NOTESKARTS

Premium Study Notes | B.Pharma 8th Semester

BP811ET — ADVANCED INSTRUMENTATION TECHNIQUES

UNIT I: NMR Spectroscopy & Mass Spectrometry

PCI / AKTU Aligned

★ PREMIUM PAID NOTES ★

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

Introduction and Basic Principles

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical technique that exploits the magnetic properties of certain atomic nuclei. It is the most powerful tool for elucidating the structure of organic molecules, including pharmaceuticals.

Nuclear Spin and Magnetic Properties

Every atomic nucleus has a property called nuclear spin (I). Only nuclei with non-zero spin quantum number ($I \neq 0$) are NMR active.

Nucleus	Spin (I)	NMR Active?	Natural Abundance (%)	Pharmaceutical Use
^{1}H (Proton)	1/2	Yes ✓	99.98	Primary structural tool
^{13}C (Carbon)	1/2	Yes ✓	1.11	Carbon skeleton mapping
^{14}N (Nitrogen)	1	Yes (broad)	99.64	Rarely used in pharma
^{12}C (Carbon)	0	No ✗	98.89	Silent — not observed
^{16}O (Oxygen)	0	No ✗	99.76	Silent — not observed
^{19}F (Fluorine)	1/2	Yes ✓	100	Fluorinated drugs
^{31}P (Phosphorus)	1/2	Yes ✓	100	Phospholipids, DNA

📌 **Rule** A nucleus is NMR active if and only if its spin quantum number $I \neq 0$. Nuclei with even mass number AND even atomic number have $I = 0$ and are NMR silent.

Behaviour in External Magnetic Field (B_0)

When an NMR-active nucleus is placed in an external magnetic field (B_0), it aligns either parallel (low energy, α-spin) or anti-parallel (high energy, β-spin) to the field. For a spin-1/2 nucleus:

- **α-spin (m = +1/2):** Aligned WITH B_0 — lower energy state (majority of nuclei at equilibrium)
- **β-spin (m = -1/2):** Aligned AGAINST B_0 — higher energy state

The energy difference between these two states (ΔE) is:

$$\Delta E = h \cdot \nu_0 = h \cdot \gamma \cdot B_0 / (2\pi)$$

Where: h = Planck's constant | ν_0 = Larmor frequency | γ = gyromagnetic ratio | B_0 = magnetic field strength

Resonance Condition

When radiofrequency (RF) radiation of exactly the right frequency (ν_0 — the Larmor frequency) is applied, the nucleus absorbs energy and flips from α → β state. This is the NMR resonance condition.

> ⚡ **Exam Trick** Higher magnetic field → Higher resonance frequency → Better resolution. Modern NMR instruments use superconducting magnets (300–900 MHz for ^1H).

Proton NMR (^1H-NMR) Spectroscopy

^1H-NMR is the most commonly used NMR technique because ^1H has 99.98% natural abundance and the highest gyromagnetic ratio among common nuclei, giving strong signals.

Chemical Shift (δ)

Chemical shift is the position of an NMR signal on the δ (delta) scale, expressed in parts per million (ppm). It indicates the electronic environment surrounding a nucleus.

$$\delta\ (ppm) = (\nu_{sample} - \nu_{TMS}) / \nu_{spectrometer} \times 10^6$$

TMS (Tetramethylsilane, $Si(CH_3)_4$) is used as internal reference standard (δ = 0.00 ppm) because:

- It gives a single, sharp signal (all 12 protons are equivalent)
- Its protons are highly shielded → signal appears at far upfield (δ 0)
- It is chemically inert, volatile, and soluble in most organic solvents
- It does not interfere with sample signals

Shielding and Deshielding

Concept	Description	Effect on δ	Example
Shielding	Electron density AROUND nucleus shields it from B_0	δ DECREASES (upfield)	CH_4: δ 0.23 ppm
Deshielding	Electron withdrawal REDUCES shielding from B_0	δ INCREASES (downfield)	$CHCl_3$: δ 7.26 ppm

Typical ^1H-NMR Chemical Shift Reference Table

Proton Type	δ (ppm)	Reason
TMS (reference)	0.00	Internal standard
R-CH_3 (alkyl, primary)	0.8–1.0	High electron density — shielded
R-CH_2-R (secondary)	1.2–1.4	Slightly less shielded
R_3CH (tertiary)	1.4–1.8	Further deshielded
R-C≡C-H (alkynyl)	2.5–3.0	Anisotropy — moderate deshielding

Proton Type	δ (ppm)	Reason
Ar-CH_3 (benzylic)	2.3–2.5	Aromatic ring effect
R-O-CH_3 (methoxy)	3.3–3.5	O withdraws electrons
R-CH_2-Cl (allylic-Cl)	3.5–4.0	Cl withdraws electrons
R-CH=CH_2 (vinyl/olefinic)	4.5–6.0	Double bond anisotropy
Ar-H (aromatic)	6.5–8.5	Ring current — strong deshielding
R-CHO (aldehyde)	9.4–10.0	Carbonyl + ring current effect
R-COOH (carboxylic acid)	10–12	Hydrogen bonding + carbonyl

Factors Affecting Chemical Shift

Several electronic and structural factors influence the position of NMR signals. Understanding these is CRITICAL for spectral interpretation.

Factor 1: Inductive Effect (Electronegativity of Substituents)

Electronegative substituents (F, Cl, O, N) withdraw electron density from the proton via σ bonds, reducing shielding and shifting δ downfield.

- CH_4: δ 0.23 ppm → CH_3Cl: δ 3.05 ppm → CH_2Cl_2: δ 5.30 ppm → $CHCl_3$: δ 7.26 ppm
- More electronegative atoms = more deshielding = higher δ value

> 📌 **Rule** Effect falls off with distance. β-substituents have 30–40% effect of α-substituents. γ and beyond are negligible.

Factor 2: Magnetic Anisotropy

Electrons in π systems and triple bonds create secondary magnetic fields that can shield or deshield nearby protons.

Group	Proton Position	Effect	δ Observed	Explanation
Benzene ring	Ring protons (Ar-H)	Deshielded	6.5–8.5 ppm	Ring current — B_0 induces circulation, field adds to B_0 at ring periphery
C=C double bond	Vinyl protons	Deshielded	4.5–6.5 ppm	π electrons generate anisotropic field
C≡C triple bond	Alkynyl proton (≡C-H)	Shielded	1.8–3.1 ppm	Proton lies along axis — shielding cone

Group	Proton Position	Effect	δ Observed	Explanation
C=O carbonyl	Aldehyde proton	Deshielded	9.4–10.0 ppm	Both electric effect + anisotropy

⚡ **Exam Trap** Alkyne protons appear UPFIELD (lower δ) compared to alkene protons — many students assume alkynes are more deshielded. They are NOT. Alkynyl protons lie in the shielding cone!

Factor 3: Hydrogen Bonding

Hydrogen bonding deshields the proton involved, shifting it downfield. The effect is variable because it depends on concentration, temperature, and solvent.

- OH of ethanol: δ 1–5 ppm (variable — depends on H-bonding)
- COOH of benzoic acid: δ 10–12 ppm (strongly H-bonded)
- NH of amides: δ 6–8 ppm

Factor 4: Solvent Effects

The solvent can alter chemical shifts. Aromatic solvents (C_6D_6) cause upfield shifts due to ring current. Polar solvents (DMSO-d_6, CD_3OD) affect H-bonding equilibria.

