<td>8.9</td>
<td>52025</td>
<td>9726</td>
<td>205354</td>
</tr>
<tr>
<td>11</td>
<td>10.1</td>
<td>12863</td>
<td>2278</td>
<td>10856</td>
</tr>
<tr>
<td>4</td>
<td>11.6</td>
<td>41204</td>
<td>4155</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>12.1</td>
<td>8774</td>
<td>1218</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>14.3</td>
<td>15547</td>
<td>2881</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>16.7</td>
<td>3616</td>
<td>2514</td>
<td>0</td>
</tr>
</tbody>
</table>
Today’s samples

1. A turpentine standard has been run for you today. Identify peak 1 to 6 which constitute the alkane series and record their retention times in Table 3.
2. Using this as a template, and by comparison with Table 2, record the retention times for each of the remaining peaks 7-12.
3. For each of the samples, identify peaks 1-12 by their retention times. These should be very close to the retention times identified for the turpentine standard but may differ a little so use your judgement. Record the peak height for each peak in Table 3.
4. Plot the peak height data for EV2 against the peak height data for EV3.
5. Repeat the process on a separate graph for EV4.

Table 3 Retention time and peak height data for standards and samples

<table>
<thead>
<tr>
<th>Peak i.d.</th>
<th>Retention time for turpentine standard / min</th>
<th>Peak height / counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EV2 (carpet)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Questions

1. Compare the two graphs. What does this show?
2. How could you obtain an objective estimate of the relationship between the peak height data?
3. How could you identify the type of solvent?
Further Reading

Appendix 1
SCHOOL OF Geography, Earth and Environmental Sciences
COSHH and RISK ASSESSMENT

Fibre Analysis 1 - Optical Microscopy for Forensic Science

Assessment Date: 08/10/09
Assessment no: 1

ASSESSMENT OF RISK:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Hazards</th>
<th>Probability</th>
<th>Severity</th>
<th>Risk Factor</th>
<th>Controls</th>
</tr>
</thead>
</table>

COSHH ASSESSMENT:

n/a

FIRST AID FOR ANY OF THE ABOVE CHEMICALS
FIBRE ANALYSIS 1 - OPTICAL MICROSCOPY FOR FORENSIC SCIENCE

Objectives
Upon conclusion of the experiment the student should be able to:

- use optical microscopy to measure observe type shape, colour and fluorescence, and measure the size of fibre samples

Evidence you will examine

<table>
<thead>
<tr>
<th>Evidence Code</th>
<th>Description</th>
<th>Control</th>
<th>Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV5</td>
<td>Fibre samples from the carpet at the crime scene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6A</td>
<td>Fibre samples from Paul’s shoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6B</td>
<td>Fibre samples from Niamh’s shoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6C</td>
<td>Fibre samples from Sofie’s shoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6D</td>
<td>Fibre samples from Gareth’s shoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6E</td>
<td>Fibre samples from Dean’s shoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6F</td>
<td>Fibre samples from Lesley’s shoes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Procedure
The fibres have been prepared by placing short lengths of fibres into a resinous mounting medium. The normal medium is DPX with a refractive index of 1.515. Then a coverslip is placed on top and allowed to dry off. You should compare each of the recovered samples with the control samples.

1) Match & Measure
Carefully record at three measurements for the width of each retrieved fibre, calculate the mean and range. Then measure your ‘control’ and see if there is a suitable match in size. Make a sketch of the shape and morphology of the fibre. Make a note of the colour

2) Polarisation
The microscope here is set up for differential interference contrast (DIC). Below the specimen is a polariser, which polarises the light. The specimen (if it is anisotropic) may twist that light, which is then analysed at the analyser to produce Newtonian, colours -similar to oil or diesel layering on water on a sunny day, i.e. the colours of the rainbow. Again you can compare the control and retrieved fibres.

3) Fluorescence
You have a fluorescence microscope capable of both UV and blue excitation using incident illumination (EPI). Fluorescence is very specific in that the wavelength that will ‘excite’ a fluorescent chemical is specific to a few nm and the emission wavelength is also specific. Use the green incident light to illuminate the fibres and see if there is any BRIGHT fluorescence. Compare control and recovered samples. Try other colours of incident light.

