Activity 5: Thin-layer chromatography

The compounds in essential oils can be separated and analysed using thin-layer chromatography. This activity can be performed with extracted or commercially bought oils. Orange and spearmint oil are described here but lavender and clove oils can also be analysed if desired. Students can compare the chromatograms from the oils with chromatograms from known pure chemical standards, such as limonene and carvone, which are present in orange and spearmint oil respectively.

Materials

- Essential oils extracted in activity 4 or commercially bought oil
- Glass Pasteur pipette
- Hexane (C_6H_{14})
- Spotting tile
- Stationary phase: thin-layer chromatography plate pre-coated with silica gel, about 5 cm x 1.2 cm
- Eluent (mobile) phase: 0.5 ml 10% ethyl acetate ($CH_3COOC_2H_5$) in hexane (C_6H_{14})
- Glass vial
- Fine paint brush or capillary tube drawn out to produce a pointed tip
- Potassium permanganate staining reagent[w1] ($KMnO_4$) (to detect limonene and carvone)
- p-anisaldehyde staining reagent[w1] ($CH_3OC_6H_4CHO$) (to detect carvone)
- Forceps
- Paper towel
- Hot air gun or hairdryer

Optional:

- Ultraviolet light source of 254 nm

Safety note: Eye protection should be worn. Do not look directly into an ultraviolet lamp. Take care using the hot air gun as the metal part can burn.

1. Using a pipette, add a few drops of hexane to the extracted oil or commercially bought oil in a well of the spotting tile.

Supporting material for:

Allan A, Worley B, Owen M (2018) Perfumes with a pop: aroma chemistry with essential oils. *Science in School* **44**: 40–46. www.scienceinschool.org/2018/issue44/distillation

2. Using a fine paint brush or pointed capillary tube, apply the hexane/oil solution to a thin layer chromatography plate, 1 cm from the bottom. The smaller the diameter of the spot the better – try to keep it to 1–2 mm. One application should be enough.
3. Add enough eluent to cover the bottom of a new glass vial. Place the chromatography plate into the glass vial with the solvent, cover the top and remove the plate when the eluent has risen to about 0.5 cm from the top of the plate. Allow to dry briefly.
4. To stain the plates, dip the plate in the p-anisaldehyde or permanganate staining reagents. Using forceps, remove any drips on a paper towel and heat with a hot air gun. Stop heating when spots appear (figures 1 and 2).
5. To identify the spots, perform thin layer chromatography under the same conditions as for pure chemicals. You can then compare the chromatograms for the oil and for the chemical, for example comparing orange oil and limonene (figure 1), or spearmint oil and carvone. One drop of the chemical in about 1 ml of hexane will work before applying them to the plate.

Optional:

Spearmint carvone can also be detected using ultraviolet light if the plate has a fluorescent indicator present in the silica gel. Follow steps 1–3 before viewing the plate under a ultraviolet light source (figure 3).

Figure 1: Thin layer chromatography plates with orange oil (left) and limonene (right) stained with permanganate

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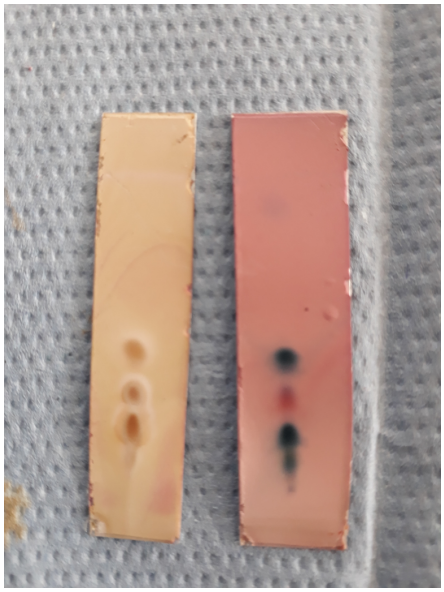

Figure 2: Thin layer chromatography plate with spearmint oil stained with permanganate (left) and p-anisaldehyde (right). The spot second from top is the terpene carvone

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Figure 3: Thin layer chromatography plate with spearmint oil visualised with ultraviolet light. The visible spot is carvone, the molecule mainly responsible for the spearmint aroma

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Discussion

After the practical work, discuss the following questions with your students:

- Why is chemical staining or ultraviolet light required to visualise the essential oil compounds on the chromatogram?
- How could you identify the compounds using the spots on the chromatogram?

Web reference

w1 – A description for making potassium permanganate and p-anisaldehyde staining reagents for thin layer chromatography plates is available on the McMaster University website. See: www.chemistry.mcmaster.ca/adronov/resources/Stains_for_Developing_TLC_Plates.pdf

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