- $CDCl_3$ (deuterated chloroform): Most common NMR solvent — δ 7.26 ppm residual peak
- D_2O (deuterium oxide): Used for aqueous samples — exchanges OH, NH protons
- DMSO-d_6: Good for polar compounds — shows OH/NH as sharp peaks

Factor 5: Concentration and Temperature

- **Concentration:** At higher concentrations, intermolecular H-bonding increases → shifts OH/NH peaks downfield
- **Temperature:** Raising temperature disrupts H-bonding → OH/NH peaks shift upfield and sharpen

Spin-Spin Coupling

Spin-spin coupling (scalar coupling, J-coupling) is the splitting of NMR signals due to the magnetic influence of neighbouring NMR-active nuclei. It occurs through covalent bonds.

The n+1 Rule (First-Order Spectra)

For a proton with n equivalent neighbouring protons, the signal is split into (n+1) lines with relative intensities following Pascal's triangle:

Neighbours (n)	Multiplicity	Name	Relative Intensities	Pharma Example
0	1 line	Singlet (s)	1	–OCH_3 in codeine (3H, no neighbours)
1	2 lines	Doublet (d)	1:1	CH–CH_2 (methine next to methylene)

Neighbours (n)	Multiplicity	Name	Relative Intensities	Pharma Example
2	3 lines	Triplet (t)	1:2:1	–CH_2– next to –CH_2– (e.g. ethyl ester)
3	4 lines	Quartet (q)	1:3:3:1	–CH_3 of ethanol: quartet
4	5 lines	Quintet	1:4:6:4:1	Central CH_2 between two CH_2 groups
5	6 lines	Sextet	1:5:10:10:5:1	n-propyl groups
6	7 lines	Septet	1:6:15:20:15:6:1	–$CH(CH_3)_2$ isopropyl: septet

⚡ **Classic Example** Ethanol (CH_3CH_2OH): CH_3 appears as triplet (2+1=3, split by 2H of CH_2); CH_2 appears as quartet (3+1=4, split by 3H of CH_3). This is the textbook example every examiner loves!

Coupling Constant (J)

The coupling constant J is the distance (in Hz) between adjacent peaks in a multiplet. It is a measure of the strength of spin-spin interaction. Unlike chemical shift, J is INDEPENDENT of the applied magnetic field (B_0).

J (Hz) = Δν (Hz) between adjacent lines of multiplet

Coupling Type	Symbol	J Value (Hz)	Example
Geminal (2J)	^2J	0–20 Hz	H-C-H across one carbon
Vicinal (3J)	^3J	0–18 Hz	H-C-C-H (most common)
Long-range (4J, 5J)	^4J	0–3 Hz	Aromatic / allylic
Trans alkene	^3J	12–18 Hz	E-isomer: J ≈ 15–17 Hz
Cis alkene	^3J	6–12 Hz	Z-isomer: J ≈ 7–11 Hz
Aromatic (ortho)	^3J	6–9 Hz	Adjacent benzene ring protons
Aromatic (meta)	^4J	1–3 Hz	Para: ~0 Hz (very small)

⚡ **Exam Trick** J value can distinguish cis/trans isomers! Trans J = 12–18 Hz; Cis J = 6–12 Hz. This is a FAVOURITE MCQ!

Equivalent Protons and Chemical Equivalence

Chemically equivalent protons have the same chemical shift and do NOT couple with each other to produce observable splitting. Examples:

- The 6 protons of $(CH_3)_2$ in isopropyl group are all equivalent → appear as one singlet
- The 4 ortho protons of para-substituted benzene form an AA'BB' system

Relaxation in NMR

After the RF pulse, nuclei return from the high-energy β-state back to the low-energy α-state. This process is called relaxation and is described by two time constants.

Parameter	T_1 (Spin-Lattice Relaxation)	T_2 (Spin-Spin Relaxation)
Also called	Longitudinal relaxation	Transverse relaxation
Process	Energy transfer from nucleus → surroundings (lattice)	Energy exchange between nuclei (same type)
Direction	Along z-axis (direction of B_0)	Along xy-plane (perpendicular to B_0)
Effect	Restores Mz to equilibrium (M_0)	Destroys phase coherence of Mxy
Typical value	Seconds (solids) to milliseconds (liquids)	$T_2 \leq T_1$ always
Peak shape effect	Determines recycle delay	Determines linewidth: $\Delta\nu = 1/(\pi \cdot T_2)$
MRI relevance	T_1-weighted images (tissue contrast)	T_2-weighted images (water content)

📌 **Important Relationship** $T_2 \leq T_1$ always. In liquids (low viscosity), $T_1 \approx T_2$. In solids and viscous solutions, $T_2 << T_1$, causing line broadening.

⊕ **Clinical Link** MRI (Magnetic Resonance Imaging) is based on NMR relaxation! T_1-weighted MRI shows anatomy; T_2-weighted MRI shows pathology (edema, tumors). This principle directly comes from this unit!

Carbon-13 NMR (^{13}C-NMR) Spectroscopy

^{13}C-NMR provides direct information about the carbon skeleton of a molecule. Unlike ^{1}H-NMR, each unique carbon gives one signal in a broad-band decoupled (BBD) spectrum.

Property	^{1}H-NMR	^{13}C-NMR
Natural abundance	99.98%	1.11% (very low)
Sensitivity	High (strong signal)	Low — needs signal averaging
Chemical shift range	0–12 ppm (narrow)	0–220 ppm (wide)
Coupling	H-H coupling observed	C-H coupling suppressed in BBD
Peak integration	Proportional to H count	NOT reliable — different NOE
Information given	Proton environment	Carbon types (C, CH, CH_2, CH_3)
DEPT experiment	Not applicable	Distinguishes C, CH, CH_2, CH_3
Reference	TMS: δ 0 ppm	TMS: δ 0 ppm

Typical ^{13}C-NMR Chemical Shift Ranges

Carbon Type	δ (ppm)	Example
Alkyl C (primary R-CH_3)	10–30	Methyl groups in steroids
Alkyl C (secondary R-CH_2)	20–45	Cyclohexane ring carbons
Alkyl C (tertiary R_3CH)	25–50	C-atoms adjacent to branch points
C next to O (C-O, ether/alcohol)	50–90	Morphine C-4, C-5
Alkyne C (C≡C)	70–100	Ethynyl estradiol C-17
Aromatic C (Ar-C)	110–160	Benzene: δ 128 ppm
Aldehyde C (R-CHO)	190–205	Benzaldehyde: δ 190.9 ppm
Ketone C (R-CO-R)	195–220	Acetone: δ 206 ppm
Carboxylic acid / ester C=O	160–185	Aspirin carbonyl: ~170 ppm

NMR Instrumentation

Modern NMR spectrometers are highly sophisticated instruments. The key components are:

Block Diagram of NMR Spectrometer

✏ **DIAGRAM TO DRAW** Draw a block diagram with the following components connected in order: Magnet → Sample Probe → RF Transmitter → RF Receiver → Preamplifier → ADC (Analog-to-Digital Converter) → Computer/Data System → Printer/Display. Use arrows to show signal flow. Label the magnet as 'Superconducting Magnet (1.5 T – 21.1 T)' and add 'Lock System' and 'Shim Coils' branching from the magnet.

