Chemistry in Action Laboratory Manual

By
Dr Stephen Summerfield
Department of Chemistry

June 2006

Loughborough University

Analytical Chemistry Techniques: Overview

Further information on analytical techniques used in these laboratory exercises is available in four separate techniques guides:

Part 1: Introduction to Analytical Chemistry:
A basic introduction to analytical science in general, and analytical chemistry in particular, including an overview of the analytical process, quality assurance, and guidance on solution preparation including volumetric and concentration calculations. It also includes a bibliography of useful texts for the analytical chemist.

Part 2: Introduction to Molecular Spectroscopy:
An introduction to the different types of molecular spectroscopic analysis, including UV-Vis, fluorescence, IR, MS and NMR, describing the basic principles of each technique and practical considerations including sample preparation. It is illustrated with simple diagrams, photographs of equipment and information to aid interpretation of spectra.

Part 3: Introduction to Chromatography:
An introduction to chromatographic analysis, describing the different types of chromatography, including TLC, GC, HPLC and ion chromatography, their application areas and basic principles of operation. It is illustrated with simple diagrams, photographs of equipment and chromatograms illustrating practical aspects of the technique.

Part 4. Introduction to Atomic Spectrometry:
An introduction to the different types of atomic spectrometric analysis, including ICP-AES, ICP-MS, XRF and AAS, describing the basic principles of each technique, their application areas and modes of operation, including practical comparison of the techniques. It is illustrated with simple diagrams and photographs of equipment.

Contact Details

Dr. Stephen Summerfield s.summerfield@lboro.ac.uk
Dr. Helen Reid h.j.reid@lboro.ac.uk

Author (s)	Stephen Summerfield & Helen Reid
Owner (s)	Loughborough University
Title	Chemistry in Action - Laboratory Manual
Classification	F180, Laboratory manual
Keywords	ukoer, sfsoer, analytical chemistry, analytical science, forensic science, forensic analysis
Description	Laboratory manual describing 11 x 3 hour experiments. Students are presented with a case scenario of a suspicious death, which they must investigate via a series of laboratory experiments. Students work in groups and co-ordinate their results in a follow-up workshop.
Creative Commons licence (url)	http://creativecommons.org/licenses/by-nc-sa/2.0/uk/
Language	English
File size	397 KB (388 KiB)
File format	pdf

Contents

General Laboratory Rules

Specific safety information is included in bold type at the head of each experiment. These are in addition to the general rules listed below which apply to all work carried out in the laboratory.

1. Safety spectacles must be worn at all times.

2. Laboratory coats must be worn at all times.

3. Eating, drinking and smoking are not allowed in laboratories.

4. Assume all chemicals are toxic unless you are certain that they are not. Toxic chemicals may be absorbed by ingestion, inhalation or contact with the skin.

5. Mouth pipetting is not allowed. Always use a pipette filler. Take particular care when fitting a filler to a glass pipette: you should hold the pipette firmly near its junction with the filler as you fit it, thus reducing the risk of the pipette breaking and causing injury.

6. Do not weigh chemicals on pieces of paper. Always use a weighing boat or other suitable container, obtaining the sample weight by difference.

7. Always seek advice on the use of unfamiliar equipment if you are uncertain of how to use it and be particularly careful with syringes, spectrometer cells etc.

8. Never dispose of waste organic solvents down the sink. Always use the waste solvent bottles provided.

Forensic Science

Forensic science is the application of analytical science to the legal process. The data produced need to be right first time, provide convincing evidence in an adversarial court system and be understandable to a jury.

Physical Evidence

Evidence is defined as any material that can provide, through scientific examination and analysis, information relating to a crime. The evidence may:

1. Prove that a crime has been committed
2. Provide leads to the investigators
3. Link the crime scene and/or the victim to the suspect
4. Corroborate or refute a suspect's statement or alibi
5. Identify a suspect
6. Induce a confession from a suspect
7. Exonerate the innocent
8. Form part of expert testimony provided by a forensic scientist in court.

Forensic samples are very varied and can be found in various locations including in or on tools, clothing, carpets, vegetation, and the human body. Weather, washing, high temperature and contamination may change analytes. When dealing with very small samples such as glass fragments, paint chips, a few fibres and blood specks, non-destructive analysis of high sensitivity is needed to permit further examination and retain valuable items.

The Chain of Custody

This is the single most important aspect of all forensic chemistry. The progress of the exhibit from crime scene to court must be documented rigorously at every stage. Every person handling evidence must sign and date the label and be prepared to testify in court to validate integrity of evidence. If the chain of custody is broken at any point, the evidence must be excluded from the court.

The Crime Scene

The access to the crime scene is limited to essential and authorised persons in order to preserve the scene. During the systematic search for evidence protective clothing must be worn to avoid contamination. Accurate records are essential. The item, its condition, the precise location with respect to a permanent position, date, and time are recorded. Evidence is photographed wherever possible in situ before moving and fingerprinting.

All items must be packaged separately to avoid cross contamination. The container should be marked with the initials of the collecting officer, date, location where it was found, case number and description. Plastic envelopes are excellent for small non-biological objects. Paper envelopes are used for dry biological evidence (e.g. dried body fluids). If the sample is not dry it should be dried in the air before packing. Small containers are frequently suitable. Garments and large exhibits can be placed in bags, rolled in paper or boxes. Remember laboratory analysis is irrelevant if evidence is lost or contaminated beforehand

Recovery of Trace Materials

1. Any small piece of evidence should be collected with tweezers and put into coin envelopes (if damp) or into small plastic bags.
2. Shaking garments onto paper is the simplest method of collecting glass and paint fragments.
3. Brushing with a new clean toothbrush onto a piece of paper is useful for collecting powders and debris.
4. Fibres and hairs are recovered by using Sellotape sequentially on fabrics, window ledges and almost any dry surface. These are then stuck onto clear acetate sheets and stored in sealed polythene bags.
5. The evidence vacuum is a small powerful vacuum cleaner, equipped with filters, used for the recovery of minute particulates from firearm discharges or drug dealing. It should be performed before fingerprinting.
6. Swabbing using sterile cloth patches is used to collect small amounts of smeared materials, such as blood, semen etc. before blood typing or DNA profiling.
7. Oil, grease cosmetics etc. are extracted from fabrics in a suitable solvent.
8. Liquids can be pipetted, swabbed or absorbed on a suitable material.

