CHEMICAL FOOD ANALYSIS
A PRACTICAL MANUAL

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These 8 practical exercises will take 10 weeks. There are no practical exercises scheduled in Week 1.

Course References

The following references are a list of chosen references available in UQ libraries that may be useful for preparing for practicals and for practical report preparation. Some of the references listed below are specifically highlighted as part of particular practical exercises.

NOTE, the references listed below or highlighted in each practical exercise are not meant to be exhaustive, and students should make use of the UQ libraries for finding other references of importance. Do not just rely on the references mentioned in this practical manual.

Since you have 2 weeks to write your final reports, then all UQ libraries become accessible, including the Gatton campus library.

AOAC International. (1995). 'Official Methods of Analysis'. 16th Ed.
(AOAC International: Gaithersburg, MD)

Aurand, L. W., Woods, A. E. and Well, M. R. (1987). 'Food Composition and Analysis'.
(AVI: New York).

Australian and New Zealand Food Standards Code (available in UQ Biological Sciences Library).

Australian Food Composition Tables (available in UQ Biological Sciences Library).

Belitz, H. D. and Grosch, W. (1999) 'Food Chemistry' 2nd Ed.
(Springer-Verlag : New York).

Charalambous, G. (1990) 'Flavors and Off-Flavours'.
(Elsevier: New York).

Christian, G.D. (1994) 'Analytical Chemistry' 5th Edition
(John Wiley and Sons Inc. : New York, USA)

Clark, N. (1992). 'Food Chemistry'.
(Food Trade press : Westerham, England)

Coultate, T. P. (1996). 'Food - The Chemistry of its Components' 3rd Ed.
(Royal Society of Chemistry: London).

D'Arcy, B.R. 'Web Notes for Food Chemistry and Analysis':

http://www.fst.uq.edu.au/staff/bdarcy/food2001/food2001content.htm

http://www.fst.uq.edu.au/staff/bdarcy/food2002/food2002content.htm

de Mann, J. M. (1999) 'Principles of Food Chemistry' 3rd Ed.
(Aspen Publishers, Inc.: Gaithersburg, Maryland, USA).

Dewdney, P. A., Burke, C. S. and Crawford, C. (1975). 'Commercial Use of Enzymes in the Food Industry'.
(BFMIRA : Leatherhead, Surrey)

Dickinson, E. and Stainsby, G. (1982). 'Colloids in Foods'.
(Applied Science : London).

Fennema, O. (1996). 'Food Chemistry' 3rd ed.
(Marcel Dekker: N.Y.)

Gruenwedel, D. W. and Whitaker, J. R. (1984) 'Food Analysis: Principles and Techniques, Vol. 1 and 2'.
(Marcel Dekker: New York).

Heimann, W. (1980). 'Fundamentals of Food Chemistry'.
(Ellis Horwood: Chichester, England).

Holland, B., Welch, A. A., Unwin, I. D., Buss. D. H., Paul, A. A. and Southgate, D. A. T. (1991) 'McCance and Widdowson's The Composition of Foods'.
(Royal Society of Chemistry: Cambridge).

James, C.S. (1995) 'Analytical Chemistry of Foods'
(Blackie Academic and Professional: London)

Kirk, R. S and Sawyer, R. (1991) 'Pearson's Chemical Analysis of Foods' 9th Ed.
(Longman Scientific and Technical; Harlow, Essex, UK).

Lawrence, J. R. (1984). 'Food Constituents and Food Residues: Their Chromatographic Determination'.
(Marcel Dekker: New York).

Lee, F. A. (1983). 'Basic Food Chemistry' 2nd Ed.
(AVI: Westport, Conn.).

Maarse, H. and Van Der Heij, D. G. (1994) 'Trends in Flavour Research'.
(Elsevier: New York).

Maynard, (1970). 'Methods in Food Analysis', 2nd Ed.
(Academic Press, London).

Miller, D. D. (1998) 'Food Chemistry: A laboratory manual'.
(John Wiley & Sons. Inc. : New York, USA).

Morton, I. D. and McLeod, A. J. (1982). 'Food Flavours - Part A – Introduction'.
(Elsevier: Amsterdam).

Nielsen, S. S. (ed.) (1998) 'Food Analysis' 2nd Ed.
(Aspen Publishers, Inc.: Gaithersburg, Maryland, USA).

Paul, A. A. and Southgate, D. A. T. (1988). 'The Composition of Foods' 4th Rev. Ed.
(Elsevier: Amsterdam).

Pomeranz, Y. and Meloan, C. E. (1994) 'Food Analysis: Theory and Practice' 3rd Ed.
(Chapman and Hall: New York, USA).

Robinson, D. S. (1987). 'Food-biochemistry and nutritional value'.
(Longman: London).

Schwimmer, S. (1981). 'Source Book of Food Enzymology'.
(A. V. I. : Westport, Conn.).

Skoog, D.A. and Leary, J.J. (1992). 'Principles of Instrumental Analysis'.
4th Edition. (Saunders College Publishing: New York)

Skoog, D. A. and West, D. M. (1986). 'Principles of Instrumental Analysis'.
(Holt, Rinehart and Winston, Inc.: New York).

Souci, S.W. Fauchman, W. and Kraut, H. (2000) 'Food Composition and Nutrition Tables'.
6^{th} Edition.
(Medpharm Scientific Publishers: Stuttgart).

Wong, D. W. S. (1989). 'Mechanism and Theory in Food Chemistry'.
(Van Nostrand Reinhold : New York).

PRACTICAL EXERCISE NO. 1

DETERMINATION OF HYDROXYMETHYLFURFURAL IN HONEY

1.1 INTRODUCTION

The presence in honey of hydroxymethylfurfural (HMF) was originally considered as evidence of its adulteration with acid-converted invert syrup. More recently, HMF content has been widely used as an indication of the heating history of honey. It has also become apparent that HMF can be produced in appreciable amounts in honey stored at ambient temperatures. Many honey standards set maxima for HMF in honey. Average levels approximately 5 mg/100 g.

HMF can be determined by a method (White, 1979) in which the UV absorbance of a clarified aqueous honey solution is determined against a reference solution of the same honey in which the 284 nm chromophore of HMF is destroyed by bisulphite. **See Problem set on the Chemical Food Analysis Web site.**

Destruction of the chromophore eliminates the background absorption of the honey. The difference spectrum between sample (without bisulphite) and reference (with bisulphite) closely resembles the HMF absorption band between 250 and 330 nm (with a maximum at 284 nm) and allows easy quantification of the HMF content using the literature value for the absorptivity of HMF. The method which follows is taken from that of White (1979).

1.2 METHOD

NOTE: Use only clean glassware and do not contaminate solutions or use the same pipette for more than one solution.

1.2.1 Reagents and Apparatus

(a) Carrez solution I: Dissolve 15 g potassium ferrocyanide $[K_4Fe(CN)_6.3H_2O]$ in water and dilute to 100 mL.

(b) Carrez solution II: Dissolve 30 g zinc acetate $[Zn\ (CH_3CO_2)_2.2H_2O]$ in water and dilute to 100 mL.

(c) Sodium bisulphite ($NaHSO_3$): 0.20% in water. Prepare fresh daily. Technical grade is adequate.

(d) Recording spectrophotometer.

1.2.2 Procedure

Dissolve honey (5 g; weighed to the nearest 1 mg in small beaker) in water (25 mL) and quantitatively transfer to a 50 mL volumetric flask (including washing the residue in the beaker with small amounts of water). Add 0.50 mL Carrez solution I, mix, add 0.50 mL Carrez solution II, mix, and dilute to volume with water. A drop of alcohol may be added to suppress surface foam. Filter through paper rejecting the first 10 mL filtrate.

Pipette 5 mL filtrate into each of two 18 x 150 mm test tubes and then pipette 5 mL water into one (sample) and 5 mL 0.20% bisulphite into the other (reference). Mix well (vortex mixer) and determine the absorbance of the sample against the reference in 1 cm cells at 284 nm and 336 nm using **quartz cells.**

If the absorbance is too high for accuracy (>0.6), dilute the sample solution as needed with water (probably 1:1) and the reference solution to the same extent with 0.1% $NaHSO_3$.

Multiply the absorbance values by the appropriate dilution factor before calculation.

1.2.3 Calculation

$$\text{HMF (mg/100 g honey)} = \frac{(A284 - A336) \times 74.87}{W}$$

where W = wt of honey sample (g)

A284, A336 = absorbance readings

$$\text{Factor} = \frac{126 \times 100 \times 1000 \times 100}{16830 \times 1000} = 74.87$$

where 126 = MW of HMF
16830 = molar absorptivity of HMF at 284 nm

1.3 QUESTIONS

1.3.1 Determine the HMF content of the honey samples provided.
Compare the values obtained with literature values and comment on the result.

1.3.2 What is a chromophore? How does chemical structure affect the ability of a chromophore to absorb light at various wavelengths?

1.3.3 Draw a schematic diagram of a UV - VIS spectrophotometer and briefly describe the function of all the components. A photocopy is not acceptable.

1.4 REFERENCES

Crane, E. (1990). 'Bees and Beekeeping'. (Heinemann Newnes: Oxford).

Pomeranz, Y. and Clifton, M. (1994). 'Instrumental Food Analysis'. 3rd ed. (Chapman & Hall: New York).

White, J.W. (1979). 'Journal of the Association of Official Analytical Chemists' Vol 62, No. 3, p. 509.

PRACTICAL EXERCISE NO. 2

DIASTATIC ACTIVITY OF HONEY

2.1 INTRODUCTION

The main constituents of honey are water, glucose, fructose, sucrose, mineral matter and proteins. A number of enzymes are also present including diastase (amylase) and invertase. Diastase, being more heat sensitive than invertase, has been used by honey importers as an indicator of overheating of honey, ie. if the diastase activity is below a defined level, the honey has received unacceptably high heat treatment during its processing.