Results
Record your observations for each fibre, then compile a checklist to compare each of the recovered fibres. Set out your results in a table similar to that shown below:
<table>
<thead>
<tr>
<th>Fibres</th>
<th>Sketch</th>
<th>Features</th>
<th>Colour</th>
<th>Polarisation</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EV6F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ASSESSMENT OF RISK:

<table>
<thead>
<tr>
<th>Activity</th>
<th>Hazards</th>
<th>Probability</th>
<th>Severity</th>
<th>Risk Factor</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pouring and moving solvents</td>
<td>Spillage, Evaporation, Combustion</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>Do not leave solvents in open containers. Limit sample size. Do not take near to ignition source or naked flame. Work only in fume cupboards with large quantities. Wear gloves. Do not inhale the vapour.</td>
</tr>
</tbody>
</table>

COSHH ASSESSMENT:

**Chlorobenzene**: Causes irritation to the respiratory and gastrointestinal tracts, and skin; vapour causes eye irritation; prolonged or repeated skin exposure may cause dermatitis or skin burns. Prolonged or repeated exposure may cause liver, kidney, or lung damage.

**Pyridine**: Harmful if breathed in or swallowed, or if it is absorbed through the skin. May reduce male fertility and chronic exposure may cause serious harm.

**Formic acid**: Contact with the eyes can cause serious long-term damage; the concentrated acid may cause serious skin damage and is readily absorbed into and through the skin; very harmful if inhaled.

**Chloroform**: Inhalation may be fatal, is toxic if swallowed, prolonged or repeated exposure may be harmful, repeated skin contact might lead to dermatitis and reproductive harm.

**Methanol**: Toxic if ingested or inhaled; very flammable; wide explosion limits for an air-methanol mixture.

**Ammonia**: Contact with the eyes can cause serious long-term damage; skin contact may cause burns; concentrated solutions can release dangerous amounts of **ammonia vapour** into the air. This presents a significant hazard if inhaled.

FIRST AID FOR ANY OF THE ABOVE CHEMICALS

**Mouth**: If swallowed DO NOT induce vomiting, seek medical advice immediately.

**Skin**: Wash immediately with plenty of soap and water.

**Eyes**: Wash with plenty of water and seek medical advice.

**Lungs**: Remove from exposure, rest and keep warm. In severe cases seek medical advice.
FIBRE ANALYSIS 2 – TLC AND FTIR

Objectives:
Upon conclusion of the experiment the student should be able to:

- perform a micro-scale solvent extraction of a fibre
- perform a TLC of extracted fibre dyes
- use FTIR to identify fibre types

Evidence you will examine
EV5: Fibre samples from the carpet at the crime scene
EV6(A-B): Example of fibre samples collected from the shoes of Paul and Niamh
EV6(C-F): Example of fibre samples collected from the shoes of Sofie, Gareth, Dean and Lesley

Fibre Analysis
When fibres are analysed for forensic purposes a hierarchy of operations are performed in the following order:

1. The initial examination is done under a microscope to determine the fibre type, for example cotton or wool.
2. Colour is determined.
3. Physical features such as cross-sectional shape are distinguished.
4. The width of the fibre is measured.
5. The fibre is examined to see if it contains any delustrant – this is used by manufacturers to determine how bright and shiny a finished garment is.
6. The fibre is examined under ultraviolet and blue light.

If the fibres can be said to match after these examinations the following two procedures are performed:

7. UV/Visible microspectrophotometry is used to more accurately determine the colour.
8. If the fibre is large enough the colour can be extracted from it and a thin-layer chromatography test can be done to identify the dyes mixed by the manufacturer to give the fibre its finished colour and hue.
9. For manmade fibres an infra red spectrometry can be performed to tell whether the fibre is made of polyester, nylon or acrylic.

You will be performing the last two procedures in this practical, on fibres which have already been matched using light microscopy.