The Laboratory

The procedures used in the laboratory must prevent contamination and be secure. Detailed, permanent records at every stage of examination ensure no confusion of results. Full documentation of all procedures used with checks undertaken by colleagues and the use of quality control samples is essential. The key is the competence and integrity of the scientist.

Table 1: Types of physical evidence

Type	Notes
CONTACT TRACES	
Fibres and textiles	Collecting fibres by adhesive lifts.
Glass	Trace and large sections left at the scene or on suspect.
Oils, greases & cosmetic products	Transferred between objects & individuals.
Paint & paint products	Paint fragment transfer from a car accident etc…
Soil and minerals	Hand picking and brushing.
DOCUMENTS	
Questioned documents	Hand-written, typed, copied or computer generated.
Serial number	Altered or eradicated on vehicles, firearms or other objects.
FIRE/EXPLOSION	
Explosives (bulk)	Liquid or solid material.
Explosives (trace)	Swabbing dry or with solvent, solvent washing, headspace analysis, vacuum sampling or adhesive lifts.
Fire investigation	Liquid, solid material and burned material.
Firearms and ammunition	Firearms, ammunition, casings etc.
MARKS	
Finger and palm prints	Visible and latent prints lifted from various surfaces.
Tyre and footprints	Prints and impressions found on surfaces.
Tool marks	Impressions or scrapes produced on surfaces.
TOXICOLOGY	
Body tissues	Various organs from autopsy with blood, urine and stomach contents.
Blood splatters	Take photographs and measure precisely.
Body fluids	Blood, semen and saliva (liquid or dry) on clothing, fabrics or objects collected by sterile cloth patches or swabs.
Drugs and controlled substances.	Brushing, extracting or vacuuming of trace samples. Bulk sample in the form of tablets, capsules, powders and other preparations.
Hair	Collected from crime scene, victim or suspect usually by adhesive lifts.

The Scenario

SUSPICIOUS DEATH

Mr John Naylor telephoned the police in a distraught state to report his wife (Mrs Sandra Naylor) missing. Mr Naylor stated that he had just returned home and found a note apparently written by his wife threatening suicide.

A woman's body was subsequently found on a nearby beach near the mouth of the River Dribble. Mr John Naylor later identified the body as his missing wife, Mrs Sandra Naylor.

Post Mortem
Death was due to drowning but the circumstances leading up to her death are unclear.

Further Enquiries
Suspicion is thrown upon the husband, who is discovered to have been having an affair with another woman and stands to gain financially from his wife's death.

Investigation

You will investigate two of the four different aspects of the case in groups and will be pooled within each group. Then at the end your conclusions will be drawn together to determine what happened according to the evidence.

Section A: Water analysis: Where did Sandra drown?

You are provided with a sample of the liquid found in the woman's lungs and you will need to identify the source: river water, sea water, bath water or swimming pool water? One possible method is to compare the water from the lungs with water samples from the various sources by their ionic content. This could involve monitoring either cations or anions. In this study you will compare the latter using ion-chromatography. This is essentially an ion-exchange separation using a suppressed ionisation detector. You will also compare the sodium content of the samples using flame atomic emission spectrometry.

Section B: Drug analysis: Was Sandra drugged?

You will need to establish whether she has traces of drugs in her body that may have contributed to her death. You will examine extracts of her blood plasma for traces of drugs using high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) or thin layer chromatography (TLC) and compare any found with various tablets found in connection with the case.

Section C: Drug identification

The tablets will be identified using infrared spectrometry and comparison with a computer database of known compounds. You will relate this to any drug found in the plasma extracts which you will identify by gas chromatography-mass spectrometry (GC-MS).

Section D: Alcohol and suicide note

The presence or otherwise of alcohol in her body will be investigated by analysis of a post-mortem blood sample using gas liquid chromatography.

There is some suspicion that the suicide note may have been faked. Whilst the signature appears genuine, it may be that an innocuous note has been altered to make its contents more sinister. You will examine the note microscopically for evidence of alteration and, in particular, to see if there is evidence of more than one pen having been used. Detailed comparison of inks and pens will be carried out using thin layer chromatography (TLC).

A: Water Analysis

You are provided with a sample of the liquid found in the woman's lungs and you will need to identify the source: river water, seawater, bath water or swimming pool water?

This is done here by determining:

1. Anions by ion chromatography
2. Phosphates by UV-Vis spectroscopy
3. Cations by flame emission spectrometry.

Results from the lung water will be compared with water samples of known origin.

A1: Anions in Water Samples by Ion Chromatography

Safety note: The experiment presents no extra hazards beyond normal laboratory precautions.

NOTE: This analysis will have already been done for you using the method, equipment and samples described below. You are presented with the chromatograms for interpretation.

Equipment

Dionex ion chromatograph fitted with a post-column suppression and a conductivity detector and AS14 column.

Mobile phase solutions

All solutions use 18mΩ ultra pure water.

Eluent

0.0840 g of sodium hydrogen carbonate (1 mM) and 0.3710 g of sodium carbonate (3.5 mM) in 1 litre of water.

Suppressor regenerator solution

1.5 ml of sulphuric acid in 2 l water.

Test water standards

River water	(diluted 20 fold)		
Tap water	(diluted 5 fold)		
Swimming pool water	(diluted 20 fold)		
Bath water	(diluted 50 fold)		
Sea water	(diluted	2000	fold)

Sample

Sample of solution from deceased's lungs.