EEC regulations prescribe the Diastase Activity to be not less than 4 DN units where;

1 DN unit = 1 mL of 1% starch hydrolysed by the diastase in 1 g of honey in 1 hour at 40°C.

In this practical exercise the diastase activity of a honey sample will be determined and the effect of heating of the honey on diastase activity will be investigated.

The principle of the method (AOAC Methods (1980)) is that a buffered mixture of soluble starch and honey solution is incubated and the time required to reach a specified end point (as defined by the absorbance of starch-iodine complex at **660 nm**) is determined spectrophotometrically. Results are expressed as mL of 1% starch hydrolysed by the enzyme in 1 g of honey in 1 hour (Diastase Number Gothe scale).

2.2 REAGENTS

2.2.1 Iodine stock solution Dissolve 8.80 g Iodine (highly corrosive - caution) in 30-40 mL H_2O containing 22.0 g KI, and dilute to 1 L with distilled H_2O.

2.2.2 **Iodine solution** (0.00035M) Dissolve 20 g KI and 5.00 mL Iodine stock solution in H_2O and dilute to 500 mL. **Prepare fresh for each practical day.**

2.2.3 **Acetate buffer solution** (pH 5.3; 1.59M). Dissolve 87 g of sodium acetate (NaOAc. $3H_2O$) in 400 mL H_2O, add about 10.5 mL acetic acid and dilute to 500 mL. Adjust pH to 5.3 with sodium acetate or acetic acid if necessary.

2.2.4 **Sodium Chloride solution** (0.5M). Dissolve 14.5 g NaCl in H_2O and dilute to 500 mL.

2.2.5 **Starch solution**. Weigh 2.000 g soluble starch and mix with 90 mL H_2O in a 250 mL conical flask. Rapidly bring the mixture to boiling point, swirling the solution as much as possible. Reduce the heat and boil gently for 3 minutes, cover, and let cool to room temperature. Transfer to a 100 mL volumetric flask and dilute to volume. Observe the details of the preparation closely to limit the variation in the absorbance values of the starch-iodine blank.

2.3 METHOD

2.3.1 Standardization of the Starch Solution.

Pipette 5 mL starch solution into 10 mL water and mix well. Pipette 1 mL of this solution with a serological pipette into several 50 mL graduated measuring cylinders containing 10 mL of dilute iodine solution (0.00035M). Mix well, and determine the H_2O dilution necessary to produce an absorbance value of 0.76+0.02 at **660 nm** in 1 cm cells (water blank). (NOTE: It should be necessary to add water to a final volume of about 45-50 mL to give the required absorbance). The standardization should be repeated when changing the starch source.

2.3.2 Determination of Diastase Activity.

Weigh 5.0 g **fresh** honey sample into a small beaker, dissolve it in 20-30 mL distilled water at room temperature and 5.0 mL acetate buffer solution, and transfer to 100 mL volumetric flask containing 3.0 mL NaCl solution. Dilute to volume with H_2O. (NOTE: The honey solution must be buffered before addition to the NaCl solution).

Pipette 10 mL of the above honey sample solution and 5 mL starch solution prepared in (Section 2.2.5) into two **separate** clean dry 22 x 150 mm test tubes. Place both tubes in a water bath at **40°C** for 15 minutes to equilibrate, then mix the two by pouring the contents of the tubes back and forth several times and replace into the 40 °C water bath. Start a stopwatch when mixing commences. After 2.5 minutes remove a 1 mL aliquot with a 1 mL serological pipette and add rapidly to 10.0 mL iodine solution in a 50 mL graduated measuring cylinder. Mix, dilute to the previously determined volume and determine the absorbance (A) in the spectrophotometer set at λ = 660 nm as described for the blank. Note the reaction time as being from the time of mixing of starch and honey to the time of addition of the aliquot to iodine. The serological pipette may be left in the test tube for subsequent use. Continue taking aliquots at 2.5 minute intervals until an absorbance value of < 0.235 is obtained.

Plot absorbance (A) against time (minutes) on rectilinear graph paper or using Excel. Draw a best-fit straight line through the starting absorbance value (5 minutes) and as many points as possible. From the graph determine the time (t minutes) for A to reach 0.235.

$$\text{Diastase Number (Gothe scale)} = \frac{300}{t}$$

If Time Permits:

2.3.3 **Effect of heating on Diastase activity.** Heat about 10 g honey in a clean dry beaker to 80°C in a boiling water bath. Hold at **80°C** for 5 minutes and cool to room temperature. Take 5.0 g of this heated honey sample and determine the Diastase Number as in Section 2.3.2.

2.4 RESULTS AND DISCUSSION

2.4.1 Compare the Diastase Number results with published values. Comment on the effect of heating on DN.

2.4.2 Explain why $DN = \frac{300}{t}$

2.4.3 What other methods can be used to determine overheating of honey? Comment on the validity of DN as a measure of overheating.

2.5 REFERENCES

Chandler, B.V. (1974). 'Composition of Australian Honeys' (CSIRO).

Crane, E. (1980). 'A Book of Honey'. (Oxford Univ. Press: Oxford).

Graham, J. M. (1992). 'The Hive and the Honey Bee'. (Dadent: Hamilton, Illinois)

Horwitz, W. (editor) (1980). 'Official Methods of Analysis of the Association of Official Analytical Chemists'. (13th ed). (AOAC: Washington, D.C.).

PRACTICAL EXERCISE NO. 3

INTRODUCTION TO ADVANCED CAPILLARY GAS CHROMATOGRAPHY (GC)

3.1 AIM

To become familiar with high resolution gas chromatography through hands-on experience.

3.2 SETTING UP THE INSTRUMENT - LINEAR FLOW VELOCITY

3.2.1 Determine the temperature to start injections of hexane (AR grade) based on the boiling point of hexane.

3.2.2 Inject 1 µl of hexane and measure its retention time in secs (t_m)

3.2.3 t_m is the dead time of the column.

Calculate µ, the linear flow velocity,

$$\mu = \frac{\text{length of column (cm)}}{t_m \text{ (sec)}} = \frac{L(cm)}{t_m(sec)}$$

µ = ...cm/sec

If µ is not in the appropriate range for the carrier gas being used:

ie., N_2 10 cm/sec He 25-30 cm/sec H_2 40-70 cm/sec

then adjust the column head pressure to change µ.

Re-inject and repeat the calculation of µ.

3.2.4 Convert the final **µ** into cm/min and calculate the column flow rate (F),

$$F \text{ (mL/min)} = \pi r^2 \mu = \pi r^2 L/t_m$$

where r = radius of column in cm

π = 3.141

NOTE: units ml/min

Example Calculation: μ = 21 cm/sec

d = 0.32 mm column

r = d / 2

$$F = \frac{\pi . (0.032 \text{ cm})^2 (21 \times 60 \text{ cm/min})}{2^2}$$

$$= 1 \text{ cm}^3/\text{min}$$

Now calculate the column flow rate for the GC system you are working on.

3.2.5 Measure the carrier gas flow rate at the split vent with a bubble flow meter.

Split vent flow rate = ...cm^3/min

Work out the split ratio for your GC system:

$$\textbf{Split ratio} = \frac{\textbf{Column Flow Rate + Split Vent Flow Rate}}{\textbf{Column Flow Rate}}$$

Adjust the split ratio knob to give a split ratio of approximately **50:1**

(This will not change the flow through column, ie., μ stays constant.)

Now record all the instrument parameters including the GC instrument name, type of carrier gas, injector mode, injector temperature, septum purge flow rate, detector temperature, column type and specifications, column head pressure and flow rates of the detector gases.

3.3 OPTIMIZING THE COLUMN TEMPERATURE

3.3.1 Inject 1 μL of the test sample provided which contains a hydrocarbon mixture of C_{12}, C_{14}, C_{16} and C_{18} in iso-octane.

3.3.2 Now optimize the column temperature to give good separation in less than 10 minutes (preferably in less than 5 minutes). This will involve adjusting the oven temperature prior to each new injection. Record the optimum temperature.

3.4 INJECTION REPRODUCIBILITY AND DETERMINING THE BEST INJECTION TECHNIQUE

Take up 1 µL of the above test mixture and perform the following injections:

- a fast injection, less than 1 second,
- a slow injection over 10 seconds.

What have you noticed about the individual peak widths with each injection?

Take up 1 µL of the above mixture and include an air gap before and after the sample plug. Perform the following injections:

- a fast injection, less than 1 second,
- a slow injection over 10 seconds.

Summarise the best injection technique based on what you have observed above. What are you trying to achieve?

3.5 DETERMINING COLUMN PERFORMANCE (EFFICIENCY)

Now that the GC conditions have been set up and the best injection technique has been determined, inject 1 µL of the above hydrocarbon test mixture. Calculate the theoretical plates of the column (column efficiency) using the last peak (C_{18}) to perform your calculations.

$$\textbf{Theoretical Plates (N)} = 5.54\ (t_r - t_m / W_{1/2H})^2$$

Repeat the calculation using the C_{16} peak.

3.6 DETERMINING THE RETENTION OR CAPACITY FACTOR, K'

Calculate the **k'** (capacity factor) for the C_{16} peak.

$$k' = \frac{t_r - t_m}{t_m}$$

3.7 DETERMINATION OF GC FINGERPRINT FOR FLAVOUR MIXTURES

Record the GC chromatogram for the food oils provided:

These should include lemon oil, orange oil, lime oil etc.