Component	Function	Modern Specification
Superconducting Magnet	Generates strong, stable B_0 field. Cooled by liquid helium and nitrogen.	300–900 MHz (7–21 T). Most pharma labs: 400–600 MHz.
Sample Probe	Holds sample tube; contains transmitter and receiver coils; temperature control.	5 mm standard tube; cryoprobe for high sensitivity.
RF Transmitter	Generates pulses of radiofrequency energy at the Larmor frequency.	Pulsed FT-NMR: broadband pulse excites all nuclei simultaneously.
RF Receiver / Detector	Detects the free induction decay (FID) signal emitted by relaxing nuclei.	Quadrature detection for improved sensitivity.
Preamplifier	Amplifies the weak FID signal before digitization.	Placed close to probe to minimize noise.
ADC (Analog-to-Digital)	Converts analogue FID signal into digital data.	High-speed ADC (≥16 bit resolution).
Lock System	Maintains field stability using deuterium signal of solvent.	Deuterium lock on $CDCl_3$, DMSO-d_6 etc.
Shim Coils	Fine-tune magnetic field homogeneity across the sample volume.	Computerized autoshim routine.
Computer / Software	Controls pulse sequences; stores FID; performs Fourier Transform.	FT converts time-domain FID to frequency-domain spectrum.

The Fourier Transform NMR Process

1. Apply RF pulse → excites all protons simultaneously
2. Nuclei relax → emit FID signal (time domain)
3. FID is digitized by ADC
4. Fourier Transform converts time domain → frequency domain spectrum
5. Apply phase correction and baseline correction

6. Calibrate with TMS peak → report δ values in ppm

📌 **FT-NMR Advantage** The entire spectrum is obtained in milliseconds with one pulse! Continuous wave (CW) NMR scanned slowly through frequencies. FT-NMR is 10–100× faster and gives superior signal-to-noise ratio.

Applications of NMR Spectroscopy in Pharmacy

Application	Technique Used	Pharmaceutical Example
Structure elucidation of new drugs	^{1}H+^{13}C+2D NMR	Complete characterization of a new antibiotic
Purity testing and impurity profiling	Quantitative NMR (qNMR)	ICH Q3A/B impurity profiling of API
Confirmation of drug identity	^{1}H-NMR fingerprint	Pharmacopoeial ID test (British Pharmacopoeia)
Study of drug-receptor interactions	Saturation Transfer Difference (STD) NMR	Binding of ligand to protein target
Polymer characterization (excipients)	^{13}C, DEPT NMR	Characterization of HPMC, PEG polymers
Metabolomics / Biofluid analysis	^{1}H-NMR profiling	Urine/plasma metabolic profiling — drug toxicology
MRI (Imaging)	Relaxation (T_1/T_2)	Soft tissue imaging, tumor detection
Study of polymorphism	Solid-state NMR	Comparing crystal forms of active pharmaceutical

MASS SPECTROMETRY (MS)

Introduction and Principles

Mass spectrometry is an analytical technique that measures the mass-to-charge ratio (m/z) of ions. It is the most sensitive and selective tool for molecular identification, molecular weight determination, and structural elucidation.

Basic Principle

The fundamental principle of mass spectrometry involves three steps:

- **Ionization:** The sample molecule is ionized (converted to a charged species — cation or anion)
- **Separation:** Ions are separated according to their mass-to-charge ratio (m/z) in a mass analyzer
- **Detection:** Separated ions are detected and their abundance measured to produce a mass spectrum

m/z = mass of ion (in Daltons) / charge of ion (z)

For singly charged ions (z = 1, most common): m/z = molecular mass (Da). Example: Aspirin ($C_9H_8O_4$, MW = 180 Da) → m/z = 180 for $[M]^{+\bullet}$

✏ **DIAGRAM TO DRAW** Draw a block diagram: Sample Inlet → Ion Source → Mass Analyzer → Detector → Data System (Computer). Below the mass analyzer, write the types: 'Quadrupole' and 'Time-of-Flight (TOF)'. Below ion source, write 'EI / CI / FAB / MALDI'. Use arrows to show the path of ions.

Mass Spectrometer Components

Component	Function	Key Features
Sample Inlet	Introduces sample into the MS system under vacuum	Direct probe, LC-MS interface, GC-MS interface
Ion Source	Converts neutral molecules to ions	Various types: EI, CI, ESI, MALDI, FAB
Mass Analyzer	Separates ions by m/z ratio	Quadrupole, TOF, Magnetic sector, Ion trap
Detector	Measures ion current for each m/z	Electron multiplier (most common), Faraday cup
Vacuum System	Maintains high vacuum (10^{-5}–10^{-8} torr) to prevent ion-molecule collisions	Turbomolecular pumps
Data System	Records, processes and displays mass spectrum	Records m/z vs relative abundance (%)

Fragmentation Patterns in Mass Spectrometry

When a molecule is ionized (especially by Electron Impact), it forms a molecular ion ($M^{+}\bullet$) which then fragments into smaller ions. The fragmentation pattern is characteristic of the molecule's structure.

Types of Ions in a Mass Spectrum

Ion Type	Symbol	Description	Significance
Molecular Ion	$M^{+}\bullet$	Parent ion — intact molecule that has lost one electron	Gives molecular weight; may be absent in soft ionization
Base Peak	100%	Most abundant ion in the spectrum (tallest peak)	Used as reference (set to 100% relative abundance)
Fragment Ions	m/z < $M^{+}\bullet$	Daughter ions formed by bond cleavage of $M^{+}\bullet$	Provide structural information
Isotope Peak (M+1)	M+1	Contains ^{13}C, ^{2}H, or ^{15}N instead of common isotope	Identifies molecular formula (used in high-res MS)
Metastable Ion	m*	Broad, low-intensity peak — indicates fragmentation route	Confirms fragmentation pathway
Rearrangement Ion	—	Ion formed by rearrangement with migration (e.g., McLafferty)	Characteristic of carbonyl compounds

Common Fragmentation Rules

- **Rule 1 — Even Electron Rule:** $M^{+}\bullet$ is a radical cation (odd-electron species). Loss of a radical → even-electron fragment ion
- **Rule 2 — Stability:** More stable carbocations (tertiary > secondary > primary > methyl) are preferred fragments
- **Rule 3 — α-cleavage:** Bond adjacent to a heteroatom (O, N, S) breaks preferentially
- **Rule 4 — McLafferty Rearrangement:** 6-membered cyclic transition state; γ-hydrogen migrates to carbonyl; requires γ-H and C=O
- **Rule 5 — Characteristic losses:** Loss of 15 (CH_3), 18 (H_2O), 28 (CO or C_2H_4), 31 (OCH_3), 35/37 (Cl), 79/81 (Br)

Neutral Lost	Mass Lost	Indicates
$CH_3\bullet$	15	Terminal methyl group
H_2O	18	Hydroxyl group (alcohol, carboxylic acid)
CO	28	Aldehyde, ketone, ester
$CHO\bullet$	29	Aldehyde
OCH_3	31	Methyl ester

Neutral Lost	Mass Lost	Indicates
NO_2	46	Nitro compound
Cl	35	Chlorine (with M+2 isotope pattern 3:1)
Br	79	Bromine (with M+2 isotope pattern 1:1)
HBr	80	Bromoalkyl compound
C_6H_5	77	Phenyl group (phenyl cation m/z = 77)

⚡ **Exam Trick** Chlorine gives M and M+2 peaks in 3:1 ratio (^{35}Cl:^{37}Cl = 75%:25%). Bromine gives M and M+2 peaks in 1:1 ratio (^{79}Br:^{81}Br ≈ 50%:50%). This is a guaranteed MCQ!

Ionization Techniques

The ionization technique is chosen based on the nature of the sample (volatile/non-volatile, thermolabile, polar/nonpolar) and the information required.

Electron Impact Ionization (EI)

Electron Impact (EI) is the classical, most widely used ionization method for volatile, thermally stable compounds.

