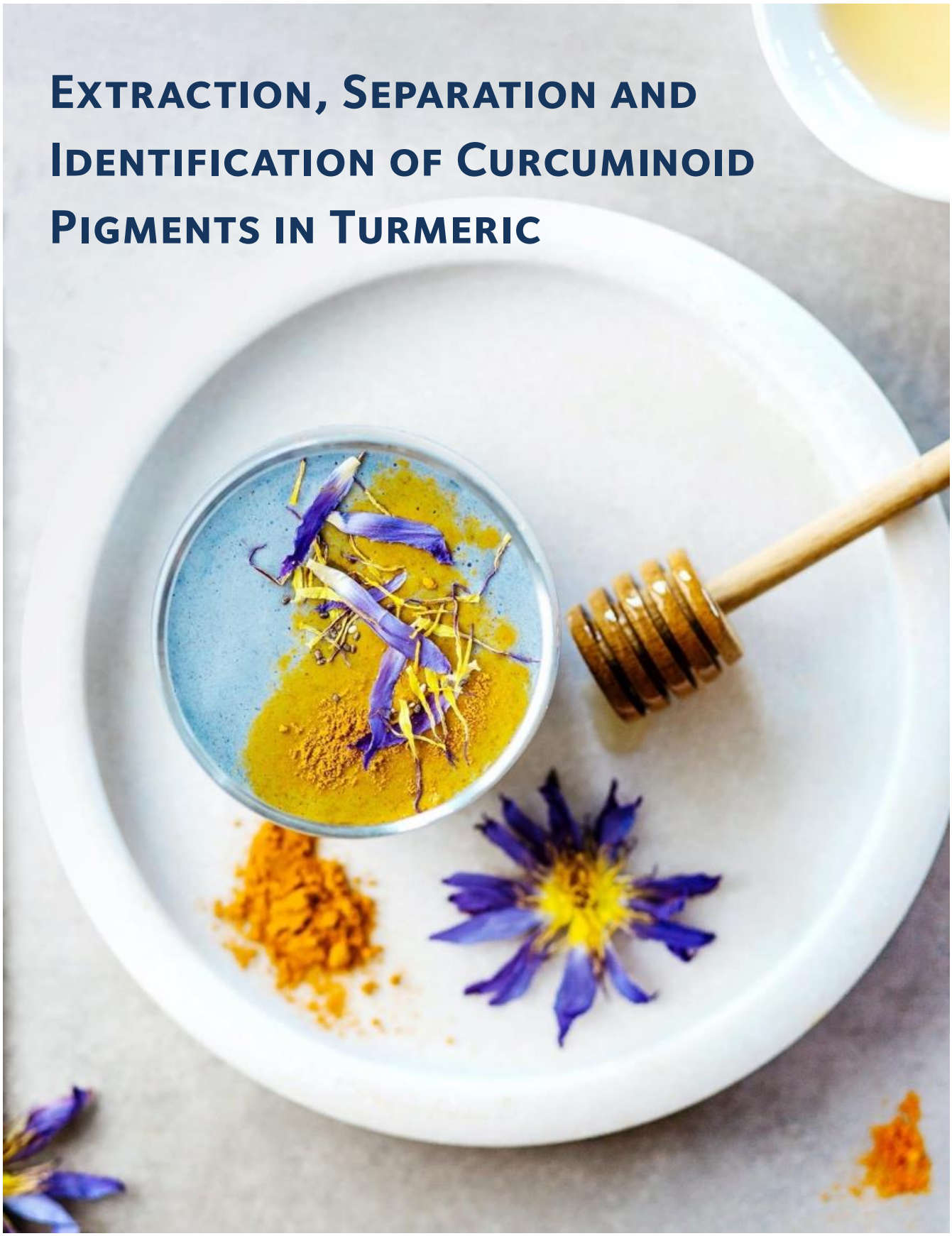
# Extraction, Separation and Identification of Curcuminoid Pigments in Turmeric



*Length of Practical: 1 hr*

## Objectives

- Extract and separate the dyes (curcuminoids) in turmeric.
- Understand the theory of TLC and use this to separate the products of extraction from turmeric.
- Identify unknown curcuminoids using a combination of experimental data analysis.

