Analytical techniques (A Level)

Thin-layer, gas and liquid chromatography, carbon-13 NMR, and proton NMR including chemical shift, integration, the n + 1 rule, TMS, deuterated solvents and D2O exchange.

37.1 Thin-layer chromatography

Chromatography separates a mixture between a **stationary phase** and a **mobile phase**.

- **Stationary phase:** a thin layer of a solid, such as **aluminium oxide** or silica, on a glass or plastic plate.
- **Mobile phase:** a **solvent** (polar or non-polar) that moves up the plate by capillary action.
- Spots of the sample are placed on a pencil **baseline**; the plate stands in a shallow layer of solvent in a covered tank. Remove it when the **solvent front** is near the top and mark it. Colourless spots are shown with **ultraviolet light** or a locating agent.

$$R_f = \frac{\text{distance moved by the substance}}{\text{distance moved by the solvent front}}$$

R_f is between 0 and 1 and, under the same conditions, is **characteristic** of a substance, so it identifies compounds by comparison with known samples.

Substances differ in R_f because of how strongly they are **adsorbed on the stationary phase** (more polar compounds stick more, and move less) and how **soluble** they are in the mobile phase (the more soluble, the further they move).

Calculate Rf from the baseline

Measure from the baseline to the **centre of the spot** and from the same baseline to the solvent front. Use matching distance units. The ratio depends on stationary phase, solvent mixture and other conditions, so compare unknown and standard on the same plate when possible. One matching R_f supports identity but does not prove it uniquely: different compounds may happen to travel similarly.

Worked example: the solvent front moves 8.00 cm and an unknown spot centre moves 5.20 cm. $R_f = 5.20/8.00 = 0.650$. A standard at 5.20 cm under the same conditions is consistent with the same compound. A second solvent system or another analytical method can distinguish accidental matches.

Keep the initial spots above the solvent surface so they do not simply dissolve into the reservoir. Use pencil for the baseline because ink components may separate. Mark the solvent front promptly before evaporation makes its location uncertain. A small concentrated spot helps avoid broad overlapping bands. A covered chamber limits evaporation and gives more reproducible solvent conditions.

Note Academy

TRY IT

The baseline is 1.00 cm from the plate bottom, spot centre 4.00 cm from the bottom and front 7.00 cm from the bottom. Find Rf.

Answer: Spot travel is 3.00 cm and front travel is 6.00 cm, giving **0.500**. Dividing 4.00 by 7.00 incorrectly measures from the bottom rather than the baseline.

Explain separation rather than memorising polarity slogans

On a polar silica/alumina stationary phase, stronger adsorption tends to slow a compound. Greater preference for the mobile solvent tends to carry it further. Both interactions matter, and changing solvent composition can change relative travel. A blanket "polar compounds always have low R_f" ignores the mobile phase and is unreliable across different systems.

Worked interpretation: a mixture gives three resolved spots, including one that matches a standard. It contains at least three distinguishable components under that method, and one may match the standard. A sample with one spot may be pure, or may contain components that co-migrate or are invisible to the chosen locating method. Describe the evidence with its practical limit.

37.2 Gas / liquid chromatography

In **gas–liquid chromatography (GLC)**:

- the **stationary phase** is a **high boiling point, non-polar liquid** coated on a solid support in a column;
- the **mobile phase** is an **unreactive carrier gas** (helium or nitrogen);
- the mixture is vaporised, and the components emerge at different **retention times** (the time from injection to the peak reaching the detector).

A component with stronger **interaction** (attraction) with the stationary phase, or a higher boiling point, has a **longer retention time**. The **area under each peak** is proportional to the amount of that component, so the **percentage composition** is found from the areas: $\%A = \dfrac{\text{area of A}}{\text{total area}} \times 100.$ Identification is by comparing retention times with known samples (or by passing the output to a mass spectrometer).

Reading a chromatogram quantitatively

A detector records signals as compounds leave the column. Retention time is the horizontal position, measured from injection, not the time between successive peaks. Peak area reflects the detected amount under the stated calibration; a tall narrow peak can have less area than a short broad peak. Use area rather than height for percentage calculations when the question specifies area proportionality.

Note Academy

Worked example: three peak areas are 24.0, 36.0 and 60.0 arbitrary units. Total is 120.0, so the mixture proportions are 20.0%, 30.0% and 50.0% under the stated equal-response assumption. Real detector response factors can differ between compounds, so supplied calibration information takes priority over a simple area fraction.

> **TRY IT**
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> Peak A has area 45.0 and peak B 15.0. Calculate A's proportion and explain why peak heights alone are insufficient.

Answer: A is $45.0/(45.0 + 15.0) \times 100 = 75.0\%$ under the proportional-response model. Width contributes to area, so height alone is not a complete measure of detected amount.

