NMR spectroscopy, accurate mass and chromatography

High resolution mass spectrometry, carbon-13 and proton NMR including chemical shift, peak area and splitting, and paper, thin-layer, gas and high-performance liquid chromatography.

High-resolution mass spectrometry

A high-resolution mass spectrometer measures m/z to several decimal places. Because atomic masses are not whole numbers, **different molecular formulae with the same nominal mass have different accurate masses**, so the molecular formula can be identified.

Accurate relative atomic masses used: $^{1}H = 1.0078$, $^{12}C = 12.0000$, $^{14}N = 14.0031$, $^{16}O = 15.9949$.

> **TRY IT**
>
> CO ($M = 27.9949$) and N_2 ($M = 28.0062$) both have a nominal mass of 28. A peak is measured at 28.0060. Which is it?

The measured value is closest to 28.0062, so it is N_2.

To calculate an accurate mass, add the accurate masses of each atom in the formula, using four decimal places.

Carbon-13 NMR

Nuclear magnetic resonance spectroscopy uses radio waves to detect the behaviour of nuclei with spin in a magnetic field. ^{13}C NMR gives information about **carbon environments**.

- **One peak per distinct carbon environment.** Carbons that are in an identical environment by symmetry give a single peak.
- The **chemical shift**, δ (in ppm, relative to tetramethylsilane, TMS, at 0), shows the kind of carbon.

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Carbon environment	δ / ppm
C–C (alkyl)	5–40
C–Cl, C–Br	25–80
C–O (alcohols, ethers)	50–90
C=C (alkene) and aromatic	100–150
C=O (carboxylic acid, ester)	160–185
C=O (aldehyde, ketone)	190–220

The number of peaks shows the number of different carbons. Propane has two peaks (the two CH_3 carbons are equivalent), but propan-1-ol has three.

Proton NMR

Proton NMR gives information about the **hydrogen atoms** in a molecule. Samples are dissolved in a solvent without hydrogen (such as $CDCl_3$ or CCl_4), with **TMS** as the reference.

What a spectrum shows

1. **Number of peaks** – number of different **proton environments**.
2. **Chemical shift**, δ – the type of environment.
3. **Relative peak area** (integration, often shown as a number or a trace) – the **ratio of the numbers of protons** in each environment.
4. **Splitting pattern** (high resolution) – the number of protons on **adjacent** carbon atoms.

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Proton environment	δ / ppm
$R-CH_3$, $R-CH_2-R$	0.9–1.7
$CH_3-C=O$ or $CH_2-C=O$	2.0–2.9
CH_2-Hal	3.0–4.2
CH_2-O (alcohols, esters)	3.3–4.3
O–H (alcohols)	1.0–5.5 (variable)
C=C–H	4.5–6.0
Aromatic H	6.0–9.0
Aldehyde, $-CHO$	9.4–10.0
Carboxylic acid, $-COOH$	10–12

The $(n+1)$ rule

A proton or group of equivalent protons with n **protons on the adjacent carbon(s)** gives a peak split into $n+1$ lines.

Adjacent protons	Splitting	Name	Relative intensities
0	1	Singlet	1
1	2	Doublet	1 : 1
2	3	Triplet	1 : 2 : 1
3	4	Quartet	1 : 3 : 3 : 1

Equivalent protons on the same carbon do not split each other. Protons on $-OH$ typically show as a singlet because of exchange.

> **TRY IT**
>
> Predict the proton NMR spectrum of ethyl ethanoate, $CH_3COOCH_2CH_3$.

Three environments in the ratio **3 : 2 : 3**. CH_3CO-: singlet at about δ 2.0 (no adjacent H). $-OCH_2-$: **quartet** at about δ 4.1 (3 adjacent H on the CH_3). $-CH_3$: **triplet** at about δ 1.3 (2 adjacent H).

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Work from the splitting to the structure. A quartet at 4 ppm with a triplet next to it is $-O-CH_2-CH_3$. A singlet with area 3 at 2.1 ppm is a methyl on a carbonyl. A $(CH_3)_2CH-$ group shows a doublet (6H) and a septet (1H).

Deuterium exchange: shaking with D_2O makes the O–H or N–H peak **disappear**, which identifies it.

Chromatography

Chromatography separates the components of a mixture using a **mobile phase** and a **stationary phase**. Components differ in how strongly they are **adsorbed** on the stationary phase and how soluble they are in the mobile phase.

Paper and thin-layer chromatography (TLC)

- **Paper**: the stationary phase is water held in the paper. **TLC**: a thin layer of silica or alumina on a plate.
- Spot the sample on a **pencil** baseline, place in a covered container with the solvent below the line, let the solvent rise, and mark the solvent front. Spots may need a locating agent (UV light or ninhydrin for amino acids).

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

R_f is always **less than 1**. Different R_f values arise from the different affinities of the components for each phase. Compare with known standards, using the same solvent and conditions.

Gas chromatography (GC) and HPLC

These are **column chromatography** methods. Components pass through a column and emerge at different **retention times**.

- **GC**: the mobile phase is an inert **gas** and the stationary phase is a liquid on a solid support in a column. Suited to volatile substances.
- **HPLC** (high-performance liquid chromatography): a **liquid** under high pressure through a column of fine particles.

Both can be linked to a **mass spectrometer** (GC-MS), which identifies each component as it leaves the column. Applications include **forensics**, **drug testing in sport**, and detecting pollutants.

Note Academy