Standard Anion Mixture containing in order of elution

Fluoride	1 ppm
Chloride	2 ppm
Nitrite	2 ppm
Bromide	10 ppm
Nitrate	10 ppm
Chlorate	5 ppm
Phosphate	10 ppm
Sulphate	10 ppm

Method

1. Dilute the deceased's sample 10 fold with pure water (18 MΩ).
2. Inject 20 µl of the diluted seawater into the sample port and start the separation run by Pressing RST. Sample is injected 0.2-min later- listen for the valve. Carefully wash the syringe. After 20 min stop the integrator, you should see one major component.
3. In turn inject samples of the diluted test waters that are provided and the diluted deceased's sample that you prepared. If this last solution gives peaks off- scale dilute 1ml by 100 times and re-examine. Finally run a sample of the Standard Anion Mixture.
4. Compare the results from all the samples to identify where the woman drowned.
5. At the end of the lab session, to shut down the ion chromatograph:
   Stop pump. Detector Autoset OFF. Cell OFF. Turn off power to system.
   Turn off helium cylinder. Turn off nitrogen cylinder. Switch off degas system.

Thinking about making valid measurements

You analyse a sample and get a peak at the same retention as the chloride standard. How confident are you that the peak from the sample is due to chloride?

Improving your technique

A comprehensive list of practical tips is given in the basic skills training guide (IC) that can be obtained from the RSC or LGC. A few of the important ones are listed below.

- Choose a suitable column and mobile phase for the analysis – the manufacturer's catalogue or web site will contain this type of information.
- Mobile phase should be free from dust and dissolved gases removed.
- Always use a solvent reservoir filter.
- Ensure there is sufficient mobile phase to complete the analysis.
- The sample to be analysed needs to be soluble in the mobile phase.
- Never use a syringe with a sharp tip because it will damage the injector.
- Investigate flow rate fluctuations – may be due to blockages or leaks within the system.
- After use, always flush water, buffered solutions, acids etc, out of the system using an appropriate solvent.

A2: Sodium in Water Samples by Flame Atomic Emission

A natural gas/air flame is used: clearly the burner of the flame emission instrument in particular becomes very hot. Instruction must be obtained on the lighting of this flame.

The chemicals used in this experiment pose little hazard provided that routine laboratory precautions are taken to avoid ingestion and skin contact.

Standard solutions

Stock standard solution contains 500 ppm of potassium (as KCl) and 250 ppm of sodium (as NaCl). Preparation of the combined 10, 8, 6, 4 and 2 ppm Na and 20, 16, 12, 8 and 4 ppm K standards

1. Pipette 2.0, 1.6, 1.2, 0.8 and 0.4 ml of the joint standard solution into respective 50-ml volumetric flasks.
2. Dilute to 50 ml with distilled water. These correspond to 10, 8, 6, 4 and 2-ppm standards of sodium, and twice these amounts of potassium.

NOTE: Sodium does not interfere in the flame photometric determination of potassium and potassium only interferes in sodium determination if the K: Na ratio is more than 10:1.

Test solutions

River water, tap water, swimming pool water, bath water, seawater.

Sample

Sample of solution from deceased's lungs.

Procedure

1. The flame should already be lit: if not, consult a demonstrator.
2. Select the sodium filter and aspirate a sample of deionised water.
3. Adjust the "blank" control until the LED reads zero.
4. Transfer a little of the most concentrated standard solution to a clean plastic cup, swirl, discard and refill.
5. Aspirate this solution and adjust the "fine" sensitivity control until the LED reads 100 (if necessary, adjust the "coarse" setting to bring the reading on-scale).
6. Aspirate deionised water again and re-adjust the blank to zero if necessary.
7. Aspirate the series of standards in turn (using plastic cups as before) and obtain a series of readings for the standard solutions.
8. Without altering the sensitivity settings, obtain readings for the test solutions and lung water sample.
9. If the readings are offscale, dilute the samples accordingly.
10. Use the standard results to plot a calibration curve, with sodium concentration on the x-axis and photometer reading on the y-axis.
11. Use this graph to determine the sodium content of the test and sample solutions from the readings taken for these samples.

If time permits, repeat the procedure with the potassium filter selected.

Thinking about making valid measurements
To give evidence in court you have to show that the analytical method is producing valid results. Are you certain that any sample pre-treatment has not invalidated the analysis?

Improving your practical technique

- The instrument should be allowed to warm up before use.
- A wavelength check should be carried out to check the instrument is correctly set up.
- Instrument needs to be calibrated using a set of standards of differing concentration for quantitative analysis.
- Solid material must be removed from the sample before analysis.
- Check that liquid is being drawn up at reasonable speed.
- Consider doing duplicate samples.
- Analyse a blank sample (i.e. solvent blank).
- In many cases, samples may need to be diluted so that the measured emission is between the lowest and highest standard on the calibration curve.

A3: Determination of Phosphate by a Colorimetric Method

Normal laboratory safety rules should be observed throughout this experiment. Lab coats and safety spectacles must be worn at all times. Mouth pipetting is forbidden. Benches must be kept clean and tidy. Eating, drinking and smoking are not allowed in the laboratory.

The vanadate-molybdate reagent used in this practical is in strong acid solution. Handle with care, avoid skin contact and clear up any spillages immediately.

Introduction

In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form the heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdo-phosphoric acid is formed. The intensity of the yellow colour is proportional to phosphate concentration. Absorbance may be measured at 400, 420 or 470 nm depending on the sensitivity required, the absorbance at 400 nm being ten times that at 490 nm for a given P concentration. However, due to potential interference from ferric ions at low wavelengths, 470 nm is normally chosen.

In this experiment, you will determine the phosphorus content of various samples of waters. No sample pre-treatment is required, except for dilution and addition of the colorimetric reagent.

To avoid any possible phosphate contamination from detergents, it is very important that you use only the acid-washed glassware supplied in the cupboard for this experiment.

Apparatus

UV/visible spectrophotometer & glass cells
Volumetric flasks: 13 x 50 ml
Beakers: 6 x 100 ml
Pipettes: 10 & 25 ml bulb pipettes

Reagents

Sample of water from the lungs of the victim.
Samples of water from the bath, swimming pool, tap, river and sea.
Vanadate-molybdate reagent: a mixture of ammonium molybdate [$(NH_4)_6Mo_7O_{24}$] and ammonium metavanadate [NH_4VO_3] in HCl.
Standard phosphate solution: KH2PO4 containing 50.0 µg P per ml.