Identification of Fibre Type by FTIR
FTIR spectrometry can be used on synthetic fibres only. Analysis is performed on fibres previously identified to their generic types (using various other microscopy techniques). Using FTIR it is possible to extend the ID of the fibre to the level of sub-type e.g. acrylic type 2 or 3, polyamide 6 or 66. This can be useful with the comparison of recovered and control samples.
1. Prepare a diffuse reflectance (DRIFT) sample cup with KBr
2. Place the sample cup in the FTIR spectrometer and acquire the DRIFT spectrum
3. Place several fibres on the surface of the KBr in the DRIFT sample cup and repeat the spectrum acquisition.
4. Subtract the spectrum obtained in (2) from (3).
5. Identify the fibre type by comparison with library spectra of known fibres of acrylic, nylon and polyester.

**TLC of Extracted Dyes**

The aim here is to extract the dye from the fibres using an appropriate solvent, identify the dye type, then perform TLC on the extracted dye using an appropriate solvent system.

**Extraction**

The correct extraction procedure will depend on the type of fibre and dye.

1. Using the FTIR identification of fibre type and the information in Table 1, select the appropriate extraction scheme.
2. Insert at least 6 fibres, each of length 6 mm approx., into a melting point tube and push to the bottom using a piece of stiff wire.
3. Inject the appropriate extraction solvent into the tube, ensuring that it covers the fibres at the bottom. A GC injection syringe is ideal for this.
4. Seal the tube by flaming the end in the flame of a small Bunsen.
5. Place the tube into a melting point apparatus and heat at the temperature and for the time given in Table 1.

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Extraction procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester</td>
<td>Try chlorobenzene @ 130 °C for 10 min, if good extraction then it is a disperse dye. (this is most likely). If little or no extraction then try DMF:formic acid (1:1) @ 100 °C for 10 min, if good extraction then it is a basic dye.</td>
</tr>
<tr>
<td>Nylon (polyamide)</td>
<td>Try chlorobenzene @ 150 °C for 10 min, if good extraction then it is a disperse dye. If little or no extraction then try pyridine:water (4:3) @ 100 °C for 20 min, if good extraction then it is an acid or basic dye. If little or no extraction then it is a reactive or diazo dye.</td>
</tr>
<tr>
<td>Acrylic</td>
<td>Try formic acid:water (1:1) @ 100 °C for 20 min, if good extraction then it is probably a basic dye.</td>
</tr>
</tbody>
</table>

**TLC Elution**

1. Fill the TLC separation tank (a deep Glass Petri-dish) with an appropriate elution solvent to a depth of 0.5 cm. Choose the solvent system based on the fibre and dye type for your control sample using Table 2.
2. Mark the origin (very lightly in pencil) along the long side of a TLC plate about 1.0 cm from the base of the plate.
3. Spot the extracted samples onto the TLC plate along the origin, using a micro-capillary tube. It is important not to scratch or damage the plate as this will cause an uneven solvent front. Try to make the smallest diameter spots possible by drying between applications with a hair dryer. Spot several times in the same place to build up a high concentration of dye. You should spot the samples in the following order along the origin:

<table>
<thead>
<tr>
<th>standard dye mix</th>
<th>control EV5</th>
<th>recovered EV6 A-B</th>
<th>recovered EV6 C-F</th>
<th>standard dye mix</th>
</tr>
</thead>
</table>

The standard dye mixture is a quality control sample to ensure that the TLC method is working properly.

4. Place the plate in the separation tank which has been pre-saturated with elution solvent. **Make sure that the origin is above the solvent surface.** Replace the tank cover and allow the chromatogram to develop until the solvent front is approx. 0.5 cm from the top of the plate.

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Dye type</th>
<th>Eluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester</td>
<td>Disperse</td>
<td>9:1 chloroform:acetone</td>
</tr>
<tr>
<td>Nylon</td>
<td>Acid or Basic</td>
<td>11:7:1:1 chloroform:methanol:ammonia:water</td>
</tr>
<tr>
<td>Acrylic</td>
<td>Basic</td>
<td>11:7:1:1 chloroform:methanol:ammonia:water</td>
</tr>
</tbody>
</table>

5. Dry the plate and examine under white and UV light.
6. Make a sketch or take a digital photograph of the plate to include with your report.

