# Experiment: Extraction and analysis of fruit components

This experiment involving the extraction and analysis of fruit components was designed by Dr Dawei Deng at HortResearch, Hamilton.

## Samples:

kiwifruit, oranges and lemons

## Reagents and Solutions:

Ethyl acetate
Hexane
Ethanol
Molybdophosphoric acid

Developing solution: 4:1 hexane/ethyl acetate (i.e., add together 80ml of hexane and 20ml of ethyl acetate, and mix). You will need 10ml per student or group of students.
Visualising solution: 5% Molybdophosphoric acid solution in a spray bottle (i.e., add 5g of molybdophosphoric acid to 100ml of ethanol, stir until all the solid has dissolved and then allow to stand for a while before transferring the top, clear solution to a bottle with a spray).

## Apparatus per student / group of students:

Beaker (250ml)
Capillary tubes (commercially available)
Pre-coated silica gel plates (commercially available) – use strips approximately 4cm x 8cm
Test tubes
Measuring cylinder
Knife

## Apparatus per class:

Aluminium foil
Spray bottle
Solvent containers and bottles
Oven

## Procedure:

**Extraction:**

* Take about 5g (half a thumb size) of each fruit sample and chop to small pieces (the smaller the better
* Put the chopped samples into separate test tubes and label (for example “K” for kiwifruit)
* Add ethyl acetate to the tubes (just covering all of fruit pieces), and shake for 10-15 minutes.

**Analysis using Thin Layer Chromatography (TLC)**:
Preparing the TLC plate:

* Get a pre-coated silica gel TLC plate that is approximately 4cm wide and 8cm long. Be careful to hold it on the sides, and not to get your fingerprints on the silica coat.
* Use a pencil (not pen) and ruler to draw a straight line parallel to the shorter side of the plate, about 1.5cm from one end of the plate. Then mark three equal interval points on the line. Mark “K” for kiwifruit, “O” for orange and “L” for lemon under the points.

**Transferring the samples:**

* Place a capillary tube into a test tube containing the solution of kiwifruit, and allow the solution to rise in the capillary. This will happen spontaneously
* Once the capillary is ‘loaded,’ hold it vertically just above the “K” point on pencil line on the plate.
* Lower the capillary until its end just touches the plate. You will observe that some of the solution flows from the capillary onto the plate. Leave the capillary in contact with the plate only briefly so that the spot is no larger than 1mm in diameter. Then lift the capillary from the TLC plate and allow the solvent to completely evaporate from the spot.
* Make a second deposit on the same spot with the capillary and allow the solvent to completely evaporate.
* Repeat 3-4 times.
* Do the same for the solutions of orange and lemon on the “O” and “L” points on the pencil line.
* Place the plate flat on a clean dry surface for 5 minutes and allow the solvent to completely evaporate.

**Developing the TLC plate:**

* Pour developing solution (4:1 of hexane/ethyl acetate) into a beaker or small wide-mouth glass bottle to a depth of about 5mm
* Pick up, by hand or using tweezers, the TLC plate at the top (the end opposite where the pencil line is drawn). Place it carefully in the beaker as vertical as possible. Make sure that the two vertical edges do not touch the sides of the beaker.
* Tightly cover the beaker with aluminium foil
* When the developing solution has moved to within 5-8mm of the top of the plate, remove the plate from the beaker and quickly draw a pencil line marking the solvent front.
* Place the plate flat on a clean dry surface and allow the solvent to completely evaporate.

**Visualising the samples:**

Check the plate. You may find some colour spots, which are visible components in the fruits. To see those invisible components, you need do the following:

* Spray 4% visualising solution on the dry plate and place it in a 120°C oven for a few minutes.
* Remove the plate, using tweezers, once dark spots appear on the plate.

**Analysing the results:**

* Compare the dark spots for each fruit sample. Each spot represents a different molecule found in the fruit. Can you see that different fruits contain different molecules? Are there any that are the same?
* Measure the distance of solvent movement (i.e. the distance from the bottom pencil line to the pencil line marking the solvent front). Also measure the distances that each of the component molecules have moved (i.e. the distance from the bottom pencil line to the centre of each spot).
* Calculate the Rf value of each substance. This is the ratio of substance advance distance to solvent advance distance:

Rf = Ls/Ln where Ls = length of the distance of solvent advance
Ln = length of the distance of advance of the substance

Different components will give different Rf values. Those components with the same Rf value will have the same, or very similar, chemical structures.