Basic Procedure for Installing Fused-Silica Capillary Columns

- Cool all heated zones and replace spent oxygen and moisture traps.
- Clean and deactivate injector and detector sleeves if necessary.
- Replace critical injector and detector seals.
- Replace the septum.
- Set detector and make-up gas flow rates.
- Carefully inspect the column for damage or breakage.
- Cut 10 cm from each column end using a ceramic scoring wager or sapphire scribe.
- Install a nut and an appropriately sized ferrule on both column ends.
- Cut an additional 10 cm from each column end to remove ferrule shards.
- Mount the capillary column in the oven using a bracket that does not allow the column to become scratched or abraded.
- Connect the column to the inlet at the distance indicated in the gas chromatograph's instrument manual.
- Set the approximate column flow rate by adjusting the head pressure (see the column manufacturer's literature).
- Set split-vent, septum-purge, and any other applicable inlet gases according to the instrument specifications.
- Confirm flow by immersing the column outlet in a vial of solvent such as acetone or methylene chloride.
- Connect the column to the detector at the distance indicated in the instrument manual.
- Check for leaks at the inlet and outlet using a thermal conductivity leak detector if available or use isopropanol and pasteur pipette.
- Do **not** use soaps or liquid-based leak detectors.
- Set the injector and detector temperatures and turn on the detector when temperatures have equilibrated.
- Do not exceed the phase's maximum operating temperature.
  Inject a non-retained substance to set the proper dead time.
- Check system integrity by ensuring that the dead-volume peak does not tail.
- Condition the column for 2 hours at the maximum operating temperature to stabilize the baseline. To determine the column's maximum temperature, consult the column manufacturer's literature.
- Run test mixtures to confirm proper installation and column performance.
- Calibrate the instrument and inject samples.

PRACTICAL EXERCISE NO. 4

METAL ANALYSIS OF FOODS USING ATOMIC ABSORPTION SPECTROSCOPY (AAS)

4.1 INTRODUCTION

The iron content of wine is important for wine makers because at high levels, iron may cause cloudiness or colour change. Levels of iron over 7-10 mg/L are generally associated with this haze formation (Amerine and Ough, 1974). Iron levels as low as 1.0 mg/L have resulted in reduced clarity. Iron is involved in the solubility and stability of certain protein fractions in wine. If the iron equilibria of wine is altered, the solubility of the protein is affected and protein-metal hazes form (Boulton et al., 1998). Iron in wine may accelerate oxidation. In white wine, colloidal ferric phosphate precipitates to form a milky white opaqueness. In red wines, the precipitation of ferric tannate forms a blue black film. Iron contamination occurs during storage in vessels from contact with fittings composed of iron or iron alloys (Amerine and Ough, 1974).

The enzyme polyphenol oxidase (phenolase) that is involved in the oxidation of polyphenols during the fermentation step of the black tea making process, contains copper as its prosthetic group. This fermentation step is very important for the development of the full flavour of black tea. Copper solutions are also sprayed on tea plants. Limits for copper content (dry weight basis) are set in countries such as the UK and Kenya (Macrae et al., 1993).

The classic methods for determining the major metals and anions, which include generally a digestion and/or ashing of the foodstuff followed by a gravimetric, titrimetric, or colorimetric analysis, have generally been sufficient for elements in rather large quantities, eg. calcium, magnesium, phosphates, and sulphates. However, to actually determine small differences in concentration of various metals, or, even more importantly, the presence and concentration of the trace minerals, the classical methods are not usually sufficiently sensitive to yield such information.

Atomic absorption spectroscopy provides one of the most useful and convenient means for the determination of metallic elements in solution from a wide variety of samples. The sample must be **solubilized** in aqueous or other solvents in order that it can be aspirated into the flame of the atomic absorption spectrophotometer. An alternative to this solubilization process is the use of the graphite or carbon rod furnace which is gaining wide acceptance for solid sampling.

The analysis of the metallic elements in foods such as beverages, eg. juices, beer, tea, coffee etc, is easily accomplished by direct aspiration into the atomic absorption flame or direct injection into the graphite furnace. However, foods such as animal and plant tissues, and fluids with considerable solids must be

treated to either decompose the solids or extract the metals. Ashing and/or wet digestion are the most commonly used methods. Ashing is usually performed at temperatures less than 500°C and digestion is usually accomplished by mixtures of nitric, sulphuric and perchloric acids.

In this exercise the copper content of tea and the iron content of wine will be assayed. The Australian and New Zealand Food Standards Code prescribes copper limits for several foods with a general limit of 10 mg/kg for all solid foods not specifically prescribed. Although copper is an oxidation catalyst in small amounts and is emetic in large doses, it is a necessary trace element in human nutrition.

When an aqueous solution of inorganic ions are aspirated into a flame they are reduced to an elemental state. Thus, within the flame is **"plasma"** containing a significant concentration of elemental particles.

Radiation from a suitable source, in this case a hollow cathode lamp, is passed through the flame containing the atomized sample after which the resultant beam of light enters the selective slit of the spectrophotometer. A blank is run, usually of the solvent used in the samples (distilled water). This allows a compensation factor to be determined to allow radiation from the flame to be accounted for in the absorbance readings.

In the flame plasma, the sample atoms absorb radiation at a wavelength characteristic to only atoms of a particular element. The wavelength (l) of the light beam produced by the hollow cathode lamp corresponds to the energy required to allow transition of electrons within the sample atoms from the ground state (E) to the excited states (E1, E2, E3). The resultant absorption in energy from the incident beam is detected by the spectrophotometer as a decrease in energy of the beam. The amount of energy absorbed by the sample atoms is proportional to the number of atoms within the flame and therefore the concentration of metal within the aspirated sample.

The absorbance value for a sample is determined by comparing the value obtained from the blank sample (distilled water) and the value obtained from the sample:

ABS (corr) = ABS (sample) - ABS (blank)

With very complex samples, the chemical and physical properties of standards prepared by normal methods may not match those of the sample. Under these circumstances, chemical and physical interferences may make it impossible to obtain accurate results. Provided that sufficient sample is available, the analysis can be performed using the **method of standard additions**. Note that it does not **overcome** interferences; it is designed to compensate for interferences by making the standards behave in the same manner as the sample. One method for compensating for background interference will also be investigated : selection of an alternative resonance line (wavelength).

Each student individually does both Parts A and B. However, each group will prepare only one set of 6 copper standards and one set of 5 iron standards (for external standards method only). Each student will then individually prepare 2 tea samples for copper analysis, 1 wine sample for iron analysis by external standard method, and 5 wine solutions for iron determination by the standard additions method.

PART A

Determination of Copper in Tea by AAS

4.2 AIM FOR PART A

To use the external standard method for determining the copper content in tea by Atomic Absorption Spectroscopy (AAS).

4.3 COPPER REAGENTS SUPPLIED

Stock Copper Solution: 100 ppm solution as commercially supplied.

4.4 PROCEDURE FOR COPPER DETERMINATION OF TEA USING THE EXTERNAL STANDARD METHOD

This practical exercise is to be performed individually.

4.4.1 Preparation of Copper Standards

Note: All glassware must be cleaned thoroughly prior to use to remove any contaminants.

By dilution (with distilled water) of the 100 ppm solution supplied, use **ONLY** a burette and volumetric flasks to prepare **100 mL each of 0, 1, 2, 3, and 4 ppm** Cu solutions. These are external standard solutions.

4.4.2 **Dry Ashing of Black Tea Leaves**: Ash 5 g of the dry food **(black tea leaves**) at 500-550^{o}C for 1 hour. After cooling, moisten the ash with 10 mL of 8M nitric acid and evaporate on a steam bath to near dryness. Then carefully and quantitatively transfer the moist render to a 50 mL volumetric flask and dilute to the mark with distilled water. Filter ready for analysis by AAS.

4.4.3 **Tea Infusion:** Prepare a tea infusion by adding 800 mL of boiling water to 10.0 g of tea leaves, and let stand for 5 minutes. Be sure to use the same black tea samples (same brand) as for the dry ashing determination. Cool, dilute to 1000 mL (in a volumetric flask) and filter ready for analysis by AAS.

4.4.4 Aspirate the Cu standards in turn, note the absorbance readings and construct a calibration graph of absorbance *versus* Cu concentration.

4.4.5 Aspirate the tea samples and determine the copper content (in mg/kg) of the tea leaves given to you. **Be sure to use the appropriate dilution factor when converting the final concentration (ppm or mg/L) obtained from your graph to the mass of Cu in the original tea leaves (mg/kg).**

Comment on the actual copper content of tea leaves (mg/kg dry weight) determined by dry ashing and the amount (mg/kg dry weight) determined using the infusion and why they are different? Comment on the percentage extraction by the hot water.

4.5 PROCEDURE FOR COPPER DETERMINATION IN A TEA INFUSION USING THE STANDARD ADDITIONS METHOD

4.5.1 Prepare the set of 7 standard addition solutions (**100 mL**) as follows:

Solution No.	Filtered Tea Infusion Solution (mL)	Spiking Solution 100 ppm Cu	*Final Conc. of Actual Added Cu [mg/L or ppm]
I	92	0 mL	**0.0**
II	92	0.2 mL	**0.2**
III	92	0.5 mL	**0.5**
IV	92	1 mL	**1.0**
V	92	2 mL	**2.0**
VI	92	3 mL	**3.0**
VII	92	4 mL	**4.0**

***The solutions are to be made up to the mark in 100 mL volumetric flasks with distilled.**

4.5.2 Obtain the AAS **absorbance** measurements for all the above **seven** solutions.

4.5.3 Plot a standard additions graph (concentration of added Cu (ppm) *versus* absorbance), and determine the **Cu concentration** in **the tea infusion** (cup of tea). You will need to research how this is done (see Web notes).

4.5.4 Discuss any differences in the results (Cu conc. in tea infusion) obtained by the external standard method *versus* the standard additions method.

4.5.5 Superimpose one graph on the other, and see if the graphs for the standard addition solutions and the external standard solutions are parallel? Why?

PART B
Determination of Iron in Wine by AAS

4.6 AIM FOR PART B

To compare the external standard method with the standard additions method for determining the iron content in wine by Atomic Absorption Spectroscopy (AAS).