Results

Compare the chromatograms of the control and recovered fibre extracts and identify the origin of the fibres. Use this information in your final report to draw conclusions about the presence of the suspects at the crime scene.

Further Reading

SCHOOL OF Geography, Earth and Environmental Sciences  
COSHH and RISK ASSESSMENT  

DRUGS OF ABUSE  

Assessment Date: 08/10/09  
Assessment no: 1  

ASSESSMENT OF RISK:  

<table>
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<th>Activity</th>
<th>Hazards</th>
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<td>3</td>
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</tr>
<tr>
<td></td>
<td>Evaporation</td>
<td></td>
<td></td>
<td></td>
<td>Limit sample size</td>
</tr>
<tr>
<td></td>
<td>Combustion</td>
<td></td>
<td></td>
<td></td>
<td>Do not take near to ignition source or naked flame</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Work only in fume cupboards</td>
</tr>
</tbody>
</table>

COSHH ASSESSMENT:  

**Chloroform:** inhalation may be fatal, is toxic if swallowed, prolonged or repeated exposure may be harmful, repeated skin contact might lead to dermatitis and reproductive harm.  

**Wagner's reagent:** 1. Iodine - very toxic if swallowed or inhaled; may lead to reproductive damage; may be absorbed through the skin. 2. Potassium iodide - may irritate the lungs or eyes.  

**Zwikker's reagent:** 1. Copper (II) sulfate pentahydrate - HARMFUL, TOXIC. May impair fertility. Harmful if swallowed. Risk of serious damage to eyes. Irritating to respiratory system, and skin. May cause sensitization by skin contact. 2. Pyridine - harmful if breathed in or swallowed, or if it is absorbed through the skin. May reduce male fertility and chronic exposure may cause serious harm.  

FIRST AID FOR ANY OF THE ABOVE CHEMICALS  

**Mouth**  
If swallowed DO NOT induce vomiting, seek medical advice immediately.  

**Skin**  
Wash immediately with plenty of soap and water  

**Eyes**  
Wash with plenty of water and seek medical advice  

**Lungs**  
Remove from exposure, rest and keep warm. In severe cases seek medical advice.
DRUGS OF ABUSE

Objectives:
Upon conclusion of the experiment the student should be able to:
- perform a solvent extraction of an unknown drug
- perform spot tests to identify the category of drug
- use FTIR to identify the extracted and unextracted drug

Evidence you will examine
EV7: Capsule taken from a packet of painkillers in Sofie’s handbag.
EV8: Sample of white wine from discarded bottle

Introduction
Drug analysis involves both the identification and quantification of drugs of abuse. FTIR provides a rapid, relatively cheap and definitive method for identification of drugs of abuse. The spectrum can be analysed by considering the six strongest absorptions occurring between 400-4000 cm$^{-1}$. This is known as the principle peaks method of drug identification. A second method involves the use of the whole spectrum, especially the fingerprint region, (400-1500 cm$^{-1}$) and an on-line library search. On-line library searches are by no means foolproof and, especially where a formulation rather than a pure drug are being analysed, they need to be treated with caution. Quantification is normally performed using GC-MS or HPLC-MS

Procedure
Classification using spot tests
When handling tablets, powders or solutions thought to contain drugs of abuse, gloves should be worn at all times.

You are provided with a capsule containing the powdered drug (EV7), mixed with various other compounds that make up the formulation. Make careful note of the physical description of the capsule.

The spot-test procedure for a wide range of drug classes is given in Appendix 1. You will perform the following two spot tests:

Wagner’s reagent – place 10-20 mg of the powder from one of the capsules into a test-tube and add 1-2 ml of Wagner’s reagent. Formation of a precipitate indicates a positive reaction.

Zwikker test – place 10-20 mg of the powder from one of the capsules into a test-tube and add a few drops of Zwikker A solution followed by a few drops of Zwikker B. The appearance of a blue-violet colour indicates a positive reaction.