Preparation of Calibration Standards

Prepare a series of calibration standards by transferring 0, 5, 10, 15 and 20 ml aliquots of the standard phosphate solution to 50 ml volumetric flasks, adding 10 ml of the vanadate-molybdate reagent to each and diluting to the mark with deionised water. The standards thus diluted contain 0, 5, 10, 15 and 20 µg P per ml (the one containing no phosphate acts as a reagent blank).

Procedure

1. Shake the 'bath water sample' to ensure thorough mixing, then transfer a 25-ml aliquot to each of two 50-ml volumetric flasks.
2. Add 10 ml of the vanadate-molybdate reagent to one of these flasks - the second acts as a sample blank - and dilute each to the mark with de-ionised water.
3. Shake to mix.
4. Repeat 1-3 for the other water samples.
5. Leave all prepared solutions (including calibration standards) to stand for at least 10 minutes for the colour to develop fully before taking absorbance readings.
6. Measure the absorbance of each standard, sample and blank at 470 nm.
7. Plot a calibration graph of absorbance vs. phosphorus concentration (in µg/ml) for the standard solutions.
8. Use this graph to determine the phosphate (as P) concentration in each water sample (remember to allow for the dilution of the sample in your calculations) and subtract the sample blank reading from the corresponding sample.

Thinking About Making Valid Measurements

To give evidence in court you have to show that the analytical method is producing valid results.

1. Are you confident that the data you have obtained can discriminate between the samples?
2. Are you certain that any sample pre-treatment has not invalidated the analysis?
3. How might you determine the concentration of organically bound phosphorus in a water sample?

Improving Your Practical Technique

- The instrument should be allowed to warm up before use.
- A wavelength check should be carried out to check the instrument is correctly set up.
- Instrument needs to be calibrated using a set of standards of differing concentration for quantitative analysis.
- Cuvettes must be scrupulously clean especially in the region where the light beam passes through.
- Cuvettes should always be placed the same way round to so subtle differences in the cuvette shape are avoided.
- Matched cuvettes or the same cuvette should be used throughout the series of analysis.
- Sample may require degassing before analysis to remove air bubbles.
- Solid material must be removed from the sample before analysis.
- Beer Lambert law no longer applies above an absorbance of 1.
- A double beam spectrophotometer permits the reference beam to have a cuvette containing solvent.
- Measurements can easily be repeated and there should not be any significant difference between the readings (<±0.003 absorbance).
- Consider doing duplicate samples.

- Analyse blank sample (i.e. solvent blank and sample blank).
- In many cases samples may need to be diluted so that the measured absorbance is between the lowest and highest standard on the calibration curve.
- Wavelength scan should normally be performed to determine the absorbance maximum (λmax) however, the determination was not carried out at the λmax due to the potential interference from iron (Fe).
- Qualitative identification of UV/Vis is very crude but it is ideal for quantification using a coloured complex in this experiment.
- Where practicable use a more specific technique such as ion chromatography.

B: Drug Analysis of Plasma Samples

You are supplied with extracts* of the woman's blood for analysis and comparison with samples of powder found in tablet bottles at her home. Analysis is by thin layer chromatography and/or high performance liquid chromatography, depending on time and availability of equipment. You are also supplied with GC-MS data for the extracts.

**The isolation and identification of an unknown drug in a body fluid is often required as part of forensic and clinical chemistry studies. The first task is to isolate the drug compound from the polar, protein-containing aqueous matrix. Solid-phase extraction was used as follows:*

1. *Dilute 1 ml of the serum sample with water (3 ml). Acidify the diluted serum with orthophosphoric acid (87%, 10 µl). Check pH with pH paper. The solution should be about pH 3*
2. *Place a C_{18} SPE cartridge on the vacuum chamber and start the suction.*
3. *When you are ready to start wash the cartridge with 1ml of 2% methanol in water to activate the stationary phase. Without letting the cartridge dry out, immediately load the acidified serum sample onto the cartridge and run though. Then allow the cartridge to run dry under vacuum for 1 min. Collect the solution that has run through (which should contain any protonated amines that will not have been retained on the neutral column) and treat as step f.*
4. *Elute the cartridge with acidified ethyl acetate containing 1% acetic acid (1 ml). Collect the eluent – this is the acid and neutral fraction.*
5. *Dry the cartridge with vacuum (1 min).*
6. *Take the collected solutions from part c and basify with 10-M potassium hydroxide (10 µl) and check the pH with pH paper (should be approx pH 9). If it is still acidic add more potassium hydroxide.*
7. *Activate the cartridge again with 1ml of 2% methanol in water to activate the stationary phase and immediately load this basified sample (approx 5 ml) onto the cartridge, wash with 2% methanol in water (1ml).*
8. *Allow the cartridge to dry under high vacuum (1 min)*
9. *Elute with basic ethyl acetate containing 2% 2M NH_4OH (1 ml) and collect the eluate, which is the basic fraction.*
10. *Acid/neutral and basic fractions can now be analysed.*

As the time available for this experiment is limited, you are supplied with acid/neutral and basic extracts already prepared.

Samples

Two extracts from the woman's blood plasma: an acid/neutral extract and basic extract (both in methanol). Samples of powder found in tablet bottles at the woman's home. These should be dissolved in 50:50 methanol: water for analysis by HPLC. As you are not carrying out a quantitative analysis, you do not need to prepare accurate dilutions: about 10 mg of powder per 25 ml of solvent should give a suitable concentration for analysis.

B1: Analysis of Plasma Extracts and Drug Samples by TLC

An UV lamp is used to observe the TLC plate. YOU MUST NOT PICK UP, TILT OR MOVE THE LAMP WHILE YOU ARE DOING THIS AND DO NOT LOOK DIRECTLY AT THE LAMP UNDER ANY CIRCUMSTANCES.

Safety Notes:
The compounds used in these experiments as "unknown" samples are drug compounds and care must be taken to avoid ingestion and skin contact. The plasma solutions must be treated as biological hazards and all work must be carried out using gloves to avoid any contact. All biological wastes and contaminated glassware must be sterilised chemically after use (see technician).

