Comparison of the active ingredient and purity of two different commercial Aspirin Tablets

Summary of Sessions 1 and 2

In Session 1, you carried out a simple extraction of two different types of Aspirin tablet, but this provided limited quantitative information - the amount of active ingredient was not determined accurately and acetyl salicylate was not explicitly tested for.

In Session 2, you performed a Soxhlet extraction, which was shown to be more quantitative than the simplified extraction method of Session 1. By carrying out a titration on the extract, you were able to quantify the combined amount of acetyl salicylate and salicylic acid within the extract. However, the *individual* amounts of each of these components were not determined, since the titration method does not discriminate between these two analytes.

Session 3

The aim of this final session is to identify the active ingredients within the extracts obtained in Session 2 and to determine their relative amounts. To do this, you will use the following techniques:

- Melting point measurement
- Infra-Red (IR) spectroscopy
- Functional group identification via chemical tests
- Gas chromatography (GC)
- High performance liquid chromatography (HPLC)

You will also need to compare your experimental results with background data that you obtained as part of the pre-laboratory exercise for this session.

In your groups, discuss (10 mins) the literature data that you have obtained and identify the key information that will you be looking for from each method.

Methods

Functional Group Tests

Carry out chemical tests (a)–(d) on your extract and the Aspirin tablets (A and B).

(a) <u>The nitro group (–NO$_2$)</u>

Add a small sample (*ca* 0.1 g) to 5% w/v solution of titanous chloride in dilute HCl (*ca* 5 cm^3). Warm on a steam bath and, if necessary, add enough acetone to give a homogeneous solution. If the solution remains purple in colour, a nitro group is not present. If a white precipitate forms, a nitro group is present.

(b) <u>The primary (aromatic) amine group (-NH$_2$)</u>

Dissolve a small sample (*ca* 0.1 g) in 0.25 cm^3 concentrated HCl (if the material does not dissolve, it is not an amine). If the sample dissolves, dilute with water (3 cm^3) and cool the solution to 0^0 C. Add a concentrated solution of sodium nitrite, dropwise, until a slight excess is present (starch-iodide paper). Formation of a clear solution with no (or little) evolution of gas indicates that a primary aromatic amine has been converted to a diazonium salt. Confirm by adding the ice-cold solution (2 cm^3) to an alkaline solution of beta napthol. (50 mg β–naphthol in 2 cm^3 of 10% w/v sodium hydroxide solution). Formation of a red or orange azo dye confirms the presence of a primary aromatic amino group.

(c) <u>The carbonyl group (>C=O)</u>

Dissolve a small sample (*ca* 0.1 g) in the minimum volume of methanol (warm if necessary) and then add Brady's reagent (1 cm^3; 2,4-dinitrophenylhydrazine in methanol and concentrated H$_2$SO$_4$). If no precipitate forms, heat the reaction mixture in a bath of boiling water (5 min). Carbonyl compounds produce orange-red 2,4-dinitrophenylhydrazones.

(d) <u>Alcoholic hydroxyl groups (-C-OH)</u>

Dissolve *ca* 0.1 g of sample in the minimum volume of chloroform and add 4 drops of acetyl chloride (**care**- do this in a fume cupboard). Shake the mixture gently. If an alcoholic hydroxyl group is present, heat will be evolved, together with gaseous hydrogen chloride. Use alkaline litmus paper (blue) to confirm the production of HCl.

1. Tabulate your results indicating both negative and positive results. Why is it important to record negative results as well as positive ones?

2. From these functional group tests, what can you conclude about the nature of the extract?

Melting point

Determine the melting point of your extract. Do this at least three times in order to obtain a range. Compare your data with that obtained by other members of the group.

1. By comparing your melting point data with the literature values for acetyl salicylate and salicylic acid, what can you conclude about the extract ?
2. Suggest reasons why your measured melting points may vary from the literature values for acetyl salicylate and salicylic acid.

Infra Red (IR) spectroscopy

Record IR spectra of your extract and of Aspirin samples A and B.

1. Tabulate the data from the spectra including the absorption frequencies, peak shapes/intensities and any assignments.
2. What can you deduce about the composition of your extract using the IR spectra and the literature data that you have already compiled ?