4.7 PRE-PREPARED SOLUTIONS AVAILABLE FOR IRON ANALYSIS

Bulk Iron Standard: 100 ppm Fe

4.8 PROCEDURE FOR EXTERNAL STANDARD DETERMINATION OF IRON IN WINE

4.8.1 Check the required lamp is fitted and set the instrument to the recommended operating parameters using the **248.3 nm** resonance line. An **air-acetylene** flame will be used.

4.8.2 Prepare the following solutions:

2% HCl (100 mL)

0.7% HCl (500 mL)

4.8.3 Prepare the following **external standard iron solutions** using the 100 ppm bulk standard already prepared, using 0.7% HCl for the dilutions:

0, 1, 2, 3, 4 ppm **Fe**

4.8.4 Prepare a **white wine sample** in duplicate by diluting 70 mL of white wine ("Chateau Thames Embankment 2003") to 100 mL with 2% HCl.

4.8.5 **Record** the **absorbances** for the above iron standard solutions and wine sample, and **prepare** a **calibration curve** using the absorbance and concentrations of the iron standards.

4.8.6 Using this calibration curve determine the **iron concentration** in the above diluted **wine sample** and record your results. Be sure not to forget the dilution factor when you use this concentration to calculate the Fe concentration in the **original undiluted wine.**

OVERALL QUESTIONS

1. Compare your results with the literature values and the Australian and New Zealand Food Standards Code, and comment on the results.

2. Construct a schematic diagram to display the layout of an atomic absorption spectrophotometer. Include the operational conditions used in your practical and comment on the effect that may be seen by changing each of these conditions. **A photocopy is not acceptable.**

4.9 REFERENCES

Amerine, M.A. and Ough, C.S. (1974) 'Wine and Must Analysis'. (John Wiley and Sons, Inc.: New York)

Boulton, R.B., Vernon, L.S., Bisson, L.F., and Kunkee, R.E. (1998) 'Principles and Practices of Winemaking'. (Aspen Publishers: Maryland).

Eden, T. (1976) 'Tea'. (Longman Group Ltd: London).

Egan, H., Kirk, R.S. and Sawyer, R. (1981) 'Pearson's Chemical Analysis of Foods' 8th ed. (Churchill Livingstone: Edinburgh).

Ensminger, A.H., Ensminger, M.E., Konlande, J.E., and Robson, J.R.K. (1995) 'The Concise Encyclopedia of Foods and Nutrition'. (CRC Press Inc.: Florida).

Harley, C.R. (1956) 'The Culture and Marketing of Tea' (Oxford University Press, New York)

Macrae, R., Robinson, R.K. and Sadler, M.J. (Editors) (1993) 'Encyclopedia of Food Science, Food Technology and Nutrition'. (Academic Press Ltd.: London).

Pomeranz, Y. and Meloan, C.E. (1978) 'Food Analysis: Theory and Practice'. (rev. ed.). (AVI: Westport, Conn.).

Skoog, D.A. and West, D. M. (1985) 'Principles of Instrumental Analysis'. 3rd ed. (Saunders College Publishing: Philadelphia).

Souci, S.W. Fauchman, W. and Kraut, H. (2000) 'Food Composition and Nutrition Tables'. 6th Edition. (Medpharm Scientific Publishers: Stuttgart).

United States Department of Agriculture. (2002) 'Nutrient Data Laboratory'. (online). (www.usda.gov.).

APPENDIX

COPPER

Analytical Data

Cu Atomic Wt. 63.54

Aqueous: Copper metal foil or wire (99.99%)
Non-aqueous: Copper 4 - cyclohexylbutyrate
Preparation of 1000 µg/mL standard:
Dissolve 1.0000 g of copper in 50 mL of 6M nitric acid and dilute to 1 litre to give 1000 µg/mL Cu.

ATOMIC ABSORPTION
Lamp current: 3.0 mA
Flame type: Air-Acetylene (oxidizing)

WAVELENGTH nm.	BANDPASS nm.	OPTIMUM WORKING RANGE µg/mL	SENSITIVITY µg/mL
324.7	0.5	1 - 5	0.025
327.4	0.5	2.5 - 10	0.50
217.9	0.2	7.5 - 30	0.16
222.6	1	45 - 180	1.0
249.2	0.5	180 - 730	4.0
244.2	1.0	400 - 1700	9.0

INTERFERENCES

Few interferences have been reported for copper.

FLAME EMISSION SPECTROSCOPY

Wavelength: 327.4 nm
Bandpass: 0.2 nm
Flame type: Nitrous oxide-acetylene

PRACTICAL EXERCISE NO. 5

GAS CHROMATOGRAPHY (GC) QUANTIFICATION OF ALCOHOL CONTENT IN BEVERAGES USING THE INTERNAL STANDARD METHOD

5.1 INTRODUCTION

Gas Chromatography is one of the most widely used techniques in analytical chemistry. It is a sensitive technique for separating a complex mixture of relatively volatile components.

In column chromatography (see Practical Exercise No.11), the separating column, normally a glass tube, is packed with a stationary bed of large surface area, eg. alumina. A mobile phase percolates through this stationary bed. In gas-liquid chromatography (GC) the stationary phase is a liquid which is chemically coated over an inert support. The sample of a mixture of compounds is introduced into the mobile gas phase stream and is carried onto the column. Once on the column, separation of components, as in all chromatography, is a result of the difference in the various forces by which the stationary phase tends to retain each of the components. Whatever the nature of this retention, be it by partitioning (solubility), chemical bonding, polarity or molecular filtration, the column will hold back some components longer than others. In GC the important column retention factors are partition of compounds between the stationary phase and the mobile gas phase, and solubility of the compounds in the stationary phase, although some of the others mentioned do also play a smaller, incidental part.

Thus, the component compounds move down the column at a rate determined by many factors, but mainly by their "strength" of retention. Here, this refers to both their degree of solubility in the liquid stationary phase and also to their volatility. Bearing in mind that different compounds will have different solubilities in the liquid phase and different volatilities, they will progress down the column at varying rates and consequently, assuming the column is long enough, the solute components of a mixture will emerge separately at the outlet of the column. In other words, all components pass through the column at different speeds and emerge in the inverse order of their retention by column materials.

Emerging from the gas chromatographic column, the separated gaseous components enter a detecting device immediately attached to the column and this senses the presence of a compound. Here the individual components register a series of signals which are amplified, fed to a chart recorder, an electronic integrator or computer, and a trace is produced. The separated components appear as a succession of peaks above the base line. This is known as the **chromatogram.**

Detection of compounds

The detector is located immediately at the exit of the gas chromatography column and its object is to detect efficiently, accurately and with the best sensitivity possible the emergence of a compound from the column. There are a considerable number of detection systems. The detector type used in this practical is called the **flame ionization detector** (F.I.D.). The eluent from the column is mixed with hydrogen and burnt in an atmosphere of air. The flame jet or a surrounding ring forms a negative electrode and another cylinder forms a positive electrode across which a potential is applied. The thermally induced ionization of an eluent from the column results in a change of electrical resistance and the resultant change in current produced at the collector electrode is amplified and fed to a recorder. The F.I.D. response is directly proportional to the number of carbon atoms bound to hydrogen or other carbon atoms. The detector responds to most organic compounds but minimally or not at all to water, carbon disulphide, inorganic compounds and most gases.

5.2 AIM

To determine the ethanol content of a range of alcoholic beverages by GC.

5.3 PROCEDURE

This practical exercise is to be performed individually.

However, each student will be assigned one particular alcohol beverage (beer, wine or spirit) and two types (eg. 2 spirits; or light and heavy beer; or red and white wine). Each student will prepare duplicates for each of the 2 samples, and a single solution for each of the 4 internal standard solutions relating to the particular beverage. This will mean each student will analyse a total of 8 solutions. The group as a whole must analyse at least two samples of beer, wine and spirit.

Each student in the group will then share each other's results and prepare an individual report on all 3 beverages (beer, wine or spirit) with their multiple types.

5.3.1 Operating Parameters

The following operating parameters must be set-up and recorded. Use your experience of Practical Exercise No. 3 to determine the column head pressure required for the optimum flow rate (25-30 cm/sec for He) and thus for optimum resolution. Each student or group of students must set these up for the particular GC instrument being used.

GC instrument name:

Injector Mode:

Injector Temperature:

FID Detector Temperature:

Column/Oven Temperature:

Column Characteristics:

Type of Carrier Gas:

Carrier Gas (column) Flow Rate:

FID Hydrogen Flow Rate:

FID Air Flow Rate:

FID Make-up Gas Flow Rate:

Column Head Pressure:

Split Vent Gas Flow Rate:

Spit Ratio:

5.3.2 Calculation of Alcohol Concentration Using a Calibration Curve

All samples and standards are to be prepared in **10 mL volumetric flasks** and then transferred to screw-capped sample tubes.

(i) Prepare a series of standards of the following concentration using standard volumetric flasks (10 mL). Prepare **one series only** depending on what product is being investigated. Use absolute ethanol (100%) when preparing standards. The remainder of the **10 mL** is to be made up with distilled water. **The internal standard (ISTD) is *n*-propanol (*n*-PrOH).** No duplicates required here.

PRODUCT	100% ETHANOL mL	*n*-PROPANOL mL	FINAL ETHANOL CONC %(v/v)
Beer	0.2	0.5	**2**
Standards	0.4	0.5	**4**
	0.6	0.5	**6**
	0.8	0.5	**8**
Wine	0.5	1.0	**5**
Standards	0.7	1.0	**7**
	1.0	1.0	**10**
	1.5	1.0	**15**
Spirits	1.0	2.0	**10**
Standards	2.0	2.0	**20**
	2.5	2.0	**25**
	3.5	2.0	**35**

(ii) Inject appropriate volumes (1 µL) of the standards onto the column. Measure the peak areas of the EtOH and *n*-PrOH (ISTD) from the GC chromatogram and calculate the **peak area ratios** (EtOH / ISTD).