Sample

Acid/neutral extract, basic extract and drug powders

Mobile Phases

A Ethyl acetate: methanol: 0.880 ammonia solution (85:10:5)
B Ethyl acetate

Apparatus

Silica Gel GF254 TLC plates
Two TLC tanks

Procedure

1. Prepare TLC tank A (large beaker) by pouring in the Mobile Phase A to a depth of about 8 mm and TLC Tank B by pouring in Mobile Phase B.
2. Line both tanks with solvent soaked paper to provide an atmosphere saturated with solvent vapour. Remember to label TLC tanks accordingly.
3. Cover with a glass plate and leave to equilibrate for 15 minutes.
4. Cut TLC plates to a suitable size to accommodate at least 4 spots then draw a pencil line about 1 cm from the bottom edge.
5. Dissolve a small amount of each drug powder in methanol.
6. Apply 1-2 μl spots of acid-neutral extract and dissolved powder along the pencil line of plate A.
7. Run plate A in TLC Tank A.
8. Apply 1-2 μl spots of basic extract and dissolved powder along the pencil line of plate B.
9. Run plate B in TLC Tank B.
10. Allow the spots to dry thoroughly before placing the plate in the TLC tank.
11. Allow the solvent to migrate to within 1 cm of the top of the plate and mark the solvent front.
12. Air-dry the plate.

13. When examining a plate under UV light, the components show up as dark spots against a white background. Mark any spots with a pencil. Never look at the UV light directly.
14. Match the drug sample with the extracted samples. Measure the Rf (Retention Times) for all observed spots, where

$$R_f = \frac{\text{distance a solute migrates up plate}}{\text{distance the solvent front migrates up plate}}$$

15. Compare the Rf values obtained from the unknown drug substances and the plasma extracts using the two solvent systems.

Thinking about Making a Valid Measurement

How far apart do the spots need to be for the analyst to be confident that adequate separation has been achieved? What experiments could be performed to check your prediction?

Improving Your Practical Technique

- Work in a clean environment to avoid contamination.
- Only handle the TLC plate by its top corner and only lay the TLC plate down on a clean surface.
- Put identifying marks on the top of the TLC plate using a pencil.
- Consider doing duplicate samples.
- Prepare a relatively concentrated solution from the original samples otherwise you will not be able to see minor components on the TLC plate.
- Select a solvent in which the sample dissolves.
- Use reference materials (e.g. a pure substance) to help to identify the components in the sample.
- The intensity of the spots can be used as a crude guide as to the relative amount of components in the sample.

B2: Analysis of Plasma Extracts and Drug samples by HPLC

Safety Notes:
The compounds used in these experiments as "unknown" samples are drug compounds and care must be taken to avoid ingestion and skin contact. The plasma solutions must be treated as biological hazards and all work must be carried out using gloves to avoid any contact. All biological wastes and contaminated glassware must be sterilised chemically after use (see technician).

Note the detector contains a UV source which must not be viewed directly

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Apparatus

Reversed-phase HPLC system consisting of:

a Autosampler
b ODS (octadecyl silyl-) bonded silica column
c Pump set to deliver at 1 ml/min
d Mobile phase - methanol : water, 50:50
e Variable wavelength UV detector
f Integrator and printer

Method

1. Check there is sufficient mobile phase in the sample reservoir and start the pump at 1 ml/min. Check the outlet tube is placed in the outlet bottle.
2. The detector should already be turned on and set to 254 nm. Adjust the zero control so that an absorbance reading close to zero is displayed.
3. Samples were prepared by diluting an aliquot of the plasma extract or drug sample with mobile phase and pipetting into vials. Load samples into autosampler taking note of the position and sample number.
4. Follow the printed instructions next to the instrument (or consult a demonstrator) and start the analysis. Record the chromatograms.
5. Compare the retention times of the peaks observed for the various samples and see if either of the plasma extracts correlate with any of the drug samples. At the end of the lab session, turn off the pump and detector, but PLEASE LEAVE THE INTEGRATOR SWITCHED ON.

Thinking About Making A Valid Measurement

You analyse a sample and get a peak at the same retention time as the standard.

How confident are you that the peak from the sample is the same as the standard?
Why are HPLC systems with mass spectrometer detectors becoming popular?

Improving Your Technique

- Choose a suitable column and mobile phase for the analysis.
- Use HPLC grade solvents.
- Mobile phase should be free of dust and dissolved gases removed - always use a solvent reservoir filter.
- Ensure there is sufficient mobile phase to complete the analysis.
- The sample to be analysed needs to be soluble in the mobile phase.
- Never use a syringe with a sharp tip because it will damage the injector.
- Investigate flow rate fluctuations because these may be due to blockages or leaks within the system.
- After use, always flush water, buffered solutions, acids etc. out of the system using an appropriate solvent.

B3: Analysis of Plasma Extracts by GC-MS

The plasma extracts have been analysed by gas chromatography-mass spectrometry (GC-MS) and the data is available for you to inspect via a computer in the laboratory.

A demonstrator will show you how to access the files, and:

- Study the chromatograms
- Study the mass spectra of the chromatographic peaks
- Compare the mass spectra with a library of mass spectral data, to identify any drug(s) in the plasma.

C: Drug Identification

The drug powders of interest are identified by infrared spectroscopy by comparison of spectra with a database held on computer and by UV-Visible Spectroscopy.

C1: Identification of Drug Type by UV-Visible Spectroscopy

Safety Notes:
The compounds used in these experiments as "unknown" samples are drug compounds and care must be taken to avoid ingestion and skin contact.

The drug samples are suspected to be barbiturate. This is a quick method of determining the type of barbiturate present.