Gas Chromatography (GC)

You will be given gas chromatograms obtained from samples of purified acetyl salicylate and salicylic acid.

1. Note the features of the chromatograms, especially any differences with what you might have expected.
2. What conclusions can you draw about the suitability of using GC for the analysis of acetyl salicylate and salicylic acid in a mixture (e.g. your extract) ?

High Performance Liquid Chromatography (HPLC)

HPLC is a chromatographic technique which is particularly suitable for more polar, less volatile organic chemicals. As with GC, the technique exploits the different interactions between the analytes, the mobile phase and the stationary phase of the chromatography column. However, in contrast to GC, HPLC is normally carried out a room temperature, thus limiting the thermal decomposition of any compounds in the sample. The elution order and retention times of

individual components can be compared with those of standards to enable compound identification. The usual detection method used with HPLC is UV/Visible spectrometry.

Method

Accurately weigh approximately 0.003 g (3 mg) of your extract into a vial (use a 4 decimal place analytical balance) and dissolve this in 6 cm^3 CH$_3$OH/H$_2$O (60/40 v/v) Obtain a chromatogram of this solution according to the HPLC conditions given to you. Label the peaks on the chromatogram by comparing chromatograms obtained for authentic standards (supplied).

Calculations

The peak areas obtained from the chromatograms can be used to determine the absolute and relative concentrations of the individual analytes since absorbance (UV/Visible detection) is proportional to analyte concentration according to the Beer-Lambert law:

$$A = \varepsilon cl,$$

where ε is the extinction coefficient or molar absorption coefficient, c is the concentration and l is the pathlength of the sample solution.

Since ε varies between analytes, it is necessary to perform a calibration for each analyte in order to establish the individual relationships between Absorbance (or peak area) and concentration. Using the HPLC data provided for acetyl salicylate and salicylic acid, construct calibration curves for each compound.

Calculation 1. Using the peak area data from your chromatogram, together with the calibration curves, determine (a) the absolute and (b) the relative concentrations of acetyl salicylate and salicylic acid in your extract.*

Which of (a) and (b) will you need to use to calculate the amounts of acetyl salicylate and salicylic acid in your extract ?

Calculation 2. Determine the amounts of acetyl salicylate and salicylic acid in your extract.

Compare the relative amounts of acetyl salicylate and salicylic acid in your extract with those in a solution of Aspirin in CH$_3$OH/H$_2$O (HPLC chromatogram provided).

Having completed these calculations, decide whether you 'agree' or 'disagree' with the following statements, and give your reasoning.

1. The ratio between peak areas (HPLC) of acetyl salicylate and salicylic acid gives the proportion of each compound in the extract. **(DISAGREE)**

2. The ratio of the gradients of the calibration curves for acetyl salicylate and salicylic acid gives their relative extinction coefficients. **(AGREE)**

3. Salicylic acid and acetyl salicylate have the same value for the extinction coefficient at the detection wavelength. **(DISAGREE)**

4. A high molar extinction coefficient indicates a high concentration of analyte. **(DISAGREE)**

5. The peak area ratio can be combined with the gradient ratio (calibration curves) to give the relative amounts of the two analytes in the extract. **(AGREE)**

6. The Soxhlet extraction method causes little or no degradation of acetyl salicylate to salicylic acid. **(AGREE – FOUND EXPERIMENTALLY)**

Calculation 3. Use the method of statement 5 in combination with the gravimetric and titration results from Session 2 to determine the absolute amounts of acetyl salicylate and salicylic acid (and the combined amount of these two) in the original extract.