(iii) Now obtain copies of all the chromatograms for the 3 sets of standard solutions (relating to beer, wine and spirit) analysed by members of your group.

(iv) Obtain 3 **calibration curves** by plotting **peak areas ratios** (EtOH / ISTD) *versus* **% EtOH** for the 3 sets of standards (beer, wine and spirit).

(v) **De-gas the beer samples by filtering rapidly under vacuum and then placing in an ultrasonic bath until frothing ceases (maximum 15 min).**

(vi) For the **wine** samples, add **1 mL *n*-propanol** (propan-1-ol), 7 mL wine and then make up to the 10 mL mark with water **(Prepare in duplicate).**

(vii) For the **spirit** samples, add **2 mL *n*-propanol** (propan-1-ol) 6 mL spirit, and then make up to the 10 mL mark with water **(Prepare in duplicate).**

(viii) For the **beer** samples, add **0.5 mL *n*-propanol** (propan-1-ol), 8 mL degassed beer, and make up to the 10 mL mark with water **(In duplicate).**

(ix) Now obtain copies of all the chromatograms for all the sample solutions of beers, wines and spirits (ISTD added) studied by members of your group.

(x) Determine the **peak areas ratios** (EtOH/ISTD) for the duplicate samples from the GC chromatograms.

(xi) Calculate **% EtOH in each beverage** from its respective **calibration curve** in (iv), taking account of the dilution factor of each.

5.3.3 Calculation of Alcohol Concentrations using Relative Response Factors

5.3.3.1 Calculation of the Relative Response Factors

Now obtain copies of all the chromatograms 12 **Internal Standard ethanol solutions (with ISTD)** prepared and analysed by members of your group.

For the 12 **Internal Standard ethanol solutions (with ISTD)** prepared by members of your group, calculate the RELATIVE RESPONSE FACTOR (RRF) for ethanol using the following formula:

Basic formula:

$$\mathbf{RRF} = \frac{\mathbf{Conc.\ EtOH}}{\mathbf{EtOH\ peak\ Area}} \times \frac{\mathbf{ISTD\ peak\ Area}}{\mathbf{Conc.\ ISTD}}$$

The following is a guide for the presentation of results in your practical record books:

Peak Area (2% Ethanol) = _____

Peak Area (ISTD) =

Conc (2.5% Ethanol) = 2.5%

Conc (ISTD) = 5%

Repeat for all the remaining 10 ethanol Internal Standard solutions.

Now determine the **average RRF** for each type of Internal Standard Solution:

Average of **RRF's** for the 4 beer **ethanol/ISTD** solutions =
Average of **RRF's** for the 4 spirit **ethanol/ISTD** solutions =
Average of **RRF's** for the 4 wine **ethanol/ISTD** solutions =

5.3.3.2 Calculation of the Concentration of Ethanol in the Beverage Samples using RRF

Now obtain copies of all the chromatograms for all the sample solutions of beers, wines and spirits (containing ISTD) studied by members of your group.

Use the chromatograms for the internal standard sample solutions (containing ISTD, and beer, wine or spirit) to determine the peak areas of ethanol and *n*-propanol.

The concentration (in %) of ethanol in, for example, the unknown beer is obtained by substituting the appropriate values into the equation below. Be sure to choose the **correct peak areas** and the correct **average RRF** for ethanol / ISTD from Section 5.3.3.1.

Determine the alcohol concentrations of all the beers, wines and spirits analysed by members of your group in your own individual report.

Formula:

$$\text{Ethanol conc. (\%)} = \frac{\text{Ethanol Peak Area} \times \text{RRF.} \times \text{Conc of ISTD}}{\text{ISTD Peak Area}}$$

The following is a guide for the presentation of results in your practical record books:

Peak Area **ISTD** = _____

Peak Area (ethanol in XXXX heavy) = _____

Average RRF =

Concentration ISTD = 5%

Ethanol conc. (%) in XXXX heavy beer =

5.4 QUESTIONS

5.4.1 What are the advantages in using an internal standard (ISTD) method to quantify ethanol? Why is *n*-propanol a good choice in this case?

5.4.2 Construct a schematic diagram to display the layout of a gas chromatograph. Include the operational conditions used in your practical and comment on the effect that may be seen by changing some of these conditions, eg. column flow rate, column length, oven temperature and type of carrier gas. **A photocopy from a reference is not acceptable.**

5.5 REFERENCES

McNair, H. M. (1969). 'Basic Gas Chromatography'. (Varian Aerograph: California).

Skoog, D.A. and West, D. M. (1985) 'Principles of Instrumental Analysis' . 3rd ed. (Saunders College Publishing: Philadelphia).

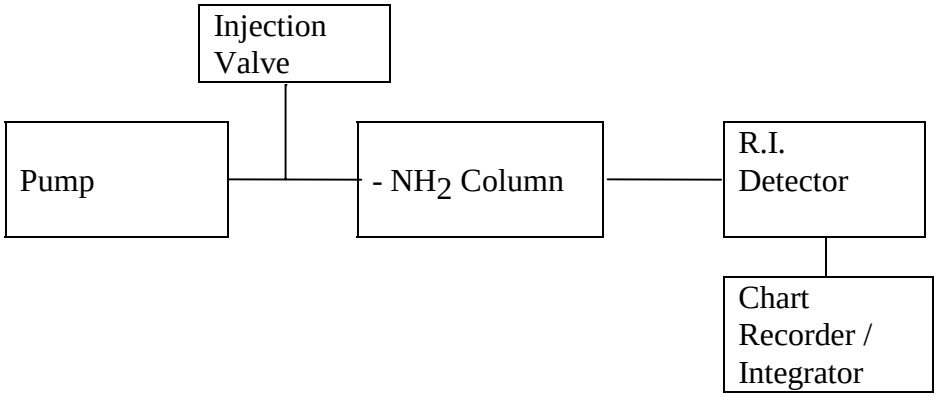
PRACTICAL EXERCISE NO 6

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) QUANTIFICATION OF SUGARS IN FOODS AND BEVERAGES USING THE EXTERNAL STANDARD METHOD

6.1 INTRODUCTION

6.1.1 The System

The system used consists of a dual-piston pump coupled with a refractive index detector. The column used is a 'Waters' carbohydrate column.



The mobile phase is an **acetonitrile/water** mixture.

Operation of the pump will be described by the Tutor. This particular type of pump requires priming and it is quite important that the mobile phase be well degassed (by using the ultrasonic bath). In the event that the pump does require priming or purging, you should consult your Tutor. The controls on the front of the pump unit are self explanatory. Note that there may be a liquid-switching valve on the front of the pump. This valve enables one to use an alternative solvent eg., for flushing compounds off the column rapidly etc.

The injection valve is a loop injector.

The refractive index detector uses a double beam optical system to make differential refractive index measurements between a reference cell (containing the eluting solvent) and a sample cell (volume 5 μL) which looks at the liquid flowing out of the chromatographic column. Whenever anything comes off the column the detector will **"see"** the change in the refractive index of the eluting stream relative to the reference. The operation of the detector will be described.

Most HPLC separations are performed to determine the amounts or concentrations of certain components in mixtures. In this exercise, the external standard method of quantification will be used to determine the quantity of some sugars in various red and white wines.

The External Standard method of quantification is most effective when the injection technique is precise and the chromatographic conditions are stable.

The sugar content of wines consists of fructose and glucose. Other beverages such fruit juice contain fructose, glucose and sucrose.

6.2 METHOD

6.2.1 Operating Conditions

Mobile Phase: 83% Acetonitrile / 17% H_2O degassed

Column: 'Waters' Carbohydrate column

Pump: P_{max} - 3,000 psi
Flow Rate 2.0 ml/min

Detector: Room temperature

Syringe Volume: 250 µL

Rheodyne Injection Loop: 25 µL (actual volume injected in HPLC)

6.2.2 Sample Solutions

Sample Solutions for study include an assortment of:

*(a) 2.0 g honey in 100 mL water

*(b) 2.0 g golden syrup in 100 mL water

*(c) 40 mL of Apple Juice (100% fruit juice) diluted to 100 mL with water

*(d) 40 ml orange fruit juice drink diluted to 100 mL with water

(e) Neat dry white wine. (**Sample Clean-up for wine**: apply the wine samples directly through a Alltech C18 solid phase extraction cartridge connected to a filter unit (0.45 µm). Collect the carbohydrate solution as the eluent.

(f) Neat sweet white wine. (**Sample Clean-up for wine**: apply the wine samples directly through a Alltech C18 solid phase extraction cartridge connected to a filter unit (0.45 µm). Collect the carbohydrate solution as the eluent.

***All samples must be filtered through a 0.45 μm filter prior to injection.**

6.2.3 Sugar Standards

Standard Solution 1: 0.05 g fructose in 10 mL Milli Q water

Standard Solution 2: 0.02 g sucrose in 10 mL Milli Q water

One set of Standard Solutions 1 and 2 are to be prepared by the whole group not by each individual student.

Standard Solution 3: 0.075 g of each of:

fructose
glucose
sucrose

in 10 mL distilled water (a single solution)

Each student will individually prepare Standard Solution 3.

All standard solutions must be filtered through a 0.45 μm filter prior to injection.

6.2.4 Experimental Procedure

(i) Set up the HPLC equipment and get it running as described by the tutor.

(ii) Prepare **single solutions** of **one juice, one spread, and one wine** as described in the previous section.

Each student will have a total of 3 samples. Each group must ensure that all six sample types (2 juices, 2 spreads, and sweet and dry wines) are done over the whole group. The group will then pool their results, with each student individually preparing their final report using the pooled group's results. Use multiple results for each sample type as replications.