Retention time and identity

Retention depends on interaction with the stationary liquid and volatility, as well as column temperature and carrier-gas conditions. A component that spends more time dissolved in the stationary phase emerges later. A higher boiling point often lengthens retention within a suitable comparison, but intermolecular interactions can change the order.

Use a standard analysed under the same conditions as supporting identification. Coupling the chromatograph to a mass spectrometer supplies mass/fragmentation evidence for each separated component. The combination is more informative than assuming one retention time uniquely identifies a molecule in every instrument.

37.3 Carbon-13 NMR spectroscopy

In ^{13}C **NMR** each carbon atom in a **different chemical environment** gives **one peak**. Carbon atoms in the same environment (equivalent by symmetry) give a single peak, so the **number of peaks** equals the **number of different carbon environments**.

Note Academy

Carbon environment	Approximate δ / ppm
C–C (alkane)	5–45
C–N, C–Cl, C–Br	25–65
C–O	50–90
C=C (alkene) and aromatic C	100–150
C=O (acid, ester, amide)	160–185
C=O (aldehyde, ketone)	190–220
C≡N	110–125

> **TRY IT**
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> How many peaks are in the ^{13}C spectrum of propanone, and of propan-1-ol?

Propanone, CH_3COCH_3: the two methyl carbons are equivalent, so **2 peaks** (one at about 205 ppm for C=O, one at about 30 ppm). Propan-1-ol has three different carbons, so **3 peaks**.

Use the number of peaks and their shifts to deduce possible structures. Benzene gives 1 peak (all carbons equivalent); methylbenzene gives 5.

Count chemical environments using symmetry

The usual syllabus ^{13}C spectrum is proton-decoupled: each distinct carbon environment gives one signal without routine proton splitting. Count environments before consulting chemical shifts. Symmetry can make distant carbons equivalent, while adjacent carbons may be different if their bonding surroundings differ.

Worked comparison: butane has two environments, because its two end methyl carbons are equivalent and its two middle methylenes are equivalent. Butan-1-ol has four different carbon environments: neither end nor the middle carbons have the same surrounding groups. 2-methylpropane has two environments, one for its three equivalent methyl groups and one for the central carbon.

Methylbenzene has five carbon environments: methyl, the ring carbon attached to it, an equivalent pair at 2/6, another pair at 3/5 and the opposite ring carbon at 4. Six ring carbons therefore need not give six signals. A candidate structure must satisfy both the number of signals and their plausible shift regions.

> **TRY IT**
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> Predict the number of carbon signals for ethanol and methoxymethane.

Note Academy

Answer: Ethanol gives two because its carbons have different environments. Methoxymethane gives one because its two methyl carbons are equivalent by symmetry. They are functional-group isomers with the same molecular formula but different carbon spectra.

Use shifts as constraints, not exact fingerprints alone

Carbonyl carbons resonate well downfield compared with saturated alkyl carbons. Carbons directly bonded to electronegative atoms are typically shifted relative to simple alkyl groups. Exact shifts depend on the full environment, so use the data table's ranges and do not reject a sound structure solely because an illustrative value differs by a few ppm.

Worked deduction: a four-carbon ester gives three carbon signals, including an ester-region carbonyl and an oxygen-linked saturated carbon. Propan-2-yl methanoate, $HCOOCH(CH_3)_2$, fits: carbonyl, O–CH and two equivalent methyl carbons give three environments. Methyl propanoate normally has four. Check proton data and the molecular formula before claiming the carbon count alone proves one structure.

37.4 Proton (^{1}H) NMR spectroscopy

A ^1H **NMR** spectrum shows peaks for hydrogens in different environments.

Information from the spectrum

1. **Number of signals (sets of lines):** the number of different **proton environments** in the simple cases studied. A split signal can contain several lines without representing several environments.
2. **Chemical shift**, δ (ppm): the environment of the proton (see the Data section for ranges). Typical values:

Note Academy

Environment	δ / ppm
$R{-}CH_3$, R_2CH_2	0.9–1.7
$CH_3{-}C{=}O$	2.0–2.5
$CH_3{-}N$	2.2–2.9
$-CH_2{-}Hal$	3.0–4.0
$-CH_2{-}O$ (alcohol, ester)	3.3–4.3
alkene $C{=}CH$	4.5–6.0
aromatic $Ar{-}H$	6.5–8.0
aldehyde $-CHO$	9.5–10.5
$-OH$ (alcohol), $-NH_2$	1–5.5 (variable)
$-COOH$	10–12

3. **Relative peak areas (integration):** the **ratio of the numbers of protons** in each environment.
4. **Splitting pattern:** the **n + 1 rule**. A proton with **n equivalent protons on the adjacent carbon** gives a peak split into **n + 1** lines: **singlet** (n = 0), **doublet** (1), **triplet** (2), **quartet** (3), multiplet (more). So ethyl, $-CH_2CH_3$, shows a quartet for the CH_2 and a triplet for the CH_3.