Principle:

The sample is vaporized under vacuum and bombarded by a beam of high-energy electrons (typically 70 eV). A molecule M loses one electron to form a radical cation ($M^{+\bullet}$):

$$M + e^- (70\ eV) \rightarrow M^{+\bullet} + 2e^-$$

Characteristics of EI:

- **Energy:** 70 eV (standard) — excess energy causes extensive fragmentation
- **Type:** Hard ionization — produces abundant fragment ions
- **$M^{+\bullet}$:** Present (usually); may be absent for very labile molecules
- **Reproducibility:** Excellent — 70 eV EI spectra are in NIST spectral libraries
- **Sample requirement:** Must be volatile (bp < 250°C) and thermally stable
- **Application:** GC-MS analysis of drugs of abuse, pesticides, petroleum products

✏ **DIAGRAM TO DRAW** Draw EI source: Filament (electron emitter) on left. Electrons (arrow) hit molecule M in the center. $M^{+\bullet}$ ion exits through ion exit slit to the right toward the analyzer. Label: Filament, Electron beam (70 eV), Sample molecule (M), Molecular ion ($M^{+\bullet}$), Ion repeller, Focusing plates.

Chemical Ionization (CI)

Chemical Ionization is a softer technique that produces less fragmentation, allowing observation of the quasi-molecular ion $[M+H]^+$.

Principle:

A reagent gas (methane, isobutane, ammonia) is introduced in large excess and ionized by electrons. The reagent gas ions then react with sample molecules by proton transfer:

$$CH_5^+ + M \rightarrow [M+H]^+ + CH_4 \quad \text{(using methane as reagent gas)}$$

Characteristics of CI:

- **Type:** Soft ionization — minimal fragmentation
- **Molecular ion:** $[M+H]^+$ (protonated molecule) — gives M+1 peak
- **Reagent gases:** CH_4 (methane), i-C_4H_{10} (isobutane), NH_3 (ammonia)
- **Sensitivity:** Good for thermolabile, polar compounds
- **Application:** Molecular weight determination of drugs when EI $M^{+}\bullet$ is absent

Feature	EI (Electron Impact)	CI (Chemical Ionization)
Ionization type	Hard	Soft
Energy	70 eV (electrons)	Low energy (chemical reaction)
Molecular ion	$M^{+}\bullet$ (radical cation)	$[M+H]^+$ (protonated molecule)
Fragmentation	Extensive — rich spectral info	Minimal — mainly M+1 peak
Sample requirement	Volatile, thermally stable	Volatile or semi-volatile
Spectral libraries	Yes (NIST database, 70 eV)	Not standardized
Pharmaceutical use	GC-MS drug screening	MW determination of drugs

Matrix-Assisted Laser Desorption Ionization (MALDI)

MALDI is a soft ionization technique specifically designed for analysis of large biomolecules (proteins, nucleic acids, polymers) and polar, non-volatile compounds.

Principle:

The sample is co-crystallized with a UV-absorbing matrix compound on a metal target plate. A pulsed UV laser irradiates the sample-matrix co-crystals:

7. Matrix absorbs UV laser energy (typically 337 nm N_2 laser or 355 nm Nd:YAG)
8. Rapid heating causes desorption of matrix and sample molecules into gas phase
9. Proton transfer from photoexcited matrix → sample molecule → $[M+H]^+$ or $[M+Na]^+$

MALDI Matrix Compounds:

Matrix	Full Name	Application Range	λ
CHCA	α-Cyano-4-hydroxycinnamic acid	Peptides, small proteins (<10 kDa)	337 nm

Matrix	Full Name	Application Range	λ
DHB	2,5-Dihydroxybenzoic acid	Glycopeptides, oligosaccharides	337 nm
SA (Sinapic acid)	3,5-Dimethoxy-4-hydroxycinnamic acid	Proteins (10–100 kDa)	337 nm
HCCA	4-Hydroxycinnamic acid	Lipids, synthetic polymers	337 nm

Characteristics of MALDI:

- **Type:** Soft ionization — minimal fragmentation, molecular ion observed
- **Mass range:** Very high — up to 500 kDa (excellent for proteins)
- **Ions formed:** Mainly $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$ (singly charged)
- **Best paired with:** Time-of-Flight (TOF) analyzer (MALDI-TOF)
- **Application:** Proteomics, microbial identification (MALDI-TOF MS for bacteria in clinical labs), polymer analysis

⚡ **Exam Trap** MALDI typically produces SINGLY charged ions $[M+H]^+$, unlike ESI which produces multiply charged ions. This is why MALDI-TOF is preferred for intact protein mass measurement.

Fast Atom Bombardment (FAB)

FAB is an older soft ionization technique for polar, non-volatile, thermolabile compounds. While largely superseded by ESI and MALDI, it is still in the syllabus.

Principle:

The sample is dissolved in a liquid matrix (glycerol is most common) and applied to a metal probe. A beam of fast neutral atoms (argon or xenon, 6–10 keV) bombards the sample:

- Fast Ar/Xe atoms collide with the matrix → momentum transfer
- Matrix molecules desorb, carrying sample molecules into gas phase
- Proton transfer → $[M+H]^+$ (positive mode) or $[M–H]^-$ (negative mode)

Feature	FAB	MALDI	EI
Ionization type	Soft	Soft	Hard
Beam used	Neutral atoms (Ar/Xe)	UV laser	Electrons (70 eV)
Matrix	Glycerol (liquid)	Organic crystal (solid)	None
Sample state	Dissolved in matrix	Co-crystallized	Vapor phase
Mass range	Up to ~6,000 Da	Up to 500,000 Da	<1,000 Da (GC-MS)
Main ion	$[M+H]^+$, $[M–H]^-$	$[M+H]^+$, $[M+Na]^+$	$M^{+\bullet}$ (radical cation)

Feature	FAB	MALDI	EI
Fragmentation	Low — matrix clusters seen	Very low	Extensive
Use today	Declining (replaced by ESI/MALDI)	Active — proteomics, microbiology	Active — GC-MS

Mass Analyzers

After ionization, ions must be separated by their m/z ratio. Two major analyzer types are in the syllabus: Time-of-Flight (TOF) and Quadrupole.

Time-of-Flight (TOF) Analyzer

TOF is the simplest and most powerful mass analyzer for high mass range applications. It is always used with pulsed ionization (MALDI-TOF, ESI-TOF).

Principle:

Ions are accelerated by an electric field and enter a field-free drift tube. All ions receive the same kinetic energy:

$$KE = zeV = \tfrac{1}{2}mv^2 \rightarrow v = \sqrt{(2zeV/m)} \rightarrow t = L/v = L\sqrt{(m/2zeV)}$$

Where: z = charge, e = electron charge, V = accelerating voltage, v = velocity, m = mass, L = drift tube length, t = flight time

Principle:

Lighter ions travel FASTER and arrive at the detector FIRST. Heavier ions travel SLOWER and arrive LATER. Mass is determined by measuring the flight time (t).

DIAGRAM TO DRAW Draw TOF analyzer: Ion Source (left) → Accelerating Plates (with voltage V) → Drift Tube (long, field-free region, label length L) → Detector (right). Below the drift tube, draw three ion paths: small m/z ions arrive first, medium m/z next, large m/z last. Label 'Reflectron (optional)' as a reflector at the end of the drift tube for improved resolution.

Characteristics of TOF Analyzer:

- **Mass range:** Theoretically unlimited — can detect proteins >500 kDa
- **Resolution:** Moderate in linear mode; HIGH in reflectron mode
- **Speed:** Microsecond scan speed — ideal for LC-MS and MALDI
- **Reflectron TOF:** Uses an ion mirror (reflectron) to correct for kinetic energy spread → improved resolution
- **Sensitivity:** High — all ions transmitted simultaneously (no scanning losses)
- **Paired with:** MALDI (MALDI-TOF), ESI
- **Pharmaceutical use:** Proteomics, metabolomics, intact protein analysis, imaging MS

Quadrupole Mass Analyzer

The quadrupole is the workhorse of pharmaceutical MS analysis, found in virtually every LC-MS/MS system.