(iii) As a group inject 250 μL (25 μL actually injected by the Rheodyne fixed loop injector) of Standards 1 and 2 to identify which peaks are fructose and sucrose. Then individually inject Solution 3 to identify glucose. Additionally, record the retention times for each sugar. Finally, determine the response factors (RF) for the three sugars using Standard Solutions 1-3 (Solution 1: RF of fructose; Solution 2: RF of sucrose; Solution 3: RF for all three sugars).

Basic Formula for Response Factor (RF):

$$\mathbf{RF = \frac{Concentration\ of\ known}{Peak\ Area\ of\ known}}$$

Conc. (glucose) = 0.75%

Peak Area (glucose) =

RF (glucose) =

Conc. (fructose) = 0.75%

Peak Area (fructose) =

RF (fructose) =

(iv) Inject your three sample solutions and obtain your 3 chromatograms. Then obtain the chromatograms for all other samples analysed by members of your group. The sample concentrations are then calculated from the **peak areas using the RF.**

Use the RF's for your own Standard Solution 3 for determining the concentration (%) of fructose, glucose and sucrose in all the honey, wine, fruit juice and the golden syrup samples, based on the pooled chromatograms obtained by the whole group.

(v) Determine the sugar content (g/100 g) of all six food samples done by members of your group using the RF and the formula below **(be sure you convert your final sugar concentrations to the actual sugar content in the original food by using the appropriate dilution factor). For the wine sample use the following method:**

Basic Formula for Determining Concentration

Amount of Unknown = Peak Area x RF

Use the following format for recording your results and calculations in your practical record books:

Peak Area (fructose in wine sample) =

RF (fructose from standard) =

Conc. (fructose in wine sample) =

Peak Area (glucose in wine sample) =

RF (glucose from standard) =

Conc. (glucose in wine sample) =

etc.

NOTE: Sugar solutions are subject to bacteriological degradation and should be freshly prepared the week prior to use and stored in a freezer, ie., they will NOT "keep" for more than a week unless stored in the freezer.

6.3 QUESTION

Construct a schematic diagram to display the layout of a HPLC instrument. Include the operational conditions used in your practical and comment on the effect that may be seen by changing some of these conditions. **A photocopy is not acceptable.**

6.4 REFERENCES

Pomeranz, Y. and Meloan, C. E. (1987). 'Food Analysis: Theory and Practice'. 2nd ed. (AVI: Conn.).

Skoog, D.A. (1985) 'Principles of Instrumental Analysis' . 3rd ed. (Saunders College Publishing: Philadelphia).

Stewart, K. K. and Whitaker, J. R. (eds) (1984). 'Modern Methods of Food Analysis'. (AVI Publishing: Westport, Conn.).

PRACTICAL EXERCISE NO. 7

THIN LAYER CHROMATOGRAPHY (TLC) OF FOOD COLOURS

7.1 INTRODUCTION

Chromatography is a physical process in which components of a mixture are separated because of their different affinities for substances in two phases, one a stationary phase and the other a mobile phase. The mobile phase can be liquid (liquid chromatography) or gaseous (gas chromatography).

Thin layer chromatography is a form of partition and/or adsorption chromatography where the stationary phase is adsorbed and absorbed water on thin sheets of powdered support material (eg. silica gel, Kieselguhr, cellulose) coated onto glass, rigid plastic film or thin aluminium foil. In some cases the powdered solid support material is actually the stationary phase. The mobile phase is a solution usually containing several liquids. A spot of sample extract is dried onto the plate. The plate is then placed in a closed chamber so that the mobile phase liquid can flow (usually upwards by capillary action) through the stationary phase. After the desired time has elapsed the plate is removed from the chamber and developed by the use of sprayed-on visualizing agents or UV light. In the case of coloured substances such as food dyes, the separation may be evident without assistance from such agents.

TLC is far more sensitive, adaptable and rapid than paper chromatography and chromatographic separations are more efficient and can be made semi-quantitative. Plates containing no organic matter can be prepared or purchased allowing the visualization of spots by charring with sulphuric acid. Visualisation under UV light after spraying the plate with a fluorescing reagent can be very effective when the separated compounds quench or modify and fluorescence. TLC is the cheapest and often the best method of isolating and identifying minor components of foods.

7.2 AIM

The aim of this experiment is to use TLC to identify food colours in some food colouring samples.

NOTE: Prepare solvents and place them in the appropriate tanks to equilibrate before preparing the tlc plate. N.B. Mix solvent solutions well to ensure homogeneity.

7.3 METHODS

7.3.1 Materials:

T.L.C. Plates: KIESELGEL G

Mobile Phase: *n*-Butanol (20 Vol), Water (12 Vol), Glacial Acetic Acid (5 Vol).

Samples:

1. Green Food Colouring (Full concentration)
2. Yellow Food Colouring (Full concentration)
3. 1% w/w Brilliant Blue
4. 1% w/w Tartrazine
5. 1% w/w supplied red food colourant
6. Green colouring and mix of standards @ 1% w/w

7.3.2 Chromatographic Method:

Apply the sample, standard food colours and commercial food dyes as evenly spaced spots along a line approximately 1 cm from the plate bottom and with no spot within 0.5 cm from the sides of the plate. The spots are generated by laying 1 - 2 drops of material at the same position using a micropipette and drying using a hairdrier between application of each drop. As small a spot as possible is required for the best final results.

The pre-mixed mobile phase should be added to a filter paper lined beaker (1 L) and allowed to equilibrate for 15 minutes.

The spotted and dried T.L.C. plate is then place in the beaker (1 L) and the mobile solvent is allowed to migrate up the plate to a height of approximately 15 cm. There are four important practical points to note at this stage:

(a) the plate should not be contacting any part of the vessel except at the top and bottom ends.

(b) the solvent in the bottom of the vessel must not be at a height greater than the spots on the plate;

(c) do not allow the solvent front to reach the upper edge of the plate.

(d) the outline of the solvent front should be traced in pencil on immediately removing the plate from the tank but before drying in the fume cupboard;

7.3.3 Qualitative Evaluation:

The qualitative identification of each of the spots in the honey sample may be achieved *via* comparison of both empirically and reference derived values called Rf values. The empirical R_f Values may be generated by measurement of the distance travelled by each component (measure from the origin to the centre of migrated spot) and the solvent front.

The dimensionless R_f Value may be calculated for each component within a sample and then identified *via* comparison to standard Rf Values:

$$R_f = \frac{\text{Distance Travelled by Component}}{\text{Distance Travelled by Solvent Front}}$$

7.4 QUESTIONS

7.4.1 Discuss the relationship between the chemical structures of the food colours and their relative R_f 's. Ensure that all relevant chemical structures are shown and discussed with regard to respective Rf values (ie. polarity, etc.).

7.4.2 Determine whether the food colours present in the samples of food colourings as determined by TLC are permitted for use in Australia according to the Australian and New Zealand Food Standards Code. (Quote the relevant sections of the code).

7.5 REFERENCES

Budauari, S. (Edit.) (1996) 'The Merck Index' 12th ed. (Merck and Co. ; New Jersey, USA).

Egan, H., Kirk, R.S. and Sawyer, R. (1981). 'Pearson's Chemical Analysis of Foods' . 8th ed. (Churchill Livingstone: Edinburgh).

Pearson, D. (1973). 'Laboratory Techniques in Food Analysis'. (Butterworths: London).

Pomeranz, Y. and Clifton, M. (1994). 'Instrumental Food Analysis'. 3rd ed. (Chapman & Hall: New York).

Windholz, M. (ed). (1983). 'The Merck Index'. 10th ed. (Rahway: N. J.).

Woods, A.E. and Aurant, L.W. (1977) 'Laboratory Manual in Food Chemistry' (AVI: Westport, Conn.).

PRACTICAL EXERCISE NO. 8

DETERMINATION OF TITRATABLE ACIDITY IN FOODS USING A POTENTIOMETRIC TITRATION

8.1 INTRODUCTION

In some cases we cannot use an indicator to detect the end point since the product is coloured (eg. beetroot). In these cases a plot of pH *versus* volume of alkali is prepared (Neutralization Curve).

8.2 METHOD

This practical exercise is to be performed individually.

NOTE: A sheet of graph paper is required for this practical exercise.

(1) Add a 10 mL aliquot of beetroot brine (by volumetric pipette) to a 250 mL beaker.

(2) Dilute to approximately 150 mL with water.

(3) Set up the apparatus as shown by the tutor.

(4) Record the initial pH of the solution.

(5) Add 1 mL aliquots of the 0.1M standard NaOH and measure the pH after each addition. If the pH reading rises by more then 0.3 of a pH unit, continue the titration but only add 0.2 mL of NaOH.

(6) Continue to add 0.2 mL of 0.1 M NaOH until the pH rises by more than 0.3 of a pH unit, then add 0.1 mL until pH of approx. 10.

(7) Plot a graph, pH vs Volume, and estimate the **pH of the end point** of the titration. This pH value will be entered into the **auto-titrator** and the titration repeated. Using this apparatus as instructed, 10 mL of beetroot brine, and 0.1M standard NaOH will be used. No water is required.

(8) Plot the first derivative Δ pH / Δ V *versus* volume added.

8.3 REFERENCES

Vogel, A. J. (1961) 'Quantitative Inorganic Analyses' (3rd ed) (Longman, London).

OPTIONAL PRACTICAL EXERCISES

PRACTICAL EXERCISE 9

DETERMINATION OF CAFFEINE IN COFFEE AND COLA DRINKS USING HPLC

PRACTICAL EXERCISE NO. 9A

QUANTIFICATION OF CAFFEINE IN VARIOUS COFFEE PRODUCTS BY HPLC

1.0 AIM

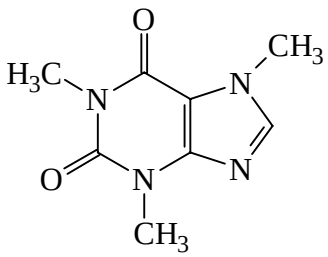
To quantify the caffeine content of a number of coffee products using an internal standard and sample clean-up by solid phase extraction.