Apparatus

UV-Vis Spectrometer
5 ml pipettes
100 ml volumetric flasks
100 ml beakers
Matched UV Silica Cells
pH paper

Reagents

Drug samples – 10 mg dissolved in 1ml methanol and made up to 10 ml with water
Distilled or deionised water
0.5 M sodium hydroxide
16% ammonium chloride
50% sulphuric acid

Procedure

1. Dilute drug solution 1:25 with water.
2. Take 2 ml of this dilute sample solution, and add 2 ml 0.5 M sodium hydroxide (pH=13).
3. Scan this solution against 0.5 M sodium hydroxide as the blank between 200 and 350 nm.
4. Determine the absorbance maximum wavelength ($_{max}$).
5. Take a further 2 ml of the dilute sample solution.
6. Add 1.5 ml 0.5M sodium hydroxide and 0.5 ml 16% ammonium chloride solution (pH=10).
7. Rescan at this lower pH of 10. Note any change in $_{max}$
8. Add 0.5ml 50% sulphuric acid to pH 13 sample to give pH <2.
9. Rescan, noting any change in max.

Thinking about making valid measurements

To give evidence in court you have to show that the analytical method is producing valid results. Are you certain that any sample pre-treatment has not invalidated the analysis? How did you ensure the integrity of the components?

Table 2: Ultraviolet spectrometry of the weak acid fraction. Wavelength of absorbance maxima (nm).

Compound	pH13	pH10	pH<2
Chlorpropamide	232	-	232
N-methyl substituted barbiturates	246	246	-
5,5-substituted barbiturates	254	239	-
Paracetamol	257	-	245
Phenylbutazone	264	264	237
Thiol substituted barbiturates	303	-	285

Improving your practical technique

- The instrument should be allowed to warm up before use.
- A wavelength check should be carried out to check the instrument is correctly set up.
- Instrument needs to be calibrated using a set of standards of differing concentration for quantitative analysis.
- Cells must be kept scrupulously clean, in particular the region where the light beam passes through the cell.
- There can be subtle differences in the cell shape, therefore always put the cell the same way round when performing quantitative measurements.
- If practicable, use matched cells or the same cell throughout the series of analyses.
- Air bubbles in the cell cause serious problems. Samples may have to be degassed before analysis.
- Solid material must be removed from the sample before analysis.
- There is an absorbance value (above 1 is not recommended) above which the Beer Lambert law no longer applies.
- There is also an absorbance below which the absorbance values are unreliable.
- If used ,an auto-flow cell sampler need to be checked : Is liquid being drawn up at reasonable speed? Is liquid exiting cell chamber at suitable speed? Has waste container been emptied?
- It may be appropriate to use a double beam spectrophotometer and to have a cell containing solvent in the reference beam.
- Measurements can be easily repeated – there should be not significant differences between the readings (e.g. $< \pm 0.003$ absorbance units). Significant differences should be investigated.
- Consider doing duplicate samples.
- Analyse a blank sample (i.e. solvent blank).
- In many cases, samples may need to be diluted so that the measured absorbance is between the lowest and highest standard on the calibration curve.
- You may need to perform a wavelength scan to determine the λ(max).
- Qualitative identification by UV-Vis is very crude, where practicable use a more specific technique such as infrared spectroscopy.

C2: Identification of Drug samples by infrared spectroscopy

Safety Notes:
The compounds used in these experiments as "unknown" samples are drug compounds and care must be taken to avoid ingestion and skin contact.

Introduction

To obtain an infrared spectrum, it is necessary to prepare a solid sample appropriately. In this case you will prepare potassium bromide discs of the unknown drug samples. The KBr disc has the advantage (over Nujol mulls) that the spectrum should contain no interfering peaks (apart from water from poorly dried sample or KBr). Consult a demonstrator for guidance on the preparation of a potassium bromide disc if you are unfamiliar with the procedure outlined below.

Procedure

1. Ensure that the instrument is switched on and that it has warmed up for at least a few minutes.
2. Make sure that the sample compartment is empty and close the lid.
3. Select the number of scans (usually ten is adequate for routine work).
4. Select ‘background’ on the menu and scan.

Preparation of KBr Disk

1. Take the spectroscopic grade KBr from the oven and cool in the desiccator.
2. Using an agate mortar and pestle grind a few mg of the drug sample to a fine powder then mix with about a 10-fold excess of dry KBr and grind the mixture to give a well-mixed powder. Keep the mixture as dry as possible during this process.
3. Check that the ‘IR Die Kit’ is complete (plunger, top and bottom die, a die holder and an anvil.
4. Press the die holder onto the anvil ensuring a proper fit.
5. Lower the base die, dull side down into the die holder to a depth of about 50-mm.
6. Place sufficient of the ground sample/KBr mixture to completely cover the polished surface of the die and tap gently to produce an even layer on the base die.
7. Lower the top die (polished side down) onto the powder.
8. Slide the plunger onto the die and press gently so the die slides to the bottom.
9. Place the assembled ‘IR Die Kit’ into the hydraulic press and tighten the top screw until it touches the top of the plunger.
10. Connect the anvil to the vacuum pump.
11. Close the hydraulic release valve on the side of the press and gently pump the handle until the pressure gauge reads between 8 and 10 tons and leave for 30 seconds.
12. Open the hydraulic release valve gently and disconnect the vacuum from the anvil when the pressure falls to zero.
13. Loosen the top screw and remove from the press.

14. Turn the 'IR Die Kit' upside down and carefully remove the anvil.
15. CAREFULLY remove the disc from the press and transfer to a sample holder.
16. Clean the 'IR Die Kit' components with a tissue and check that all parts are present. Take care not to scratch the polished surfaces.
17. If the dies or plunger stick in the die holder contact the instructor.

Running the Spectrum

1. Place sample into the beam of the FT-IR spectrometer and scan the spectrum from 4000 cm^{-1} to 600 cm^{-1}.
2. Annotate the spectrum with name, date, compound, and phase (in this case KBr Disk).
3. Having obtained the infrared spectra, use the IR Viewmaster computer program to identify the unknown drugs. Consult the detailed instructions on the use of this program next to the computer.