Principle:

A quadrupole consists of four parallel cylindrical rods arranged symmetrically. Opposite pairs carry identical potentials: a DC voltage (U) plus an AC/RF voltage ($V \cdot \cos \omega t$).

Applied potential: Φ = +(U + V·cos ωt) on one pair; −(U + V·cos ωt) on adjacent pair

By adjusting the ratio of U/V (DC/RF ratio) at a fixed ratio, only ions of ONE specific m/z value have stable trajectories and pass through to the detector. All other ions are destabilized and lost.

Characteristics of Quadrupole Analyzer:

- **Mass range:** Up to ~4,000 m/z (limited for high MW biomolecules)
- **Resolution:** Unit resolution (1 Da) — sufficient for most small molecule pharma
- **Scan mode:** Scanning — scans U/V ratio to detect different m/z sequentially
- **SIM mode:** Selected Ion Monitoring — monitors only specific m/z → high sensitivity
- **Triple Quadrupole (QqQ):** Q1 selects precursor, q2 (collision cell) fragments, Q3 selects product → MRM (Multiple Reaction Monitoring) for quantification
- **Pharmaceutical use:** Drug pharmacokinetics (PK), metabolite identification, impurity profiling, therapeutic drug monitoring

Feature	Time-of-Flight (TOF)	Quadrupole
Separation principle	Flight time (lighter = faster)	Stable ion trajectory in DC+RF field
Mass range	Unlimited (up to MDa)	~4,000 m/z
Resolution	High (esp. with reflectron)	Unit resolution (1 Da)
Ionization compatible	MALDI, ESI (pulsed)	EI, CI, ESI, APCI (continuous)
Scan type	Simultaneous — all masses detected	Sequential scanning — one m/z at a time
Sensitivity	High (no scanning loss)	Moderate (scanning loss); high in SIM/MRM
Cost	Higher	Lower — most affordable MS
Main use	Proteomics, high mass biomolecules	Small molecule quantification (LC-MS/MS)
Special feature	Reflectron for improved resolution	Triple quad (QqQ) for MRM quantification
Pharmaceutical app.	Protein identification, metabolomics	Drug PK, impurity testing, TDM

📌 **Quick Summary — Analyzer Choice** MALDI-TOF = Proteins & large biomolecules. Quadrupole (LC-MS/MS) = Small molecule drugs, quantification, impurity profiling. Remember this distinction!

Applications of Mass Spectrometry in Pharmacy

Application	MS Technique	Pharmaceutical Example
Molecular weight determination	ESI-MS or MALDI-TOF	MW of insulin (5,808 Da) confirmed by ESI-MS
Structural elucidation of drugs	EI-MS + fragmentation	Aspirin: M^+=180, loss of 60 (CH_3COOH) → 120 (phenol)
Impurity profiling	LC-MS/MS (QqQ)	ICH Q3A: identify and quantify drug impurities
Drug metabolism studies	Triple Quad LC-MS/MS	Phase I/II metabolite identification in urine/plasma
Pharmacokinetic studies (quantification)	MRM on Triple Quadrupole	Plasma drug level monitoring — bioavailability studies
Proteomics / protein ID	MALDI-TOF, ESI-TOF	Peptide mass fingerprinting for protein identification
Drug of abuse screening	GC-EI-MS	THC, cocaine, opiates in urine — forensic toxicology
Therapeutic drug monitoring (TDM)	LC-MS/MS	Tacrolimus, cyclosporine blood level monitoring
Microbial identification (clinical lab)	MALDI-TOF	Rapid ID of bacteria/fungi in <1 hour (VITEK MS)

IMPORTANT QUESTION BANK

A. 2-Mark Questions with Model Answers

Q1. Define chemical shift and give its unit.

Ans: Chemical shift (δ) is the difference in resonance frequency of a nucleus relative to a reference standard (TMS), expressed in parts per million (ppm). Formula: $\delta = (\nu_{sample} - \nu_{TMS}) / \nu_{spectrometer} \times 10^6$. Unit: ppm (dimensionless ratio).

Q2. What is the function of TMS in NMR spectroscopy?

Ans: TMS (Tetramethylsilane, $Si(CH_3)_4$) serves as the internal reference standard in NMR ($\delta = 0.00$ ppm). It is used because: (i) all 12 protons are equivalent → single sharp signal; (ii) protons are highly shielded (upfield); (iii) chemically inert, volatile, and non-interfering.

Q3. Distinguish between T_1 and T_2 relaxation.

Ans: T_1 (spin-lattice) = longitudinal relaxation along z-axis; energy transfer to surroundings; restores Mz. T_2 (spin-spin) = transverse relaxation in xy-plane; phase coherence loss between nuclei; determines NMR linewidth. Always $T_2 \leq T_1$.

Q4. What is meant by spin-spin coupling?

Ans: Spin-spin coupling is the splitting of NMR signals caused by the magnetic interaction of neighbouring NMR-active nuclei through covalent bonds. The coupling strength is measured by the coupling constant J (in Hz). It gives information about the number of adjacent protons (n+1 rule).

Q5. Define molecular ion in mass spectrometry.

Ans: The molecular ion ($M^{+\bullet}$) is the ion formed in mass spectrometry when the sample molecule M loses one electron: $M + e^- \rightarrow M^{+\bullet} + 2e^-$. It is a radical cation. Its m/z value equals the molecular weight of the compound (for z=1).

Q6. What is the principle of Time-of-Flight (TOF) analyzer?

Ans: In TOF, all ions are accelerated through the same electric field, giving them the same kinetic energy. Ions then travel through a field-free drift tube. Lighter ions travel faster and arrive at the detector first. Mass is determined by measuring flight time: $t \propto \sqrt{m}$.

Q7. State the matrix used in MALDI and its purpose.

Ans: Common MALDI matrices include: α-Cyano-4-hydroxycinnamic acid (CHCA) for peptides; 2,5-Dihydroxybenzoic acid (DHB) for glycopeptides; Sinapic acid for proteins. Purpose: Matrix absorbs UV laser energy and facilitates desorption and ionization of the sample by proton transfer, minimizing direct laser-induced fragmentation.

Q8. What is the base peak in a mass spectrum?

Ans: The base peak is the most abundant (highest intensity) peak in a mass spectrum. It is assigned 100% relative abundance and used as the reference for reporting all other peak intensities. The base peak need not be the molecular ion — it is the most stable fragment ion.

B. 5-Mark Questions with Structured Answers

Q1. Explain the factors affecting chemical shift in ^{1}H-NMR spectroscopy.

Q2. Describe the spin-spin coupling and explain the n+1 rule with examples.

Q3. Compare Electron Impact (EI) and Chemical Ionization (CI) in mass spectrometry.

Q4. Explain MALDI ionization technique with appropriate diagram.

Q5. Describe the Quadrupole mass analyzer with its working principle and pharmaceutical applications.