## Introduction and Context

The spice turmeric, that is widely used in cooking, is a powdered version of the *Curcuma longa* plant. There are at least 110 different species of this plant and turmeric is a member of the ginger family. For over 4000 years, people have been using the plant and/or powder for cooking, religious and medicinal purposes. In cooking, turmeric provides a vibrant yellow/orange colour to many dishes, such as curries, as well as providing an earthy and slightly citrus flavour. Given its use in traditional medicine, research into identifying the active ingredients has been undertaken, to assess whether turmeric can be of use in modern medicine. So far, scientists have observed anti-inflammatory effects in human dendritic cells by inhibiting the response to inflammatory cytokines (chemical signals) and anti-microbial effects of metal complexes of turmeric extracts.

Figure 1: Turmeric root and powder

It has been established that multiple phytochemicals (molecules/chemicals made by plants) present in turmeric powder are responsible for the observed health benefits. As part of this identification process, chemists need to extract, separate and investigate the properties of the individual phytochemicals from turmeric.

Isolating all the biologically active components is a significant task. Today, you are going to isolate the intensely coloured chemicals – the curcuminoids – which are responsible for one of turmeric's other common usages, namely, as a natural dye.

# Theory

## Solvent Extraction

Solvent extraction is an important technique in organic chemistry for the separation of a compound(s) from a mixture. It is often the initial method employed for isolating natural products from plant materials. Many of us carry out solid-liquid extraction every day whilst making a cup of tea – placing a tea bag in boiling water extracts the tannins, theobromine, and caffeine from the tea leaves into the water!


Figure 2: Tea brewing in a beaker

In liquid-liquid extraction, the solute is added to two immiscible (not forming a homogenous mixture) solvents, usually water and an organic solvent, and components of the solute partition themselves between the two solvents depending on their relative solubilities in each of the phases. The two layers are then separated. In solid-liquid extraction, a solid solute(s) is separated from a mixture of solids by dissolving it in a liquid phase. The insoluble solids are then removed by filtration. Further techniques are normally required to allow the isolation of pure compounds.

## Thin Layer Chromatography (TLC)

Chromatography is one of the principal techniques used for purifying mixtures of compounds. There are various forms of this which exploit different properties of the molecules within a mixture, generally based on size, solubility, charge or polarity. It relies on the interactions of the molecules between the stationary phase (usually a solid phase) and a mobile phase (commonly a liquid or a gas).

Thin layer chromatography, (TLC), similar to paper chromatography, is a commonly used technique in synthetic chemistry to enable a scientist to investigate how many components are within a mixture, by separating them apart. Given that TLC is carried out on a small scale, this is not a suitable method for purifying/separating significant quantities of materials;

however, it does enable chemists to identify conditions needed to perform larger-scale chromatography for separation. Rather than using paper as the stationary phase, a solid absorbent, such as alumina or silica, is spread as a thin layer on an inert base such as glass, aluminium foil or insoluble plastic. The polarity and water-solubility of the molecules under investigation determine whether paper or another material is the most suitable choice of stationary phase .

The mixture is 'spotted' at the bottom of the TLC plate and allowed to dry. The plate is placed in a closed vessel containing solvent(s) (the mobile phase) so that the liquid level is below the spot. The solvent ascends the plate by capillary action. This technique is usually performed in a closed vessel to ensure that the atmosphere is saturated with solvent vapour and that evaporation from the plate is minimised before the run is complete. The plate is removed when the solvent front approaches the top of the plate and the position of the solvent front recorded before it is dried. The retention factor value, **$R_f$ value**, is then calculated. The $R_f$ values are characteristic of the compound in a particular solvent at a particular temperature. The diagram below shows you how to calculate $R_f$ values.