How to Interpret an IR Spectrum

1. Note the conditions under which the spectrum was obtained.
2. Remember that the absence of peaks may be as useful as the presence of peaks.
3. Do not attempt to identify all the peaks. Go for the large peaks first.
4. Many sharp peaks of medium or strong intensity throughout the spectrum generally indicate an aromatic compound.
5. Examine 4000-2000 cm^{-1}

Table 3: Region 1 (4000-2000 cm^{-1})

Wavenumber **(cm^{-1})**	**Bond**	**Notes**
3600-3300	O–H	Alcohols, phenols
3500-3300	N–H	Amines, amides
3100-3000	C–H	Aromatic rings
2980-2800	C–H	Unsaturated C-H (e.g. alkanes) for CH3, CH2 and CH
2280-2200	C≡N	Nitriles

6. Examine 2000-1500 cm-1

Table 4: Region 2 (2000-1500 cm^{-1})

Wavenumber **(cm^{-1})**	**Bond**	**Notes**
1760-1690	C=O	The most intense band in this region. Indicative of aldehydes, ketones, esters etc.
1680-1610	C=C	Alkenes (less intense and sharper than C=O)
1360-1180	C–N	Amines, amides
1750-1650	N=O	Intense and sharp band with second band in region 3

7. Examine 1500-650 cm-1

Table 5: Region 2: the fingerprint region (1500-650 cm^{-1})

Wavenumber (cm^{-1})	**Bond**	**Notes**
1460	CH_3	Medium intensity peaks.
1370	CH_2	Medium intensity peaks

8. Tabulate your results and make the appropriate deductions after consulting a detailed correlation table.

Thinking about Making Valid Measurements

Infrared spectroscopy is a technique often used to identify organic substances. Why is it a more useful technique than ultraviolet spectroscopy?

- Large peaks below the bottom of the chart or with 'squared tips' indicate that the sample is too thick or concentrated so a new KBr disk needs to be produced.
- If the spectrum is weak and very noisy there is not enough sample. The KBr disk needs to be remade.
- If the baseline cannot be adjusted to 90% transmittance then the NaCl or KBr disk is fogged or scratched and needs to be replaced or remade.

Improving Your Practical Technique

- The instrument should be allowed to warm up before use.
- A wavelength check should be carried out to check the instrument is correctly set up using a polystyrene standard film.

C3: Analysis of Plasma Extracts by GC-MS

The plasma extracts have been analysed by gas chromatography-mass spectrometry (GC-MS) and the data is available for you to inspect via a computer in the laboratory.

A demonstrator will show you how to access the files, and:

- Study the chromatograms.
- Study the mass spectra of the chromatographic peaks.
- Compare the mass spectra with a library of mass spectral data, to identify any drug(s) in the plasma.

D: Alcohol and suicide note

You are supplied with a blood sample from the deceased for determination of alcohol (ethanol) content. This is done by headspace GC using propan-1-ol as an internal standard.

Examine a note allegedly written by the deceased shortly before she died. You are looking for signs of alteration and in particular whether more than one pen may have been used.

Examination with the naked eye and under a microscope is followed by analysis of samples of the ink and comparison of these with ink from pens found in the house.

D1: Determination of Alcohol in Serum by Headspace GC

The alcohols used in this experiment pose little hazard provided that routine laboratory precautions are taken to avoid ingestion or undue skin contact. Take care with the injection syringe to avoid puncturing the skin.

Note that the GC injection port is heated and could cause burns. Do not touch electrical connections. Ensure that the detector flame is ignited if hydrogen gas is flowing.

The serum samples must be treated as biological hazards and all work must be carried out using gloves to avoid any contact. All biological wastes and contaminated glassware must be placed in the available Biohazard containers for sterilisation/disposal.

Introduction

The use of an internal standard (where you measure the ethanol peak area *relative to the internal standard peak area* (in this case propan-1-ol)) minimises errors due to variation in injection volume. Temperature of the vapour sampled should be kept as constant as possible.

Apparatus

Gas chromatograph and capillary column
Integrator/printer
Micropipettes
500 µl headspace syringes
Headspace vials with caps

Reagents

Stock solution of ethanol: 5% w/v (5g in 100-ml de-ionised water)
Solution of propan-1-ol: 0.1% (internal standard)

Preparation of Samples and Standards

1. Prepare a series of standards containing 50, 100, 150 and 200-mg ethanol per 100 ml of water by diluting the stock ethanol solution.
2. Using a micropipette, transfer 250 µl of the propan-1-ol solution into each of eight headspace vials.
3. Transfer 250 µl aliquots of the four prepared ethanol standards into the first four vials, two 250 µl aliquots of the serum sample into vials 5 and 6 and two 250 µl aliquots of de-ionised water into vials 7 & 8 to act as a blank.
4. Label all vials clearly.
5. Seal each vial with septum and screw cap and place all the vials in the GC oven on the left bench as you enter the GC lab. This should be set to 40°C.
6. Leave the vials to equilibrate for about 15 min.

GC Analysis

1. The 500 µl headspace syringes must be prepared by pre-heating in oven and not washed with solvent.
2. Inject 500 µl from vial 7 (the blank) in a rapid and smooth motion into the GC and simultaneously press the 'Inj. A' button on the integrator.
3. Only one peak from the internal standard (the propan-1-ol) should be observed. If more than one peak is observed then repeat using Vial 8 (blank).
4. Repeat the procedure for all the standards and samples (vials 1-6).
5. From the integrator printout, you will see that each standard and sample has two peaks. The first (lower retention time) is ethanol and the second is propan-1-ol.
6. Calculate the ethanol: propan-1-ol peak area ratio for each standard.
7. Plot a calibration graph of

$$\frac{\text{Peak Area of ethanol}}{\text{Peak Area of propan-1-ol}} \text{ vs Concentration of ethanol.}$$

8. Determine the ethanol concentration in the plasma sample.

Thinking about Making Valid Measurements

At what sample concentration would you be confident (e.g. 95%) that the amount measured exceeds 80 mg per 100 ml. What experiment could you perform to check your prediction?

Improving Your Practical Technique

- Work in a clean environment to avoid contamination.
- Alcohol is a volatile solvent – keep solutions stoppered when not in use to prevent evaporation.
- Syringe technique can make significant difference to the volume injected into the GC so replicate injection should be carried out until good reproducibility is achieved.
- Remember that volumetric flasks are calibrated for solutions at 20 ºC when making up solutions to the mark.
- Chromatographs should be labelled and dated as they are produced.
- Always establish a good flat baseline with reagent blank.
- Run standards at the beginning and end of the run.
- Check calibration using one of the standards.
- Consider running the sample in duplicate or triplicate.