> **TRY IT**
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> A compound $C_4H_8O_2$ has peaks: δ 1.2 (triplet, 3H), δ 2.0 (singlet, 3H), δ 4.1 (quartet, 2H). Identify it.

The singlet 3H at δ 2.0 is $CH_3{-}C{=}O$. The quartet 2H at δ 4.1 is $-OCH_2-$ next to a CH_3 (triplet, δ 1.2). The compound is **ethyl ethanoate**, $CH_3COOCH_2CH_3$.

Standards and solvents

- **TMS** (tetramethylsilane, $Si(CH_3)_4$) is the **standard** for chemical shift measurements, set at δ = 0. It is used because it gives a **single, sharp peak** (12 equivalent protons), is **chemically inert**, is **volatile** (easily removed), and its peak is **away from most others** (a high-field position).
- **Deuterated solvents** (for example $CDCl_3$, or D_2O) greatly reduce solvent proton signals that could obscure the sample. Small residual protonated-solvent or water signals can still appear.

Note Academy

Identifying O–H and N–H protons: D_2O exchange

Add a few drops of D_2O to the sample and re-run the spectrum. The protons of **–OH and –NH** groups **exchange** with deuterium (e.g. $R{-}OH + D_2O \rightleftharpoons R{-}OD + HOD$), so their peaks **disappear** from the spectrum. This identifies which peak is the O–H or N–H proton, since they appear at variable shifts.

Signals, lines and integration are different measurements

A signal represents a chemical environment; splitting creates several **lines within that signal**. An ethyl group typically supplies two signals, a triplet and a quartet, rather than seven proton environments. Integration measures the total area of each signal including all its lines, giving the relative number of contributing protons. The tallest individual line does not directly count hydrogens.

The $n + 1$ rule applies to simple first-order coupling with an equivalent set of neighbouring protons. Equivalent protons do not split each other, and coupling to several non-equivalent sets can give more complex patterns. Exchangeable OH/NH protons often do not follow the simple adjacent-carbon rule because exchange affects their coupling. Use the simplified conditions appropriate to the given spectrum.

Worked example — ethanol: methyl H atoms are adjacent to two equivalent CH_2 protons, giving a triplet. The CH_2 protons are adjacent to three equivalent methyl protons, giving a quartet. Their integrated areas are 3 : 2. An OH signal may appear separately with variable position and shape and disappears on D_2O exchange. The OH proton should not automatically be included as another fixed splitting neighbour.

> **TRY IT**
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> A signal integrates to 6H and is a doublet; another integrates to 1H and is a septet. Suggest a fragment.

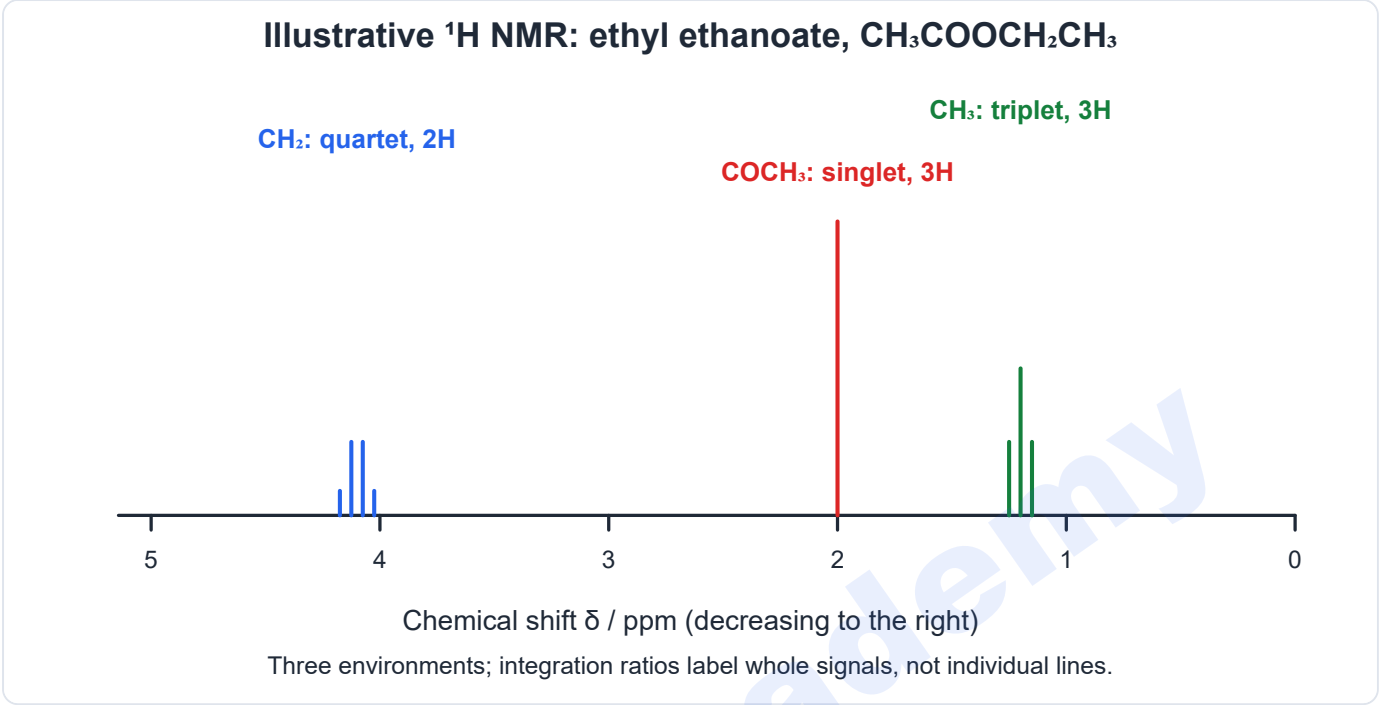
Answer: An isopropyl-like $(CH_3)_2CH{-}$ fragment under the simple coupling model. The two equivalent methyl groups are split by one neighbouring H; the methine is split by six equivalent methyl H atoms. The full structure still requires shifts and molecular formula.