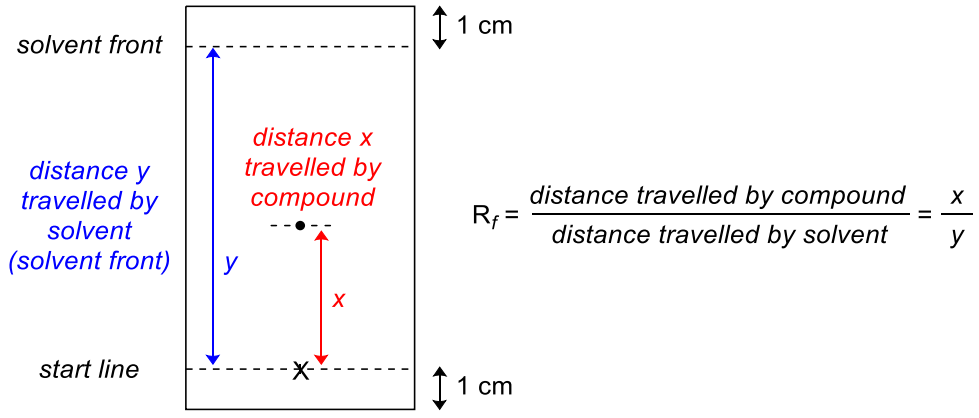



Figure 3: Labelled TLC plate

As the solvents rise up the plate, it carries the mixture of compounds with it. The progression at which the individual compounds move up the plate, and, therefore, the resulting $R_f$ values, depends on how the compounds interact with the stationary phase compared with the mobile phase (the relative strengths of the intermolecular forces). In "normal phase" TLC, a polar stationary phase (e.g. silica gel) is used with a less polar mobile phase (e.g. organic solvent(s)). Compounds that have a high affinity with the stationary phase tend be polar molecules and may have O or N atoms which can form hydrogen bonds to the stationary phase (silica gel has O-H bonds at the surface) and therefore they cling to the stationary phase surface.

These compounds spend less time in the mobile phase and therefore move slowly up the plate and have low $R_f$ values. Less polar molecules have a higher affinity to the less polar mobile phase and therefore will spend more time in the mobile phase. This means it will travel faster up the plate and have a high $R_f$ value.

Different solvents can be used to make up the mobile phase and these will lead to different separations of molecules. This practical uses solvents that are greener (ethanol, isopropanol) than those typically used in a chemistry research lab for this particular set of compounds (i.e. dichloromethane and methanol), though the separation is less effective.

## Mass Spectrometry

Whilst TLC can enable separation of the various components in a mixture, we cannot identify what each spot actually is. This is where spectroscopic measurements are needed, for example, mass spectrometry. Mass spectrometry is a useful analytical tool to help identify compounds, or confirm the structures of the products of chemical reactions. One form of mass spectrometry works on the basis that atoms or molecules that carry a charge can be deflected by a magnetic field. The force experienced by the charged particle (atom or molecule) depends upon the charge on the particle. Therefore, lighter partciles will be deflected more than heavier ones. The amount of deflection can be used to "sort" the particles by their mass (strictly, this is the mass:charge ratio, *m/z*, but we can assume here that all the particles being analysed carry a single charge). An alternative type of mass spectrometer starts by bombarding the sample with electrons to charge the molecules in the sample. This process normally results in the molecule fragmenting to produce smaller fragments.

This mixture of ions is accelerated in an electric field, so that all the species have the same kinetic energy. This results in the heavier fragments having a slower speed. After the fragment ions spread out depending on mass, the fragments of the molecule are detected electrically. This type of separation and detection is referred to as a "time-of-flight" system. The output from either type of spectrometer is a stick diagram which shows you what the spectrometer has detected at different values of *m/z*. A spectrum for pentane shows a peak for the whole molecule $CH_3CH_2CH_2CH_2CH_3^+$ at *m/z* 72, and then fragments such as $CH_3CH_2CH_2CH_2^+$ at *m/z* 57 and $CH_3CH_2CH_2^+$ at *m/z* 43:

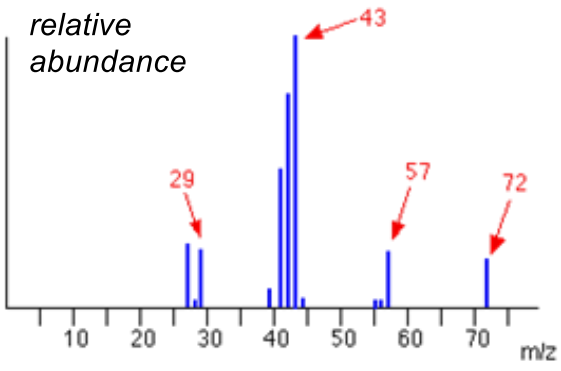



Figure 4: Mass spectrum of pentane

# Practical Work

## Health & Safety

A full risk assessment must be carried out before undertaking this practical work. We advise teachers and technicians to refer to either the CLEAPSS website or SSERC website for up to date health and safety information. We assume no liability with regard to injuries or damage to property that may occur as a result of using the information contained in these resources.

Turmeric can stain clothes, surfaces and skin. Take care to avoid permanent staining of skin and clothes by wearing gloves and a lab coat.

Handle TLC plates only at the edges, to minimise transfer of oils from the skin onto the TLC plate; TLC plates may have sharp corners, so take care handling them, using tweezers to hold them.

## Procedure

### Equipment and consumables

You will be provided with the following materials:

- Test tube and rack
- Spatula
- Glass rod
- Measuring cylinder
- Funnel
- Filter paper
- Boiling tube and rack
- Silica TLC plate
- TLC spotters / capillary tubes
- 250 mL beaker
- Watch glass
- Tweezers
- 0.5 g ground turmeric
- 10 $cm^3$ ethanol
- TLC eluent (70:30 cyclohexane : IPA)
- Opt. 0.5% boric acid in 1:1 ethanol : water

You will also need:

- Pen
- Pencil
- Ruler

## Pre-Lab Questions

Read through the procedures below and then answer the following questions:

1. When extracting the curcuminoids from turmeric, which extraction method is used? Explain how this method works.
2. In the experiment outlined below, what is the mobile phase and what is the stationary phase in the TLC separation?
3. Why is a pencil rather than a pen used to draw the base line on the TLC plate?
4. Why do you place a watch glass over the glass beaker when the TLC is developing?
5. What factors affect the $R_f$ value of a molecule? Refer to polarity and intramolecular forces in your answer.

## Procedure - Extraction

1. Weigh out 0.5 g of ground turmeric and place in a test tube.
2. Measure out 5 mL of ethanol and add to the turmeric in the test tube.
3. Gently stir for 2-3 minutes using a long spatula or glass rod. Set aside to soak, while you complete steps 1, 2 and 3 of the TLC Separation below.
4. Give a final stir for a minute. Then, filter the mixture using a funnel and fluted filter paper into the boiling tube; (you do not have to wait to filter the whole suspension, as you only need a small volume for TLC separation). Return to step 4 in the TLC Separation below.