Extraction of the drug
Many drugs are present in over the counter preparations as salts of the patent drug. This is also true of Prescription Only Medicines (POMs). These are, in general, water soluble and so can be extracted with water. In this case, by alteration of the pH, the
salt form can be converted to a neutral form (the free acid or base), and using a back extraction into a non-polar solvent can then be removed from the complex extraction mixture. You will use such a protocol in this practical.

**Capsule**
1. Carefully separate the two halves of the capsule, EV7, (the blue part is the easiest to place uppermost) and transfer the contents into a boiling tube.
2. Add distilled water (10ml) to dissolve the active component (which is present in salt form)
3. Add 1M HCl **DROPWISE** until the solution becomes **JUST** acid to litmus and mix thoroughly. This will convert the drug to its free acid form.
4. Transfer the solution into the extraction funnel provided, pipette 2 ml chloroform into it and shake thoroughly. The chloroform layer is the lower of the two. Run the chloroform into the screw-topped vial, making sure not to remove any of the aqueous layer.
5. Repeat the extraction process with a further 2 ml of chloroform.

**Wine**
1. Place a 5 cm³ sample of the suspect wine, EV8, into a boiling tube
2. Repeat steps 3-5 above.

**Capsule and Wine**
1. Using nitrogen gas, concentrate the samples by blowing the chloroform off **IN A FLOW HOOD**.
2. Once the samples are concentrated, the infra red spectrum may be obtained, using the Diffuse Reflectance (DRIFT) technique.

**Results**
1. Use the flow-chart given in Appendix 3.2 to identify the class of drug.
2. FTIR provides a rapid, relatively cheap and definitive method for identification of drugs of abuse. The spectrum can be analysed by considering the six strongest absorptions occurring between 400-4000 cm⁻¹. This is known as the principle peaks method of drug identification. A second method involves the use of the whole spectrum, especially the fingerprint region, (400-1500 cm⁻¹) and an on-line library search. On-line library searches are by no means foolproof and, especially where a formulation rather than a pure drug are being analysed, so it is good practice to back this up with other confirmatory sources. Access the paper by Manning and O’Brien¹ at [http://www.unodc.org/unodc/en/data-and-analysis/bulletin/bulletin_1958-01-01_1_page006.html](http://www.unodc.org/unodc/en/data-and-analysis/bulletin/bulletin_1958-01-01_1_page006.html) and identify the main absorption band-heads in your spectra.
1. Access the NIST Chemistry WebBook² at [http://webbook.nist.gov/chemistry/](http://webbook.nist.gov/chemistry/) and search for the candidate compounds by **Name** and **IR Spectrum**. You will then be able to download images of several spectra and compare them with your own. **NB**: make sure that you download the SOLID KBr disc spectrum. Include the spectrum of the most likely candidate in your laboratory report.
2. Discuss the advantages of solvent extracting the drug, compared to direct analysis, when using FT-IR spectroscopy.
References
Appendix 3.2
Flowchart for Drug Testing
(adapted from E Jungreis, Spot Test Analysis, 2nd Edn., 1997, Wiley, New York.)

Alkaloidal tests (Mayer's, Dragendorff's, Wagner's)

- positive
- negative

Marquis test

- positive
- negative

Zwikker test

- positive
- negative

Cobalt thiocyanate test

- positive
- negative

Mandelin's test

- positive
- negative

Barbiturates

- positive
- negative

Erlich test

- positive
- negative

Duquenois test

- positive
- negative

Opiates
- Codeine
- Heroin
- Morphine
- Opium
- Papaverine
- Nonopiates
- Meperidine
- Methapyrilene

Atropine
Cocaine
Methadone
Methylphenidate
Nicotine
Phencyclidine
Procaine
Scopolamine
Aconitine
Caffeine
Lidocaine
Methaqualone
Physostigmine
Psilocin
Quinine
Strichnine
Yohimbine

Aspirin
Benzocaine
Ephedrine
Mescaline

Lysergide
Psilocybin

Cannabidiol
Cannabinol
Tetrahydrocannabinol

Amphetamine sulfate
Methamphetamine
Thiopental
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