2.0 INTRODUCTION

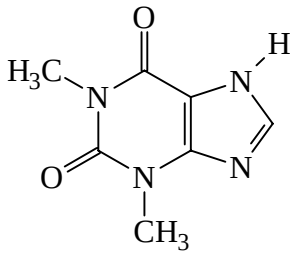
The amount of caffeine in coffee can be determined using an internal standard under isocratic conditions.

The Internal Standard method is used when the injection procedure may not be precise, the chromatographic conditions are unstable, or if there has been any sample preparation.

Theophylline is used here as an **internal standard** as the structure and chemical properties of theophylline compare favourably with caffeine.



CAFFEINE



THEOPHYLLINE

3.0 METHOD

3.1 Solutions to be analysed

All 6 solutions will be prepared in the first week of the practical and put through the HPLC in the first week and the subsequent week. Great care must taken during these preparations.

Solution 1: Caffeine (10 ppm) filtered

Solution 2: Caffeine (10 ppm) and theophylline (10 ppm) filtered

Solution 3: Caffeine (10 ppm) and theophylline (10 ppm) extracted and filtered.

Solution 4-6: Coffee solution with added theophylline (5 ppm) extracted and filtered (three commercial coffee products must be analysed).

Use the bulk caffeine and theophylline standards (100 ppm) and Milli Q water to prepare the above solutions (**10 mL**).

3.2 Preparation of standards and calibration

3.2.1 Solution 1: Caffeine (10 ppm) filtered.

This solution is used to determine the retention time for caffeine and thus to distinguish caffeine from theophylline. It must be filtered through a 0.45 μm filter prior to injection.

Detector:	UV 254 nm @ 0.1 AUFS
Starting mobile phase:	Methanol / water (35**:**65)
Column:	Reverse phase ‘Waters’ Nova Pak C18
Flow Rate:	1 mL/min
Injection Volume:	250 μL (actually 20 μL loop is used and only 20 μL goes onto the column)

3.2.2 Solution 2: Caffeine (10 ppm) and theophylline (10 ppm) filtered.

This solution is used to develop the appropriate chromatographic conditions for analysis and to calibrate the analysis by calculation of the **relative response factors** for the analyte and internal standard. Solution 2 must be filtered through a 0.45 μm filter prior to injection. Note, **3 mL** of this solution will be cleaned-up and filtered in Section 3.3 leaving **7 mL** for this analysis.

Step 1:

Develop an **isocratic** (MeOH / H_2O) separation of the two components using MeOH / H_2O (35**:**65) as a starting point. **Inject 250 μL of Solution 2.**

It may be necessary to modify this with the samples later.

Step 2:

Calculate the relative response of caffeine to theophylline from the **peak areas.** Be sure you have correctly identified which peak is due to caffeine using the results from Section 3.2.1.

Internal Standard Calibration Calculation

Calculate the **relative response factor (RRF)** for caffeine.

$$RRF = \frac{\text{Conc. of component} \times \text{Peak Area of int. std.}}{\text{Peak Area of component} \times \text{Conc. of int. std.}}$$

$$\mathbf{RRF = \frac{Conc.\ of\ caffeine \times Peak\ Area\ of\ int.\ std.}{Peak\ Area\ of\ caffeine \times Conc.\ of\ int.\ std.}}$$

3.3 Clean-up of Standard Solution by solid phase extraction

Solution 3: Caffeine (10 ppm) and theophylline (10 ppm) extracted and filtered.

Step 1: Condition the solid phase extraction column (Alltech C18) with 4 mL of milli Q water.

Step 2: Inject **three (3) mL** of the calibration solution (Solution 2) through the column and discard the eluent. The caffeine and theophylline will remain on the column.

Step 3: A 0.45 μm filter is then attached to the bottom of the Alltech C18 extraction cartridge and the caffeine/theophylline is **washed** from the column into a screw-cap sample tube by injecting **three (3) mL** of HPLC grade methanol into the Alltech C18 extraction cartridge.

This solution can then be dried and reconstituted, but **here** will be **analysed directly.**

Step 4: The Alltech C18 extraction cartridge will be reused. Clean the cartridge by flushing with methanol (3 x 5 mL) followed by water (2 x 5 mL).

3.4 Analysis of the Standard Solution

Inject 250 μL of the solution of caffeine and theophylline after extraction and filtration (solution 3) and note the areas.

The areas before extraction were determined in 3.2.2 step 2.

Determine the **% recovery** for the clean-up step for both caffeine and theophylline using the following formula:

$$\% \text{ recovery} = \frac{\text{peak area after extraction}}{\text{peak area before extraction}} \times 100$$

Are the % recoveries different. If so, what does this mean for the internal standard method that follows?

3.5 Sample clean-up by solid phase extraction

3.5.1 Preparation of a coffee solution with 5 ppm added theophylline:

Weigh out (exactly) a sample of about 2 g of coffee, dissolve in hot water, and make up to 100 mL in a volumetric flask. Filter all of this solution (No. 42 filter paper) and then further filter 5 mL through a 0.20 μL filter. Now perform a 1 in 100 dilution on a 1 mL doubly filtered aliquot, being sure to include 5 mL of the 100 ppm theophylline solution prior to making up to the mark (in a 100 mL volumetric flask). This is the sample for solid phase extraction clean-up.

3.5.2 Sample clean-up:

The three coffee samples (solutions 4, 5, and 6) are cleaned up as described in Section 3.3 steps 1-3. **Use the coffee solutions prepared above in Section 3.5.1 (100 mL with 5 ppm added theophylline) for these extractions.** Be sure to **clean** the cartridge as per Section 3.3 step 4 between each coffee sample.

3.6 Sample analysis

3.6.1 Inject **in turn** 250 μL of the solutions of each coffee solution (solutions 4, 5 and 6) prepared above in 3.5.2.

3.6.2 Calculate the **concentration** of caffeine in the coffee samples using the **RRF** calculated previously in Section 3.2.2 and the peak areas of the internal standard and caffeine from these runs, using the following formula:

$$\textbf{Conc. component} = \frac{\textbf{Peak Area component x RRF x Conc. int. std.}}{\textbf{Peak Area int. std.}}$$

Use the following format for **recording** your results and calculations in the practical record book.

Caffeine in Coffee:

Conc. Internal Standard (theophylline) = 5 ppm

Peak Area Internal Standard =

Peak Area caffeine =

RRF (caffeine) =

$$\textbf{Conc. caffeine} = \frac{\textbf{Peak Area caffeine x RRF x Conc. int. std}}{\textbf{Peak Area int. std.}}$$

Caffeine Conc of the final coffee solution =

ppm, (mg/L), (μg/mL)

Use the dilution factor from Section 3.5.1 to determine the caffeine content (g/100 g) in the three <u>solid</u> coffee products analysed.

4.0 DISCUSSION

Compare the caffeine levels between the analysed commercial coffee products and with those reported in the literature for coffee.

5.0 QUESTIONS

5.1 Calculate the **Retention** or **Capacity Factor**, **k'**, for caffeine and theophylline.

5.2 Calculate the **efficiency** (number of theoretical plates) for the last peak using the **width at half height method.**

5.3 Determine the selectivity, α, for caffeine and theophylline.

PRACTICAL EXERCISE NO. 9B

QUANTIFICATION OF CAFFEINE IN COLA SOFT DRINKS BY HPLC

1.0 AIM

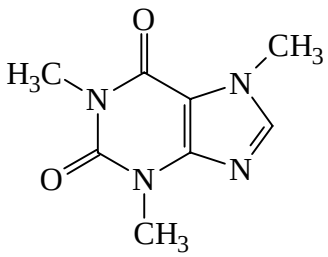
To quantify the caffeine content of cola soft drinks using an internal standard and sample clean-up by solid phase extraction.

2.0 INTRODUCTION

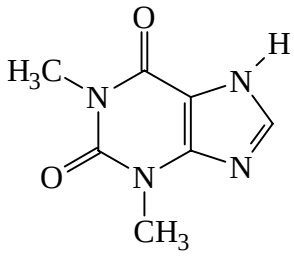
The amount of caffeine in soft drinks such as Coke and Pepsi can be determined using an internal standard under isocratic conditions.

The Internal Standard method is used when the injection procedure may not be precise, the chromatographic conditions are unstable, or if there has been any sample preparation.

Theophylline is used here as an **internal standard** as the structure and chemical properties of theophylline compare favourably with caffeine.



CAFFEINE



THEOPHYLLINE

3.0 METHOD

3.1 Solutions to be analysed

All 6 solutions will be prepared in the first week of the practical and put through the HPLC in the <u>subsequent</u> week. Great care must taken during these preparations.

Solution 1: Caffeine (10 ppm) filtered

Solution 2: Caffeine (10 ppm) and theophylline (10 ppm) filtered

Solution 3: Caffeine (10 ppm) and theophylline (10 ppm) extracted and filtered.

Solution 4: Degassed Coke with added theophylline (10 ppm) extracted and filtered.

Solution 5: Degassed **Caffeine-Free Coke** with added theophylline (10 ppm) extracted and filtered.

Solution 6: Degassed Pepsi with added theophylline (10 ppm) extracted and filtered.

Use the bulk caffeine and theophylline standards (100 ppm) and Milli Q water to prepare the above solutions (**10 mL**). All soft drinks must be **degassed** prior to mixing with the internal standard (theophylline).

3.2 Preparation of standards and calibration

3.2.1 Solution 1: Caffeine (10 ppm) filtered.

This solution is used to determine the retention time for caffeine and thus to distinguish caffeine from theophylline. It must be filtered through a 0.45 μm filter prior to injection.

