

Chromatography

How does chromatography separate and identify the substances in a mixture?

●●○ Intermediate

Analytical Chemistry

Last reviewed 4 October 2026

Before you start: it helps to understand [Classification of Matter](#) and [Functional Groups](#).

What is it?

Chromatography separates the substances in a mixture so that each one can be identified and measured. Every method has two phases:

- a **stationary phase**, which stays in place (paper, a thin layer of silica on a plate, or the inside of a column);
- a **mobile phase**, which moves through or over it (a solvent, or a gas).

As the mobile phase moves, each substance in the mixture is constantly shared between the two phases. A substance that is attracted more strongly to the stationary phase spends more time stuck there and moves **slowly**; one that prefers the mobile phase moves **quickly**.

Different substances therefore travel different distances (or take different times) and come apart.

KEY IDEA

Separation depends on the **balance of attractions**: stronger attraction to the stationary phase means slower movement. A pure substance gives **one** spot or peak; a mixture gives several.

Why does it matter?

- **Identification.** Forensic scientists identify inks, drugs and explosives; food chemists check for banned dyes.
- **Purity.** One spot on a plate is a quick check that a product is pure; chemists use columns to purify the compounds they make.
- **Measurement.** Gas and liquid chromatography measure pesticides in food, alcohol in blood and drugs in urine, often down to billionths of a gram.

How does it work?

1. Paper and thin-layer chromatography (TLC)

1. Draw a **baseline** in **pencil** (ink would itself separate) about 1 cm from the bottom of the paper or plate.
2. Put small spots of the mixture and of known **standards** on the baseline.
3. Stand the plate in a little solvent, **below** the baseline (otherwise the spots would dissolve into the solvent), and cover the container so the air stays saturated with solvent vapour.
4. Remove the plate before the solvent reaches the top, and mark the **solvent front** at once.
5. Make colourless spots visible (for example under UV light) and measure the distances.

2. The retention factor, R_f

$$R_f = \frac{\text{distance moved by spot}}{\text{distance moved by solvent}}$$

Both distances are measured **from the baseline** (to the centre of the spot) in the same unit, so R_f has **no unit** and lies between 0 and 1. Under the same conditions (same stationary phase, solvent and temperature), a substance always has the same R_f , so a spot can be identified by matching it to a standard run **on the same plate**.

3. The role of polarity

On a TLC plate of **silica** (very polar), polar compounds, such as those with $-\text{OH}$ or $-\text{COOH}$ groups, are held strongly and have **low** R_f values; non-polar compounds, such as hydrocarbons, have **high** R_f values. Using a **more polar solvent** pulls all the compounds further and raises every R_f .

4. Gas chromatography (GC)

In GC the sample is vaporized and carried by an unreactive gas (such as helium) through a long, thin **column** coated with the stationary phase. A detector at the end records a **peak** as each substance comes out.

- The **retention time**, the time from injection to the peak, identifies a substance (compare with standards run under the same conditions).
- The **area** under a peak is proportional to the **amount** of that substance. For quantities, a calibration with standards is used, as in spectrophotometry.

Liquid chromatography (HPLC) works the same way, with a liquid mobile phase pumped through a packed column.

∞ THINK OF IT LIKE THIS

Picture a crowd walking through a shopping street. Window-shoppers (strongly attracted to the shops, the stationary phase) stop often and move slowly; people hurrying to work (who prefer the moving crowd, the mobile phase) get through quickly. After an hour, the two groups are far apart, even though everyone set off together.

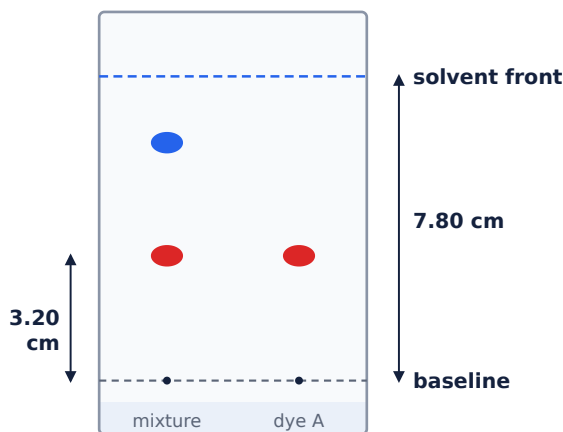
Q MORE PRECISELY

A substance distributes between the phases according to its **partition** (or adsorption) equilibrium, which depends on intermolecular forces: hydrogen bonding, dipole–dipole forces and dispersion forces. In GC, volatility matters too: compounds with low boiling points spend more time in the gas and come out first. Two substances with the same R_f or retention time under one set of conditions are **not** necessarily the same compound; confirming identity needs a second method, such as a different solvent or column, or mass spectrometry.