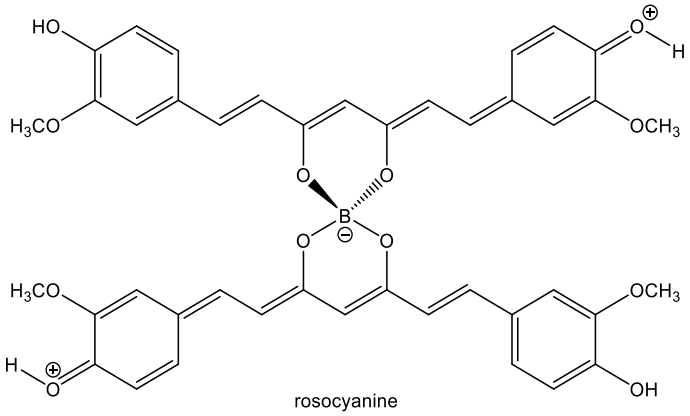
## Procedure – TLC Separation

1. Draw a horizontal pencil line around 1 cm above the bottom of the TLC plate and draw an X in the centre to mark where the sample will be spotted.
2. Measure out 10 mL of the TLC eluent (70:30 cyclohexane : IPA) into a 250 mL glass beaker. (*Ensure the solvent level is below the level of the spots*).
3. Add a folded filter paper, soaked in TLC eluent, to the TLC chamber: this minimises evaporation of the TLC eluent. Cover with a watch glass. (*See Figure 5*). Return to step 4 in the Extraction above.
4. Use a capillary tube to spot the sample on the X just drawn. Try to spot a minimal amount of sample onto the TLC plate as this will give the best result – this is best done by keeping a finger on top of the capillary tube, dipping the tube into the sample and slowly lifting your finger until a minimal amount of your sample is in the tube.

Figure 5: TLC Chamber

5. When the spot of sample is dry, stand the TLC plate in the TLC eluent using tweezers (make sure that the solvent level is not above the line with the sport on it) and cover the beaker with a watch glass.
6. When the solvent front has reached about 1 cm from the top of the plate, remove the plate and draw a horizontal line with a pencil to mark the maximum height of the solvent front.
   Allow the TLC plate to dry and calculate $R_f$ values for all spots. Record these in a table.

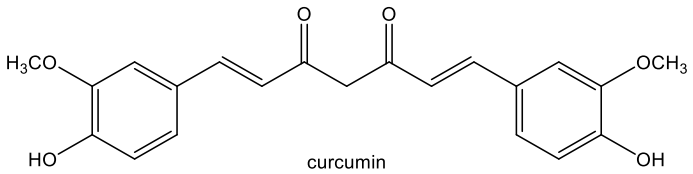
Optional: To make the spots more visible, you can dip the dried TLC plate in 0.5% w/v boric acid in 1:1 ethanol:water, then dry the TLC plate again. (The compounds react with boric acid to form more intense orange products such as rosocyanine)



rosocyanine

## Post-Lab Questions – Spectra and TLC Analysis

1. How many spots can you see on your TLC plate?
2. The principal dye component in turmeric is curcumin, whose structure is given below:



curcumin

   Comparison with a 95% purity curcumin sample in the same solvent system you are using gave curcumin an $R_f$ value of ~0.40.

   Sketch the TLC plate, and identify which spot corresponds to curcumin.