Detector: UV 254 nm @ 0.1 AUFS

Starting mobile phase: Methanol / water (35**:**65)

Column: Reverse phase ‘Waters’ Nova Pak C18

Flow Rate: 1 mL/min

Injection Volume: 250 μL (actually 20 μL loop is used and only 20 μL goes onto the column)

3.2.2 Solution 2: Caffeine (10 ppm) and theophylline (10 ppm) filtered.

This solution is used to develop the appropriate chromatographic conditions for analysis and to calibrate the analysis by calculation of the **relative response factors** for the analyte and internal standard. It must be filtered through a 0.45 μm filter prior to injection. Note, **3 mL** of this solution will be cleaned-up and filtered in Section 3.3 leaving **7 mL** for this analysis.

Step 1:

Develop an **isocratic** (MeOH / H_2O) separation of the two components using MeOH / H_2O (35**:**65) as a starting point. **Inject 250 μL of Solution 2.**

It may be necessary to modify this with the samples later.

Step 2:

Calculate the relative response of caffeine to theophylline from the **peak areas.** Be sure you have correctly identified which peak is due to caffeine using the **results** from Section 3.2.1.

Internal Standard Calibration Calculation

Calculate the **relative response factor (RRF)** for caffeine.

$$RRF = \frac{\text{Conc. of component} \times \text{Peak Area of int. std.}}{\text{Peak Area of component} \times \text{Conc. of int. std.}}$$

$$\mathbf{RRF = \frac{Conc.\ of\ caffeine \times Peak\ Area\ of\ int.\ std.}{Peak\ Area\ of\ caffeine \times Conc.\ of\ int.\ std.}}$$

3.3 Clean-up of Standard Solution by solid phase extraction

Solution 3: Caffeine (10 ppm) and theophylline (10 ppm) extracted and filtered.

Step 1: Condition the solid phase extraction column (Alltech C18) with 4 mL of milli Q water.

Step 2: Inject **three (3) mL** of the calibration solution (solution 2) through the column and discard the eluent. The caffeine and theophylline will remain on the column.

Step 3: A 0.45 μm filter is then attached to the bottom of the Alltech C18 extraction cartridge and the caffeine/theophylline is **washed** from the column into a screw-cap sample tube by injecting **three (3) mL** of HPLC grade methanol into the Alltech C18 extraction cartridge.

This solution can then be dried and reconstituted, but **here** will be **analysed directly.**

Step 4: The Alltech C18 extraction cartridge will be reused. Clean the cartridge by flushing with methanol (3 x 5 mL) followed by water (2 x 5 mL).

3.4 Analysis of the Standard Solution

Inject 250 μL of the solution of caffeine and theophylline after extraction and filtration (solution 3) and note the areas.

The areas before extraction were determined in 3.2.2 step 2.

Determine the **% recovery** for the clean-up step for both caffeine and theophylline using the following formula:

$$\% \text{ recovery} = \frac{\text{peak area after extraction}}{\text{peak area before extraction}} \times 100$$

Are the % recoveries different. If so, what does this mean for the internal standard method that follows?

3.5 Sample clean-up by solid phase extraction

Samples (solutions 4, 5 and 6) are prepared as described in Section 3.3 steps 1-3. **Use degassed soft drinks diluted 1 in 4 to prepare the starting solutions (10 mL with 10 ppm added theophylline) for these extractions.** Be sure to **clean** the cartridge as per Section 3.3 step 4 between each soft drink sample.

3.6 Sample analysis

3.6.1 Inject **in turn** 250 μL of the solutions of each soft drink prepared above in 3.5 (solutions 4, 5 and 6).

3.6.2 Calculate the concentration of caffeine in the samples using the **RRF** calculated previously in Section 3.2.2 and the peak areas of the internal standard and caffeine from these runs, using the following formula:

$$\textbf{Conc. component} = \frac{\textbf{Peak Area component x RRF x Conc. int. std.}}{\textbf{Peak Area int. std.}}$$

Use the following format for **recording** your results and calculations in the practical record book.

Caffeine in Coke:

Conc. Internal Standard (theophylline) = 10 ppm

Peak Area Internal Standard =

Peak Area caffeine =

RRF (caffeine) =

$$\textbf{Conc. caffeine} = \frac{\textbf{Peak Area caffeine x RRF x Conc. int. std}}{\textbf{Peak Area int. std.}}$$

Conc caffeine =

ppm, (mg/L), (µg/mL)

Caffeine in Pepsi:

Conc. Internal Standard (theophylline) = 10 ppm

Peak Area Internal Standard =

Peak Area caffeine =

RRF (caffeine) =

$$\textbf{Conc. caffeine} = \frac{\textbf{Peak Area caffeine x RRF x Conc. int. std.}}{\textbf{Peak Area int. std.}}$$

Conc. Caffeine =

ppm, (mg/L), (µg/mL)

Caffeine in caffeine-free coke

Conc. Internal Standard (theophylline) = 10 pm

Peak Area Internal Standard =

Peak Area Caffeine =

RRF (caffeine) =

$$\textbf{Conc. caffeine} = \frac{\textbf{Peak Area caffeine x RRF x conc. int. std.}}{\textbf{Peak Area int. std.}}$$

Conc. caffeine =

1 ppm, (mg/L), (μg/mL)

Use the dilution factor from Section 3.5 to determine the caffeine content (ppm) in the original three cola soft drink products analysed.

4.0 DISCUSSION

Compare the caffeine levels between the soft drinks and with that reported in the literature.

5.0 QUESTIONS

5.1 Calculate the **Retention** or **Capacity Factor**, **k'**, for caffeine and theophylline.

5.2 Calculate the **efficiency** (number of theoretical plates) for the last peak using the **width at half height method.**

5.3 Determine the selectivity, **α**, for caffeine and theophylline.

PRACTICAL EXERCISE NO. 10

COLUMN CHROMATOGRAPHIC SEPARATION OF PLANT PIGMENTS

(TIME 5 HOURS INDIVIDUAL WORK)

10.1 METHOD

This practical exercise is to be performed individually.

10.1.1 Sample preparation

Prepare a plant pigment extract in the following way.

(a) Pick 5 g of leaves, cut into small sections and grind with a little sand in a mortar with 25 mL of acetone.
(b) Filter the mixture into a separating funnel and grind the leaves with another 25 mL of acetone. Extract the pigments into 30 mL of petroleum spirit. Obtain a stopper for the separating funnel and ask to be shown how to extract and vent the separating funnel.
(c) Discard the lower acetone layer and vent the separating funnel as before. Wash the petroleum spirit with 3 x 10 mL portions of water. Dry the solvent layer over sodium sulphate (5 minutes), filter and evaporate to 5 mL.

10.1.2. Preparation of the Column.

(a) Make a slurry of chromatographic grade cellulose (15 g) in petroleum spirit (50 mL).

(b) Carefully pour the slurry into a column with a cotton wool plug. **Tap** the column gently whilst filling to **remove air bubbles**.

(c) Allow most of the solvent to run off leaving approximately 2-3 mm solvent over the top of the absorbent. **DO NOT allow the column to run 'dry'.**

(d) Set up the column and number the test tubes 1 - 12.

(e) Carefully introduce 1-2 mL of pigment extract onto the top of the column. Allow most of this to run onto the column and then add 1 mL solvent (petroleum spirit). Repeat the process until all the pigment is absorbed and a clear solvent layer is at the top. Do not disturb the column surface.

(f) Prepare the following solvents:

(1) 2 mL + 30 mL ($CHCl_3$: Petroleum Spirit)
(2) 4 mL + 30 mL ($CHCl_3$: Petroleum Spirit)
(3) 2 mL + 30 mL (Acetone : Petroleum Spirit)
(4) 4 mL + 30 mL (Acetone : Petroleum Spirit)
(5) 6 mL + 30 mL (Acetone : Petroleum Spirit)

(g) Develop the column by running through petroleum spirit (only) until the first yellow band (carotenes) is eluted. Gradually increase the polarity of the solvents with $CHCl_3$ and then acetone, ie. the solvents above (strictly in order No. 1 to No. 5), until the xanthophylls (yellow) and chlorophylls (green) are removed. Higher proportions of acetone to petroleum (1**:**5) will remove the final yellow green band of chlorophyll b. Collect the coloured bands as separate fractions, in separate test tubes.

NOTE: Do <u>not change</u> solvents until the respective coloured bands are completely off the column, even if this means using more than the volume of solvent listed above.

(h) Scan each fraction spectrophotometrically using the spectrophotometer in the visible region 400 nm to 700 nm. Mark each recording carefully and compare the observed spectra with published data for the pure pigments (Goodwin 1972).

10.2 DISCUSSION TOPICS

10.2.1 Comment on the purity of each fraction collected - base your comments on the spectroscopic data.

10.2.2 Why is it necessary to increase the polarity (show structures) of the developing solvent? How could a gradient elution technique simplify the procedure.

10.2.3 What type of absorbent is cellulose? Could other absorbents be used?

10.2.4 How could this procedure be modified for quantitative analysis?

10.2.5 Draw a schematic diagram of a UV - VIS spectrophotometer and include a discussion of all the components. **A photocopy is not acceptable.**

10.3 REFERENCES

Goodwin, T.W. (ed) (1967) 'Chemistry & Biochemistry of Plant Pigments' (Academic Press, London).

Goodwin, T.W. (1972) 'Introduction to Plant Biochemistry' (Pergamon Press, New York).

Heath, O.V.S. (1970) 'The Physiological Aspects of Photosynthesis' (Heinemann Educational Books Ltd. London).

Heftmann, F. (1967) 'Chromatography' (2nd Edition) (Reinhold, New York).

Pecsok, R.L. and Shields, L.D. (1968) 'Modern Methods of Chemical Analysis' (Wiley, New York).