A structured route to a molecular assignment

1. Use molecular formula/mass and any IR evidence to restrict functional groups.
2. Count proton environments and turn integration ratios into H counts consistent with the formula.
3. Recognise useful patterns such as an ethyl triplet/quartet pair.
4. Use shifts to place those fragments next to electronegative atoms or carbonyl groups.
5. Assemble the fragments, then check **every** signal and atom against the proposal.

Note Academy

Worked example — distinguish two esters: methyl propanoate, $CH_3CH_2COOCH_3$, has a 3H singlet for OCH_3 and an ethyl-like pair whose CH_2 lies next to C=O. Ethyl ethanoate has a 3H singlet for CH_3CO- and a CH_2 adjacent to oxygen, commonly near 4 ppm. Both have formula $C_4H_8O_2$ and can show a 3 : 2 : 3 integration pattern, but their shifts locate the ethyl group on opposite sides of the ester linkage.



Worked combined deduction: C_3H_6O has a carbonyl IR absorption, two carbon environments, and a proton spectrum with one 6H singlet. Propanone fits: its two methyl groups are equivalent and have no adjacent carbon-bound H to split them. Propanal would have three carbon environments and distinct aldehyde/alkyl proton signals, contradicting the data.

> **TRY IT**
>
> A $C_3H_6O_2$ ester has two proton signals, integrating to 3H each, both singlets. Suggest its structure.

Answer: Methyl ethanoate, CH_3COOCH_3. Its two methyl groups are in different environments and have no adjacent carbon-bound proton set producing ordinary splitting. A carboxylic-acid alternative would require accounting for an exchangeable proton and a different signal pattern.

Standards, solvents and exchange checks

TMS gives one reference signal because its twelve protons are equivalent. A deuterated solvent greatly reduces solvent proton signals, but small residual protonated-solvent peaks can remain. Water contamination can also add signals. Therefore “every observed small peak must be an impurity in the organic product” is too strong without checking known solvent/reference positions.

Note Academy

D_2O exchange replaces exchangeable H with deuterium, which is not detected in the ordinary proton spectrum in the same way. The disappearing signal supports OH or NH assignment. It does not mean the entire molecule or all hydrogen atoms have disappeared. D_2O/HOD-related signals and changes in exchange behaviour can complicate a real experiment; the basic syllabus deduction uses the missing analyte OH/NH signal.

> **TRY IT**
>
> A broad 1H signal disappears after D_2O addition while a 3H methyl signal remains. What does this establish?

Answer: The disappearing proton is exchangeable, consistent with OH or NH, while the methyl hydrogens are carbon-bound and retained. Further evidence distinguishes alcohol, acid, phenol or an NH-containing group.

Chapter check: compare chromatography under matched conditions, use areas rather than heights, count symmetry-equivalent NMR environments, and justify a structure against all supplied evidence. The diagrams and numerical spectra here are illustrative teaching examples, not measured sample data.

References and further reading

Scope follows section 37 of the **Cambridge 9701 syllabus for 2025–2027**. **Chemguide's Cambridge topic index** links to alternative explanations. The worked exercises and added diagrams are original teaching examples; numerical examples are illustrative unless a specific source is identified.

Note Academy