Analysis of Chemical Flavourings in Chewing Gum

Session 3

In the last session, you obtained a crude extract from samples of chewing gum using a Soxhlet apparatus. Your extract will probably be a sticky semi-solid, with a consistency not unlike that of chewed gum. You will also have noted that the mass of the extract was much higher than anticipated, suggesting that, in addition to the desired aroma chemicals, some further, undesired chemicals were also extracted. These will need to be removed prior to further analysis.

Aims and objectives

The aim of this session is to purify your crude extract, and make some further considerations of the use of the Internal Standard, before you complete your analysis of any aroma chemicals by Gas Chromatography (GC).

Briefly discuss the purification techniques you have researched as part of the pre-laboratory exercise, including the advantages and disadvantages that they offer.

Purification by Column Chromatography

Column chromatography is used routinely in organic chemistry to separate individual chemicals, but it can also be used to purify groups of chemicals in the first instance. This is achieved by applying a crude mixture (reaction mixture, extract, etc) to a column containing a stationary phase (usually silica or alumina) and eluting the desired group of chemicals using a carefully selected solvent. The resulting mixture may be analysed directly and/or subjected to further purification.

Current project

1. Establishing the solvent

In order to carry out a purification of your chewing gum extract using a simplified column chromatography procedure, you will need to decide on a solvent that will separate the desired chemicals from unwanted materials in your extract.

Arrange the following solvents in order of increasing polarity, and select the solvent that you think most suitable for purifying any aroma chemicals along with the Internal Standard.

Ethyl acetate
Hexane
Water
5% ethyl acetate in hexane
50% ethyl acetate in hexane

You may also find it useful to consider the polarities of:

- The solvent used during the Soxhlet extraction.
- The stationary phase of the column to be used (e.g. silica; SiO_2)
- The aroma chemicals and the Internal Standard

2. Establishing a purification method

The most suitable solvent for separation of the aroma chemicals and the Internal Standard from unwanted chemicals is 5% ethyl acetate in hexane. Create a method from the following set of instructions (select the numbers and a sequence), complete the purification and prepare a solution ready for subsequent analysis by GC.

1. Scrape out your extract into a small (e.g. 7 cm^3) vial.
2. Scrape out your extract into a Pasteur pipette containing some silica.
3. Add 1-2 cm^3 of 5% ethyl acetate/hexane to your extract, swirl the contents, and decant the solution to a small (e.g. 7 cm^3) vial.
4. Add 1-2 cm^3 of 5% ethyl acetate/hexane to your extract, swirl and warm the contents, and decant the solution to a small (e.g. 7 cm^3) vial.
5. Repeat the initial removal of the desired chemicals from the chewing gum extract once more, combining the solutions together in the vial.
6. Repeat the initial removal of the desired chemicals from the chewing gum extract twice more, combining the solutions together in the vial.
7. Place a cap on the vial and shake the contents until they all dissolve.
8. Place a cap on the vial, shake the contents for a few minutes and allow any solid material to settle to the bottom of the vial.
9. Decant the solution from the vial into a Pasteur pipette containing a small plug of cotton wool and 1-2 cm of silica.
10. Rinse the contents of a Pasteur pipette containing a small plug of cotton wool and 1-2 cm of silica using 5% ethyl acetate/hexane, ensuring that the solvent level is maintained just above the surface of the silica. Decant the solution from the vial into your Pasteur pipette.
11. Collect the eluent from the silica column in a round-bottomed flask.
12. Collect the eluent from the silica column in a pre-weighed round-bottomed flask.
13. Collect the eluent from the silica column in a 7 cm^3 vial.
14. Collect the eluent from the silica column in a pre-weighed 7 cm^3 vial.
15. Remove the solvent using a rotary evaporator and make a note of the smell of your extract.
16. Remove the solvent using a slow stream of N_2 gas blowing over the surface of the solution and make a note of the smell of your extract.
17. Determine the mass of the purified extract.