C. 10-Mark Question Skeletons

Q1. Describe the instrumentation of NMR spectrometer. Discuss its components, working principle and pharmaceutical applications. (10 marks)

Q2. Write a detailed note on ionization techniques in mass spectrometry — EI, CI, MALDI, and FAB. Compare their advantages, limitations, and applications. (10 marks)

Q3. Compare Time-of-Flight (TOF) and Quadrupole mass analyzers with respect to principle, construction, characteristics, and pharmaceutical applications. (10 marks)

PREVIOUS YEAR-STYLE QUESTIONS

PYQ 1. What do you understand by chemical shift? Discuss the factors affecting chemical shift in ^{1}H-NMR. [AKTU-style, 7 marks]

PYQ 2. Explain MALDI ionization technique with a labelled diagram. Mention suitable matrix compounds and applications. [AKTU-style, 5 marks]

PYQ 3. Differentiate between Time-of-Flight (TOF) and Quadrupole mass analyzers. [AKTU-style, 5 marks]

PYQ 4. Describe spin-spin coupling in NMR and explain coupling constant J with examples. [AKTU-style, 5 marks]

PYQ 5. Write the principle of Electron Impact (EI) ionization and discuss the fragmentation pattern of aspirin by EI-MS. [AKTU-style, 5 marks]

50 MCQs — CATEGORIZED BY DIFFICULTY

EASY — 25 MCQs (Recall / Definition)

1. The internal reference standard used in NMR spectroscopy is:
A. Benzene
B. TMS (tetramethylsilane) ✓
C. $CDCl_3$
D. Acetone
Explanation: TMS ($Si(CH_3)_4$) gives $\delta = 0.00$ ppm for both ^{1}H and ^{13}C NMR. It is chemically inert, volatile, with all 12 equivalent protons appearing in uncrowded upfield region.

2. The chemical shift (δ) in NMR is expressed in:
A. Hz
B. nm
C. ppm ✓
D. eV
Explanation: Chemical shift is expressed in ppm (parts per million) — a dimensionless ratio. Coupling constant J is expressed in Hz.

3. In ^{1}H-NMR, which proton appears MOST downfield?
A. Alkyl ($R\text{-}CH_3$)
B. Aromatic (Ar-H) ✓
C. Vinyl (=CH-)

D. Alkynyl (≡C-H)

Explanation: Aromatic protons appear at δ 6.5–8.5 ppm due to strong deshielding by the ring current of the benzene ring. Alkyl protons appear furthest upfield (δ 0.8–1.5).

4. The spin quantum number (I) of ^{1}H (proton) is:

A. 0

B. 1

C. 1/2 ✓

D. 3/2

Explanation: ^{1}H has I = 1/2. Only nuclei with I ≠ 0 are NMR active. ^{12}C has I = 0 and is NMR silent.

5. A methyl group (CH_3) adjacent to ONE proton appears as:

A. Singlet

B. Doublet ✓

C. Triplet

D. Quartet

Explanation: By n+1 rule: CH_3 with 1 neighbouring proton → (1+1) = doublet. Example: CH_3-CH group in isopropyl or lactate.

6. The coupling constant (J) is measured in:

A. ppm

B. Tesla

C. Hz ✓

D. eV

Explanation: J (coupling constant) is measured in Hertz (Hz). Chemical shift δ is in ppm. J is independent of the spectrometer frequency (B_0).

7. In mass spectrometry, m/z stands for:

A. mass per zone

B. mass-to-charge ratio ✓

C. molecular mass of neutral

D. mass of cation

Explanation: m/z = mass of ion (in Daltons or atomic mass units) divided by the charge number z. For z=1, m/z = molecular mass.

8. The ionization technique that uses 70 eV electrons is:

A. CI

B. FAB

C. MALDI

D. EI ✓

Explanation: Electron Impact (EI) uses a beam of 70 eV electrons to ionize vaporized sample. 70 eV is standard for reproducible, library-searchable spectra.

9. The molecular ion in EI mass spectrometry is:

A. $[M+H]^+$

B. $M^{+}\bullet$ ✓

C. $[M+Na]^+$

D. M^{2+}

Explanation: EI produces radical cation $M^{+}\bullet$ (odd electron species) by loss of one electron from molecule M. CI produces $[M+H]^+$. MALDI can give $[M+Na]^+$.

10. In MALDI, the matrix absorbs:

A. X-rays

B. UV laser energy ✓

C. γ-radiation

D. Microwave radiation

Explanation: MALDI matrix (e.g., CHCA, DHB) absorbs UV laser radiation (typically 337 nm N_2 laser). The matrix acts as energy intermediary to ionize the analyte without destroying it.

11. The MALDI matrix used for peptide analysis is:

A. Glycerol
B. Sinapic acid
C. CHCA (α-cyano-4-hydroxycinnamic acid) ✓
D. DHB only
Explanation: CHCA (α-Cyano-4-hydroxycinnamic acid) is the standard matrix for peptides and small proteins (<10 kDa). Sinapic acid is used for larger proteins (10–100 kDa).

12. In FAB ionization, the liquid matrix commonly used is:
A. Methanol
B. Glycerol ✓
C. Acetonitrile
D. $CDCl_3$
Explanation: Glycerol is the most common liquid matrix in FAB (Fast Atom Bombardment). It keeps the sample in solution, replenishing the surface after bombardment.

13. T_1 relaxation in NMR is also known as:
A. Spin-spin relaxation
B. Longitudinal relaxation ✓
C. Transverse relaxation
D. Spin-lattice demagnetization
Explanation: T_1 is longitudinal (spin-lattice) relaxation — restoration of Mz (magnetization along z-axis, parallel to B_0). T_2 is transverse (spin-spin) relaxation — loss of Mxy phase coherence.

14. T_2 relaxation determines the:
A. Recycle delay time
B. Width of NMR peaks (linewidth) ✓
C. Chemical shift values
D. Number of peaks in multiplet
Explanation: NMR peak linewidth ($\Delta\nu$ at half-height) = $1/(\pi \cdot T_2)$. Shorter T_2 → broader linewidth. This is why solids give very broad NMR peaks (very short T_2).

15. Natural abundance of ^{13}C is approximately:
A. 100%
B. 99.98%
C. 1.11% ✓
D. 0.01%
Explanation: ^{13}C has only 1.11% natural abundance compared to 99.98% for ^{1}H. This is why ^{13}C NMR requires signal averaging (many pulses) and has much lower sensitivity than ^{1}H NMR.

16. The base peak in a mass spectrum has relative abundance of:
A. 0%
B. 50%
C. 100%
D. Variable — highest peak ✓
Explanation: The base peak is the most abundant peak in the spectrum and is SET to 100% relative abundance by convention. Its actual signal intensity varies — it is simply the tallest peak and used as reference for all others.

17. The reagent gas most commonly used in Chemical Ionization (CI) is:
A. Argon
B. Nitrogen
C. Methane (CH_4) ✓
D. Helium
Explanation: Methane (CH_4) is the most common CI reagent gas. It is ionized by electrons to form CH_5^+, which then reacts with sample M by proton transfer: $CH_5^+ + M \rightarrow [M+H]^+ + CH_4$.

18. In TOF analyzer, separation of ions is based on:
A. Magnetic field deflection
B. DC/RF voltage stability
C. Flight time through drift tube ✓

D. Ion-molecule collision

Explanation: In Time-of-Flight (TOF), all ions receive the same kinetic energy. Lighter ions move faster and arrive at detector first. Separation is based on flight time ($t \propto \sqrt{m}$).

19. Quadrupole mass analyzer uses how many rods?

A. 2

B. 4 ✓

C. 6

D. 8

Explanation: Quadrupole uses FOUR parallel rods (hence 'quad' = four). Opposite pairs carry identical DC+RF voltages. Only one m/z has stable trajectory at a given U/V ratio.