D2: Extraction of inks and separation by TLC of the Suicide Note

The chemicals used in this experiment pose little hazard provided that routine laboratory precautions are taken to avoid ingestion and skin contact. Care should be taken when handling and disposing of flammable organic solvents. When using an UV lamp, do not look directly at the lamp under any circumstances.

Sample

Various pens and 'suicide' note

Reagents

Ethanol

Mobile Phases

5:3:2 ethyl acetate: ethanol: water

Apparatus

Test tubes
Test tube rack
Silica Gel GF254 TLC plates
Two TLC tanks (large beakers)
Filter paper

Visual examination of the suspect note and pens

1. Using each of the supplied pens, write specimen words on a sheet similar to that used for the note.
2. Using a low power microscope, compare these specimens with the writing on the note.
3. Look at colour of ink and thickness of lines and any other visible characteristics, such as blotchiness etc.
4. Identify which pens give marks of similar appearance to the writing on the note.
5. Try to identify any areas of the note that show signs of possible alteration.

Extraction of ink

1. Having identified areas of the note worthy of further scrutiny, carefully cut out several letters and place in small glass sample tubes.
2. Also transfer to a tube a similar-sized piece from a control section of paper free of ink to act as a blank.
3. Add 1–2 drops of ethanol to each tube.
4. Allow 15 minutes for the ink to dissolve.
5. Perform the same extraction as above with each of the specimen inks of similar colour to those on the note.

Procedure

1. Make up the eluent ethyl acetate : ethanol : water (5:3:2).
2. Pour into the chromatographic tank to a depth of less than 1 cm.
3. Cover the tank and leave to equilibrate with the solvent for about 20 minutes.
4. Cut the aluminium-backed silica gel with fluorescent indicator to appropriate size.
5. Mark the base line approx. 1-cm from the bottom of the plate with a pencil.
6. Using a narrow capillary tube, apply a small spot of each ink solution plus the blank, spacing the spots at equal distances along the base line of the TLC plate.
7. Allow to dry in the fume cupboard.
8. Stand the plate in the tank and develop it, allowing solvent to migrate to within 1 cm of the top edge of the plate.
9. Mark the solvent front with a pencil.
10. Air-dry the plate in the fume cupboard.
11. Note the appearance and positions of all spots and check under the UV lamp (take care not to look at the lamp directly) as well as any matching and non-matching inks.
12. If more than one pen was used to write the note, draw conclusions as to whether the note has been altered. If so, are you able to decipher the text of the original note?

Thinking about Making a Valid Measurement

How far apart do the spots need to be for the analyst to be confident that adequate separation has been achieved? What experiments could be performed to check your prediction?

Improving Your Practical Technique

- Work in a clean environment to avoid contamination.
- Only handle the TLC plate by its top corner.
- Only lay the TLC plate down on a clean surface.
- Put identifying marks on the top of the TLC plate using a pencil.
- Consider doing duplicate samples.
- Prepare a relatively concentrated solution from the original samples otherwise you will not be able to see minor components on the TLC plate.
- Cover the tank and line it with solvent soaked paper to provide an atmosphere saturated with solvent vapour.
- Select a solvent in which the solvent dissolves.
- Use reference materials (e.g. a pure substance) to help to identify the components in the sample.
- Allow the spots to dry before putting the spotted TLC plate in the tank.
- When examining a plate under UV light, the components show up as dark spots against a white background. Never look at the UV light directly.
- The intensity of the spots can be used as a crude guide as to the relative amount of components in the sample.

Appendix A

Weighing Liquids Accurately

1. Close all the doors of the balance and check that the spirit level is centred.
2. Tare the four-place balance.
3. Weigh volumetric flask and record the weight.
4. Take volumetric flask out of the balance.
5. Tare the balance.
6. Replace the volumetric flask on the balance.
7. Using a Pasteur pipette add the required amount of liquid.
8. Take volumetric flask out of the balance
9. Tare the balance.
10. Replace the volumetric flask on balance
11. Record the weight.

Weighing Accurately an Approximate Weight of Solid

Weighing by difference.

1. Close all the doors of the balance and check that the spirit level is centred.
2. Place weighing boat on balance.
3. Tare the four-place balance.
4. Add the required amount of solid.
5. Record the weight (weighing boat + sample).
6. Remove the weighing boat from balance.
7. Transfer the solid to the volumetric flask.
8. Tare the balance.
9. Replace the weighing boat on balance.
10. Record the weight of weighing boat plus any sample remaining.
11. Subtract the weight in ‘10’ from that in ‘5’ to give weight of sample.

Quantitative Transfer Weighing Solids Method

This has the disadvantage that any spillage requires starting again and that some solids are not easy to wash out of the weighing boat.

1. Close all the doors of the balance and check that the spirit level is centred.
2. Tare the four-place balance.
3. Place weighing boat on balance.
4. Record the weight (weighing boat).
5. Add the required amount of solid.
6. Record the weight (weighing boat + sample).

7. Quantitatively transfer the contents of the weighing boat by washing the contents into the volumetric flask.
8. Subtract weight ‘4’ from ‘6.’

Drawing a Graph

1. Collect all of the data in tabular form.
2. Decide on the most appropriate form of presentation that may include transformation to convert the data to linear form.
3. Choose a concise descriptive title together with a figure number.
4. The measured quantity (e.g. absorbance, peak area etc.) should be plotted on the y-axis and the known quantity (e.g. concentration) on the x-axis.
5. Determine which variable is to be plotted on the x-axis and which the y-axis.
6. Select appropriate scales for both axes and make sure that the numbers and the scale marks are clearly shown.
7. Decide appropriate descriptive labels for both axes with SI units where appropriate.
8. Calculate by linear regression the best-fit line.
9. Plot the points and draw the best-fit line.
10. Write a figure legend to include a key that identifies all the symbols as required.