How does this approach refine the calculation of the combined amount of acetyl salicylate and salicylic acid from the titration method alone ?
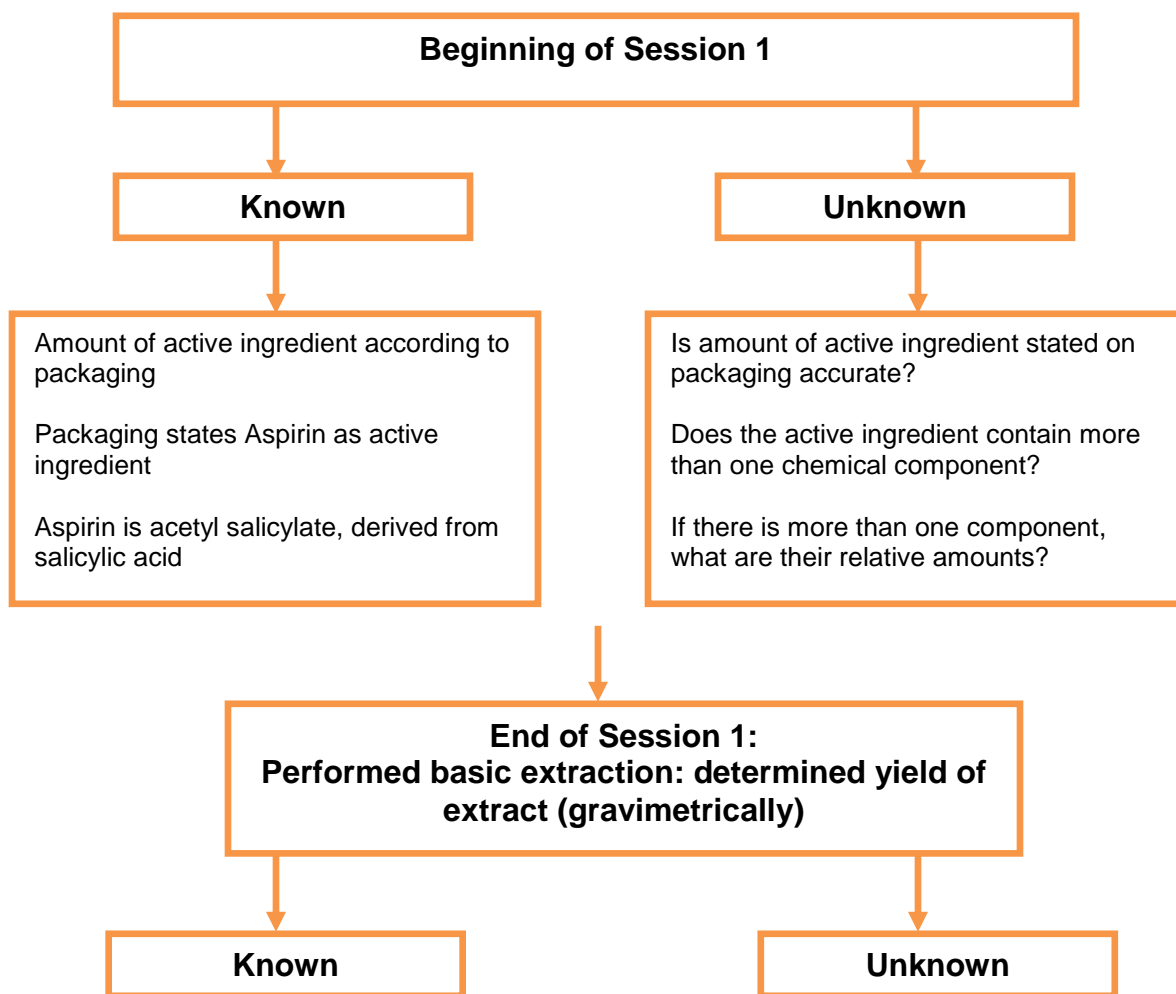

* There are two ways of doing this:

(a) Use your peak areas and the calibration curves to 'read off' the analyte concentrations.
(b) Use your peak areas combined with the 'line of best fit' to determine the analyte concentrations.

Preparation for Final Report

Over the course of these sessions, you have collected data, determined the limitations of the methods, and refined the process to improve the quality of data obtained.

As a preparation for your final report, prepare a flow diagram describing the information collected at each stage and the associated limitations. Compile information about what is known and what is not known at each stage. An example of this, corresponding to the initial stages of the investigation is shown below:

Beginning of Session 1

Known

Unknown

Amount of active ingredient according to packaging

Packaging states Aspirin as active ingredient

Aspirin is acetyl salicylate, derived from salicylic acid

Is amount of active ingredient stated on packaging accurate?

Does the active ingredient contain more than one chemical component?

If there is more than one component, what are their relative amounts?

End of Session 1:
Performed basic extraction: determined yield of extract (gravimetrically)

Known

Unknown

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