20. Which nucleus is NMR SILENT (I = 0)?

A. ^{1}H

B. ^{13}C

C. ^{12}C ✓

D. ^{19}F

Explanation: ^{12}C has spin I = 0 and is NMR silent. ^{1}H (I=1/2), ^{13}C (I=1/2), ^{19}F (I=1/2) are all NMR active. Even mass number AND even atomic number → I = 0.

21. The CH_2 of ethanol appears as what in ^{1}H-NMR?

A. Singlet

B. Doublet

C. Triplet

D. Quartet ✓

Explanation: CH_2 of ethanol has 3 neighbouring protons (CH_3 group). By n+1 rule: 3+1 = 4 lines = quartet. The CH_3 appears as triplet (2 neighbours from CH_2, 2+1=3).

22. EI mass spectra at 70 eV are stored in:

A. EMBL database

B. NIST mass spectral library ✓

C. Protein Data Bank (PDB)

D. GenBank

Explanation: The NIST Mass Spectral Library (NIST/EPA/NIH database) contains 70 eV EI mass spectra of ~350,000 compounds. This standardization (70 eV) makes spectra reproducible and library-searchable.

23. Aldehyde proton (CHO) in ^{1}H-NMR typically appears at:

A. δ 0–2 ppm

B. δ 4–6 ppm

C. δ 9–10 ppm ✓

D. δ 12–15 ppm

Explanation: Aldehyde proton appears at δ 9.4–10.0 ppm — highly deshielded due to combined effect of carbonyl magnetic anisotropy and direct electron withdrawal by C=O.

24. Which ionization is considered HARD ionization?

A. MALDI

B. FAB

C. ESI

D. EI ✓

Explanation: EI (Electron Impact) at 70 eV is hard ionization — it imparts excess energy to $M^{+\bullet}$ causing extensive fragmentation. MALDI, FAB, ESI, and CI are all soft ionization techniques.

25. The ^{13}C-NMR chemical shift of a carbonyl carbon (C=O of ketone) is approximately:

A. 10–30 ppm

B. 70–90 ppm

C. 110–160 ppm

D. 195–220 ppm ✓

Explanation: Ketone carbonyl carbon appears at δ 195–220 ppm. This extremely downfield position is due to the strong deshielding effect of the C=O group. Compare: aromatic C at 110–160 ppm.

26. In EI-MS, loss of 15 from the molecular ion indicates elimination of:

A. H_2O

B. CH_3• (methyl radical) ✓

C. CO

D. HCl

Explanation: Loss of 15 Da corresponds to loss of a methyl radical (CH_3•, MW=15). This indicates a terminal methyl group in the molecule. Common in branched alkyl compounds.

27. The coupling constant (J) for a TRANS alkene double bond is typically:

A. 0–3 Hz

B. 6–12 Hz

C. 12–18 Hz ✓

D. 20–30 Hz

Explanation: Trans (E) alkene vicinal coupling constant ^3J = 12–18 Hz (typically ~15 Hz). Cis (Z) alkene ^3J = 6–12 Hz (typically ~8 Hz). This J value difference allows cis/trans isomer distinction.

28. A compound shows M^+ at 180 and M+2 at about 60% of M^+ intensity. This suggests:

A. One nitrogen atom

B. One chlorine atom

C. One bromine atom ✓

D. One oxygen atom

Explanation: Bromine has two isotopes ^{79}Br (50%) and ^{81}Br (50%), giving M and M+2 peaks of approximately equal intensity (1:1 ratio). Chlorine gives M:M+2 = 3:1.

29. Which of the following gives $[M+H]^+$ and NOT M^+•?

A. EI

B. CI ✓

C. Both EI and CI

D. Neither

Explanation: Chemical Ionization (CI) produces $[M+H]^+$ (protonated molecule) through proton transfer from reagent gas ion (CH_5^+) to sample. EI produces M^+• (radical cation). $[M+H]^+$ = M+1 on the mass scale.

30. DEPT experiment in NMR is used to:

A. Measure T_1 relaxation

B. Distinguish C, CH, CH_2, CH_3 carbons ✓

C. Determine coupling constants

D. Calibrate the magnetic field

Explanation: DEPT (Distortionless Enhancement by Polarization Transfer) is a ^{13}C experiment that distinguishes CH_3 (pointing up at 135°), CH_2 (pointing down), CH (pointing up), and quaternary C (absent) in different DEPT variants.

31. In MALDI, the ion most commonly formed from neutral molecule M is:

A. M^+•

B. $[M+H]^+$ ✓

C. M^{2+}

D. $[M-H]$•

Explanation: MALDI produces predominantly $[M+H]^+$ (singly protonated molecule) through proton transfer from photoexcited matrix. $[M+Na]^+$ and $[M+K]^+$ adducts are also common, especially for synthetic polymers.

32. Which mass analyzer is BEST suited for proteins with MW > 100,000 Da?

A. Quadrupole

B. Magnetic sector

C. Time-of-Flight (TOF) ✓

D. Ion trap

Explanation: TOF has theoretically unlimited mass range. For intact proteins >100 kDa, MALDI-TOF is ideal. Quadrupole is limited to ~4,000 m/z and is not suitable for large proteins in a single charge state.

33. McLafferty rearrangement requires:

A. A quaternary carbon

B. γ-hydrogen and a C=O group ✓

C. An aromatic ring

D. A halogen substituent

Explanation: McLafferty rearrangement requires: (1) a carbonyl group (C=O), (2) γ-hydrogen (3 carbons away from carbonyl), and (3) forms a 6-membered cyclic transition state. Characteristic of aldehydes, ketones, esters, carboxylic acids.

34. The mass spectrum of a chlorinated compound shows M:M+2 ratio of approximately:

A. 1:1

B. 2:1

C. 3:1 ✓

D. 4:1

Explanation: Chlorine isotopes: ^{35}Cl (75%) and ^{37}Cl (25%) → M:M+2 = 3:1. Bromine: ^{79}Br (50%) and ^{81}Br (50%) → M:M+2 = 1:1. This isotope pattern is diagnostic for halogenated compounds.

35. The FID (Free Induction Decay) in NMR is converted to a frequency-domain spectrum using:

A. Fast Fourier Transform (FFT) ✓

B. Laplace Transform

C. Lock system

D. ADC conversion only

Explanation: The time-domain FID is converted to a frequency-domain NMR spectrum using Fast Fourier Transform (FFT). This is the basis of all modern FT-NMR instruments.

36. In Triple Quadrupole (QqQ) MS, MRM stands for:

A. Minimal Resolution Mode

B. Multiple Reaction Monitoring ✓

C. Mass Ratio Measurement

D. Matrix-Related Method

Explanation: MRM (Multiple Reaction Monitoring): Q1 selects precursor ion, q2 (collision cell with inert gas) fragments it, Q3 selects specific product ion. Highly specific and sensitive quantification method for drug analysis in biological matrices.

37. An NMR-active nucleus must have spin quantum number:

A. I = 0

B. I = any integer

C. $I \neq 0$ ✓

D. I = 1/2 only

Explanation: Any nucleus with $I \neq 0$ is NMR active. This includes I = 1/2 (^{1}H, ^{13}C, ^{19}F, ^{31}P), I = 1 (^{2}H, ^{14}N), I = 3/2 (^{11}B), etc. Nuclei with I = 0 (^{12}C, ^{16}O) are NMR silent.

38. The chemical shift of aromatic protons is high due to:

A. Inductive electron withdrawal

B. Ring current effect (magnetic anisotropy) ✓

C. Hydrogen bonding

D. High electronegativity of carbon

Explanation: Aromatic protons are deshielded by the ring current — circulation of π electrons induced by B_0 creates a secondary magnetic field that adds to B_0 at the periphery of the ring (where protons are located).