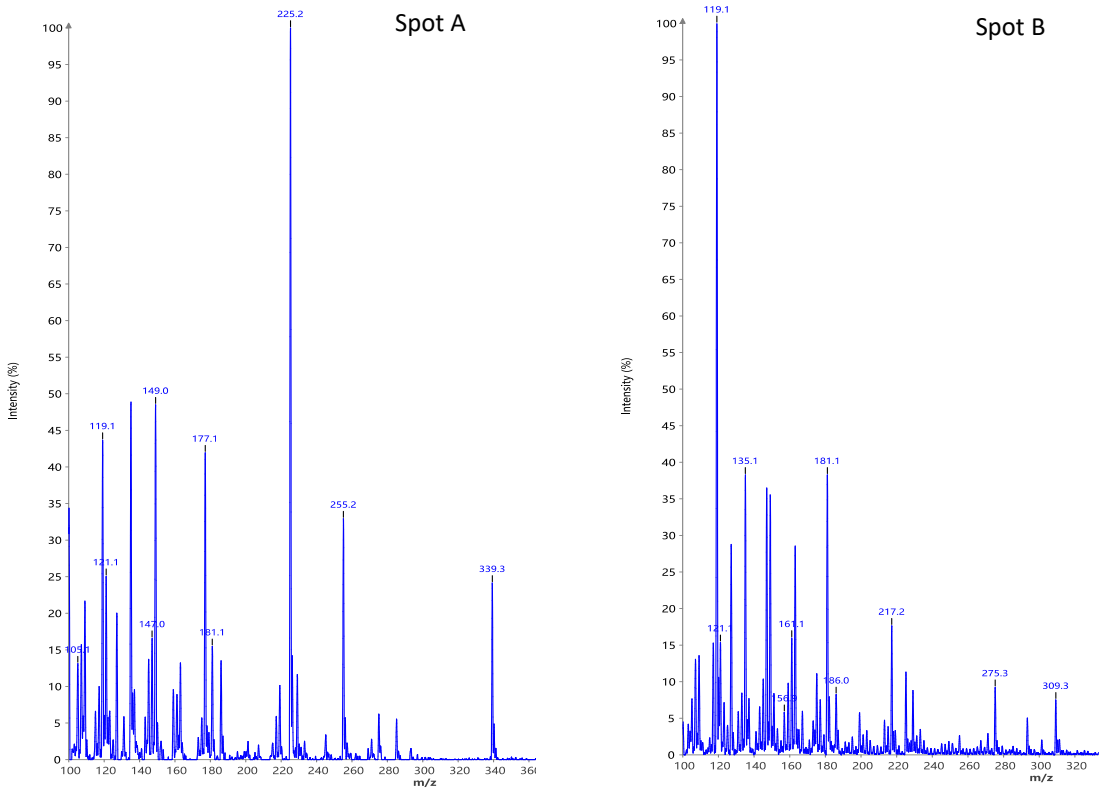
A relatively new technique of TLC-MS is available for use by undergraduates in the £6 M Analytical Suite in the Chemistry Teaching Laboratory at the University of Oxford. This technique provides an opportunity to collect MS data directly from a TLC late. Please see our video of this technique being deployed.

3. When TLC-MS is performed on the TLC plates using this solvent system, mass spectrometry data of the spots is not viable as the material is too diffuse. With a different solvent system of dichloromethane and methanol, researchers at the University of Oxford are able to produce TLC plates viable for use with TLC-MS, as shown in the video. Below is a table detailing the hazards of the solvents along with their waste streams.

| System 1:<br>Isopropanol : Cyclohexane | System 2:<br>Dichloromethane : Methanol |
|---|---|
| **Isopropanol**<br>H225 – Highly flammable liquid and vapour<br>H319 – Causes serious eye irritation<br>H336 – May cause drowsiness or dizziness<br>Standard organic waste disposal | **Dichloromethane**<br>H315 – Causes skin irritation<br>H319 – Causes serious eye irritation<br>H336 – May cause drowsiness or dizziness<br>H351 – Suspected of causing cancer<br>Requires special waste disposal |
| **Cyclohexane**<br>H225 – Highly flammable liquid and vapour<br>H304 – May be fatal if swallowed and enters airways<br>H315 – Causes skin irritation<br>H336 – May cause drowsiness or dizziness<br>H410 – Very toxic to aquatic life with long lasting effects<br>Standard organic waste disposal | **Methanol**<br>H225 – Highly flammable liquid and vapour<br>H301 + H311 + H331 – Toxic if swallowed, in contact with skin or if inhaled<br>H370 – Causes damage to organs (eyes, central nervous system)<br>Standard organic waste disposal |

Using the information in the question, consider the pros and cons of each solvent system.

4. From the TLC-MS data, two compounds closely related to curcumin (i.e. their structures are somewhat similar) have been identified. The mass spectra for these two compounds are shown below:




By referring to the mass spectra, determine the relative formula masses of the compounds in spots A and B.

N.B. The mass spectrometry data were obtained by a method that utilises chemical ionisation, so the molecule is protonated to give it a charge and form a $[M+H]^+$ species. Therefore, the *m*/*z* value of the molecular ion is one higher than expected.

5. Given the structure of curcumin shown in question 2, and the relative formula masses of the compounds in spots A and B, suggest possible structures for these two compounds related to curcumin (hint: their structures are similar to curcumin).

## Acknowledgements and copyright



This resource has been authored by members of the Department of Chemistry at the University of Oxford.