Visualise it

Thin-layer chromatography

$R_f = \text{distance moved by spot} \div \text{distance moved by solvent}$



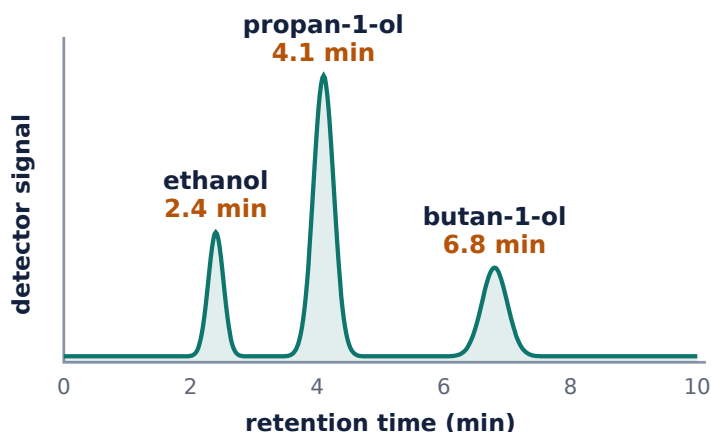
Red spot: $R_f = 3.20 \text{ cm} \div 7.80 \text{ cm} = 0.410$, same as dye A.

The blue spot ($R_f 0.782$) is a second, different dye.

Distances are measured from the baseline. The red spot matches dye A run on the same plate.

A gas chromatogram

Each peak is one compound; peak area shows how much.



Here, the lower the boiling point, the sooner the peak.

Retention time identifies each compound; peak area tells how much is present.

Worked example

WORKED EXAMPLE: Calculating an R_f value

Question: On a TLC plate, the solvent front is 7.80 cm above the baseline and a red spot is 3.20 cm above it. Find R_f .

Step 1 $R_f = \frac{3.20 \text{ cm}}{7.80 \text{ cm}} = 0.410$ (the cm cancel)

WORKED EXAMPLE: Identifying a dye with replicate plates

Question: A food dye is run on three plates beside standards. Its R_f values are 0.42, 0.44 and 0.43. Standards on the same plates: dye P 0.43, dye Q 0.52. Which dye is it?

Step 1 Mean: $\bar{R}_f = \frac{0.42 + 0.44 + 0.43}{3} = 0.43$

Step 2 Standard deviation (sample, $n - 1$): $s = 0.01$

Step 3 Report $R_f = 0.43 \pm 0.01$ ($n = 3$). Dye P (0.43) lies within this spread; dye Q (0.52) is 9 standard deviations away.

Step 4 The dye is consistent with **dye P**. Running replicates shows the result is reproducible, not a one-off reading.

 WORKED EXAMPLE: Composition from GC peak areas

Question: A GC run of a solvent mixture gives peak areas of 1250 (ethanol), 3400 (propan-1-ol) and 850 (butan-1-ol), in the same arbitrary units. Assuming the detector responds equally to each, find the percentage of each component.

Step 1 Total area = $1250 + 3400 + 850 = 5500$

Step 2 Ethanol: $\frac{1250}{5500} \times 100\% = 22.7\%$

Step 3 Propan-1-ol: $\frac{3400}{5500} \times 100\% = 61.8\%$

Step 4 Butan-1-ol: $\frac{850}{5500} \times 100\% = 15.5\%$

Step 5 Check: $22.7\% + 61.8\% + 15.5\% = 100.0\%$. ✓

Common mistake

 COMMON MISTAKE: Measuring from the bottom of the plate

Both distances in R_f are measured from the **baseline**, not from the edge of the plate. Measuring from the edge makes every R_f too large.

 COMMON MISTAKE: Comparing R_f values from different conditions

R_f depends on the solvent, the stationary phase and the temperature. Only compare with standards run **on the same plate**, or under identical conditions.

 COMMON MISTAKE: Drawing the baseline in ink or below the solvent

Ink contains dyes that separate too; a baseline below the solvent level lets the sample dissolve away into the solvent instead of travelling up the plate.

 NOTATION NOTE

- R_f is the retention factor (sometimes called the retardation factor); it is written without a unit.
- TLC = thin-layer chromatography; GC = gas chromatography; HPLC = high-performance liquid chromatography.
- The substance being analysed is often called the analyte; the mobile phase in GC is the carrier gas.

Remember this

REMEMBER THIS

- Stationary phase stays put; mobile phase moves. Stronger attraction to the stationary phase = slower.
- R_f = distance moved by spot $\div$ distance moved by solvent front, both from the baseline; no unit; compare with standards on the same plate.
- On polar silica: polar compounds have low R_f ; a more polar solvent raises all R_f values.
- GC: retention time identifies; peak area measures amount. Measure in replicate and report mean $\pm$ standard deviation.

References

1. Brown, T. L.; LeMay, H. E., Jr.; Bursten, B. E.; Murphy, C. J.; Woodward, P. M.; Stoltzfus, M. W. Chemistry: The Central Science, 15th ed.; Pearson, 2022.

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