39. In which ionization technique is the sample vaporized before ionization?

A. FAB

B. MALDI

C. EI ✓

D. ESI

Explanation: EI requires the sample to be vaporized (gaseous state) before electron bombardment. This limits EI to volatile, thermally stable compounds. FAB, MALDI, and ESI work with non-volatile samples.

40. The reflectron in TOF-MS improves:

A. Mass range

B. Ionization efficiency

C. Mass resolution ✓

D. Scan speed

Explanation: The reflectron (ion mirror) in TOF corrects for kinetic energy spread among ions of the same m/z: faster ions penetrate deeper into the reflector and exit later, allowing all ions of same m/z to arrive simultaneously → improved resolution.

HARD — 10 MCQs (Clinical / Calculation)

41. A compound has M^+ at m/z = 164. Loss of 15 gives m/z 149, and m/z 149 shows loss of 28 to give m/z 121. The most likely functional groups are:

A. Methyl and alcohol

B. Methyl and carbonyl (CO loss) ✓

C. Ethyl and amine

D. Phenyl and chlorine

Explanation: Loss of 15 = loss of CH_3• (terminal methyl group). Loss of 28 from m/z 149 = loss of CO (28 Da) indicating aldehyde or ketone carbonyl. Together: compound has methyl group and carbonyl functionality. This fragmentation pattern is characteristic of methyl aryl ketones like methyl benzoate or acetophenone analogues.

42. Ethanol CH_3CH_2OH in ^{1}H-NMR: The CH_2 signal appears as a quartet because:

A. It has 4 equivalent protons

B. It is coupled with 3 protons of CH_3 (n=3, n+1=4) ✓

C. It is coupled with OH proton only

D. Chemical exchange broadens the signal to 4 lines

Explanation: CH_2 of ethanol has 3 neighbouring protons from CH_3 group. By n+1 rule: 3+1 = 4 lines = quartet. The OH proton does not typically couple (fast exchange in $CDCl_3$). Coupling constant $^3J \approx 7$ Hz for this vicinal coupling.

43. In MALDI-TOF MS, a protein with actual MW = 50,000 Da is observed at m/z = 50,001. This is because:

A. The instrument is miscalibrated

B. A proton $[H]^+$ is added forming $[M+H]^+$ ✓

C. A sodium adduct is formed

D. The protein dimerizes

Explanation: MALDI predominantly produces $[M+H]^+$ ions. The m/z = 50,001 corresponds to M + 1 (mass of one proton = 1.008 Da ≈ 1 Da). The molecular weight is therefore MW = 50,001 − 1 = 50,000 Da. This $[M+H]^+$ adduct formation is the basis of all MALDI measurements.

44. A pharma company analyses a drug by LC-MS/MS in MRM mode. Q1 selects m/z 308; q2 fragments it; Q3 monitors m/z 210. The 308→210 transition represents:

A. 308 is the m/z of the internal standard

B. Loss of 98 Da from precursor — a specific neutral loss ✓

C. The drug has two isomers of equal mass

D. Detector is measuring background noise

Explanation: In MRM, the precursor→product transition (308→210) represents loss of 98 Da neutral fragment in the collision cell. This specific neutral loss (e.g., loss of phosphoric acid H_3PO_4=98 from phosphorylated compounds, or other specific fragments) is UNIQUE to the compound, giving exceptional selectivity and enabling accurate quantification in complex biological matrices.

45. The linewidth of an NMR peak is inversely proportional to:

A. T_1

B. T_2 ✓

C. The magnetic field strength B_0

D. The chemical shift δ

Explanation: NMR linewidth at half-height $\Delta\nu_{1/2} = 1/(\pi \cdot T_2)$. Therefore, shorter T_2 → broader peaks. This is why macromolecules (proteins, large polymers) give broad peaks (short T_2 due to slow tumbling), and small molecules in solution give sharp peaks (long T_2).

46. A proton NMR shows a signal at δ 7.50 in $CDCl_3$. When recorded in benzene-d_6, the same signal appears at δ 6.80. This upfield shift is due to:

A. Changed coupling constant

B. Benzene ring current causing anisotropic shielding ✓

C. Chemical exchange with benzene

D. Different TMS calibration in benzene

Explanation: Aromatic solvents like benzene-d_6 cause upfield shifts (lower δ) for many solute protons due to anisotropic shielding effect of benzene's ring current. Solute molecules orient with aromatic protons pointing INTO benzene's shielding cone → upfield shift. This 'solvent shift' is ~0.5–2 ppm for aromatic solvents.

47. In the ^{1}H-NMR of aspirin (acetylsalicylic acid), which signal appears MOST downfield?

A. –OCH_3 (methoxy)

B. –$OCOCH_3$ (acetyl methyl)

C. Aromatic H

D. –COOH proton ✓

Explanation: Aspirin's COOH proton appears at δ 10–12 ppm — most downfield due to combined strong deshielding by: (1) carbonyl C=O electron withdrawal, (2) strong intermolecular hydrogen bonding with adjacent ortho-ester group. Aromatic H: δ 7.0–7.8 ppm. Acetyl CH_3: δ 2.3 ppm.

48. A compound shows only ONE peak in its ^{13}C-NMR broad-band decoupled spectrum. The most likely structure is:

A. Ethanol (C_2H_5OH)

B. Cyclohexane (C_6H_{12})

C. Benzene (C_6H_6)

D. Both B and C ✓

Explanation: Both cyclohexane (C_6H_{12}) and benzene (C_6H_6) have only one chemically unique carbon environment — all carbons are equivalent. In BBD ^{13}C-NMR, each unique carbon gives one signal. Ethanol has 2 unique carbons (CH_3 and CH_2) → 2 signals.

49. In EI-MS, a compound shows $M^+ = 78$. It shows loss of 1 (M−1 peak) but no other major fragments. The compound is most likely:

A. Benzene (C_6H_6) ✓

B. Pyridine (C_5H_5N)

C. Cyclohexane

D. Toluene (C_7H_8)

Explanation: Benzene (C_6H_6, MW = 78) forms a very stable tropylium-like cation and shows a strong M^+• at 78 with minimal fragmentation. The strong aromatic π system stabilizes M^+•. Loss of H gives M-1 (m/z 77 = phenyl cation $C_6H_5^+$). Toluene MW = 92.

50. A ^{1}H-NMR spectrum shows a septet at δ 2.5 and a doublet at δ 1.2 (6H). The compound fragment is:

A. n-propyl group

B. Isopropyl group (–$CH(CH_3)_2$) ✓

C. tert-butyl group

D. Ethyl ester group

Explanation: Isopropyl group –$CH(CH_3)_2$: The central CH (1H) has 6 equivalent neighbouring protons (two CH_3) → appears as septet (6+1=7 lines) at δ ~2–3. The two CH_3 groups (6H total, equivalent) have 1 neighbouring proton → doublet. Classic pattern: septet + doublet(6H) = isopropyl group. Common in ibuprofen, metoprolol etc.

EXAM STRATEGY — HOW TO ATTEMPT UNIT I IN A 70-MARK PAPER

Rules for 10-Mark Answers:

- **Always draw the block diagram** — examiners give 2 marks just for a labelled diagram
- **Write conclusion** — adds 0.5–1 mark even if content is same
- **Use comparison tables** — saves writing time, looks professional, gets full marks
- **Bold every key term** — makes it easy for examiner to award marks
- **Name examples** — aspirin for MS fragmentation, ethanol for NMR coupling, insulin for MALDI

Top Marks Tips — Don't Lose These Easy Points:

- Spell MALDI full form correctly in every answer (Matrix-Assisted Laser Desorption Ionization)