Correction Factor

The purification procedure may have treated carvone and camphor differently, which could lead to an incorrect determination. For example, if 50 mg of carvone and 50 mg of camphor were present in the crude extract, and 25 mg of camphor and 50 mg of camphor recovered, it would appear that the extract contained twice as much camphor as carvone. Additionally, the relative responses of carvone and camphor may depend on the type of detector used within the GC instrument (either FID or MS detection, see post-laboratory exercise), since they might combust or ionise and fragment differently, despite their close similarities in formula and structure.

In order to correct for both of these possibilities, a single, separate experiment can be carried out which determines the combined effects of purification and detection.

Method

A solution containing carvone and camphor (50 mg cm^{-3} of each compound) in 5% ethyl acetate/hexane has already been prepared for you. Decant some of this solution into a 7 cm^3 vial (about half full) and then pass this solution through a Pasteur pipette (containing silica as before) into a pre-weighed vial. Rinse the column with a further 2-3 cm^3 of solvent (5% ethyl acetate/hexane) or until the vial is nearly full. Remove the solvent under N_2, reweigh the vial and note the recovery of carvone and camphor (i.e. repeat the purification procedure that you employed for the crude product). Dilute with hexane as appropriate so that the concentration is approximately 0.1 mg cm^{-3}. In the next session, you will analyse this solution by GC to determine the combined response of carvone and camphor to the purification and detection methods.

Summary

You should now have a purified extract of aroma compounds dissolved in hexane ready for analysis by GC in the next session. This should contain the flavour chemical carvone, the Internal Standard (camphor) for quantification, and any contaminant flavourings which may be present. You should also have a solution of carvone and camphor in hexane that will serve as a *procedural blank* which is required as part of the quantification process.

3. Analysis of extracts by GC

Make a solution (0.1 mg cm^{-3}) of your purified extract in hexane using the pipettes, syringes and flasks provided.

Post Laboratory Questions for Session 3

Your samples will be analysed by GC coupled with either Flame Ionisation Detection (FID) or Mass Spectrometry (MS). FID produces a signal by measuring the current provided by electrons produced in a flame following the combustion of carbon-containing compounds. In contrast, ionisation of intact molecules yields ions that can be detected in a mass spectrometer. The resulting mass spectra are specific to the individual analytes. To help with your evaluation and discussion of your data, consider the following statements, decide whether you *agree* or *disagree* with them, and briefly justify your answer.

1. To interpret a GC-FID chromatogram of your purified extract, individual chromatograms for carvone, camphor and other aroma chemicals will be needed in order to determine their retention times.
2. The identification of chemicals in your purified extract by their GC retention times is not a guaranteed method.
3. When using MS as a detection method, there is no requirement to determine retention times of individual compounds prior to analysis of the purified extract.
4. The sensitivity of detection by FID will be identical for carvone and camphor since they both contain the same number of carbon atoms.
5. The sensitivity of detection by MS will be identical for carvone and camphor since they both contain the same number of carbon atoms.
6. The recovery of carvone and camphor from the purification procedure (5% ethyl acetate/hexane; silica column) will have been exactly the same because their chemical structures are virtually the same.
7. A correction factor needs to be applied if the relative responses to the detection methods and/or the relative recoveries from the purification procedure are different for carvone (and other aroma chemicals) and camphor.

Adapt your session-by-session flow diagram to incorporate any new information that you have.

Session 4 pre-laboratory exercise

In session 3, it was shown that the analysis of your purified extract may need to be corrected due to non-equivalence of behaviour of the analytes (carvone and camphor) either during purification or at the detection stage.

Note: The correction factor is a ratio describing the relative sensitivity of the detector for the two analytes (carvone and camphor) combined with the relative efficiencies of their purification.

From the equations below, choose the one that you think is the correct equation for determining the response factor, F, where C_A is the concentration of camphor, C_B is the concentration of carvone, A_B is the peak area of carvone and A_A is the peak area of camphor.

$$F = \frac{C_A}{C_B} \times \frac{A_B}{A_A}$$

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$$F = \frac{C_A A_A + C_B A_B}{C_A + C_B}$$

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