# PharmacoLearn

**MPC201T Advanced Spectral Analysis, M.Pharm Semester 2**

## Unit 3: Mass Spectrometry: Fragmentation Rules and Interpretation

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### 3.1 The molecular ion and the ground rules

Electron ionisation knocks one electron out of the molecule, giving the molecular ion M+, a radical cation whose m/z is the molecular weight. Everything else in the spectrum is explained by how that radical cation breaks apart. Four ground rules govern all the fragmentation chemistry of this unit.

- **The nitrogen rule.** A molecule of even molecular weight contains an even number of nitrogen atoms (zero counts as even); odd molecular weight means an odd number of nitrogens. One glance at M+ therefore tests any proposed formula.
- **Charge and radical stay where they are most stable.** Cleavage is favoured when it produces the more stable cation (often resonance stabilised or heteroatom stabilised) and the more stable radical; Stevenson's rule adds that, of the two fragments, the charge preferentially remains with the fragment of lower ionisation energy.
- **The even electron rule.** Radical cations fragment to give either an even electron cation plus a radical, or a radical cation plus a neutral molecule (the rearrangement route). Even electron cations then fragment only to other even electron cations plus neutral molecules. Pathways that would create two radicals at once are not observed.
- **Cleavage happens at the chemically sensible bonds:** next to heteroatoms (alpha cleavage), at benzylic and allylic positions (resonance stabilised products), and at branch points in chains (more substituted cations are more stable).

### 3.2 The ring rule: counting unsaturation

The syllabus names it the **ring rule**; the quantity is the index of hydrogen deficiency, also called double bond equivalents. For a formula Cc Hh Nn Oo Xx (X a halogen, counted as hydrogen; oxygen and sulfur are ignored):

**DBE = C - (H + X)/2 + N/2 + 1**

Each ring and each pi bond contributes one. Worked checks: benzene C6H6 gives 6 - 3 + 1 = 4 (one ring, three double bonds). Acetophenone C8H8O gives 8 - 4 + 1 = 5 (ring, three ring double bonds, one carbonyl). Caffeine C8H10N4O2 gives 8 - 5 + 2 + 1 = 6 (two fused rings and four pi bonds). The DBE is computed before any fragment is interpreted, because it caps the structural possibilities: a formula with DBE 1 cannot contain a benzene ring, whatever the peaks seem to say, and a formula with DBE 5 and an IR carbonyl has exactly four unsaturations left for rings and double bonds.

### 3.3 Fragmentation of alkanes

A straight chain alkane's molecular ion breaks carbon-carbon bonds along the chain, and the positive charge stays on the alkyl fragment. The spectrum is a picket fence of clusters at m/z 29, 43, 57, 71, 85, each an alkyl cation CnH2n+1, spaced 14 units (one CH2) apart, with the corresponding alkane pattern of small satellite peaks. The propyl (43) and butyl (57) cations are usually the tallest because secondary character and favourable cleavage statistics combine there. The molecular ion of a straight chain alkane is visible but modest; with branching it weakens sharply, because cleavage at a branch point gives secondary

or tertiary cations so readily that few molecular ions survive. A branched alkane is read from its gaps and its enhanced peaks: cleavage at the branch throws out the largest radical preferentially and leaves the stable cation. In an unknown, a weak or absent M+ with a strong 43/57 pattern is itself evidence for a branched saturated skeleton, and the DBE from MS plus an IR with only C-H bands completes the picture.

### 3.4 Fragmentation of alcohols

Alcohols fragment by **alpha cleavage**: the bond between the alpha carbon and the next carbon breaks, the charge staying on the oxygen containing fragment as an oxonium ion, resonance stabilised between carbon and oxygen. The rules of thumb are quantitative. A primary alcohol gives the oxonium ion CH2=OH+ at **m/z 31**, often the base peak. Secondary and tertiary alcohols give the homologous ions at 45, 59 and upward, and the largest group on the alpha carbon is lost preferentially as the radical. A molecular ion that is weak or absent is typical, especially for tertiary alcohols. The second signature is dehydration: loss of water, M - 18, common in the molecular ion region for larger alcohols, sometimes followed by ethylene loss (M - 46 overall). A long chain primary alcohol such as 1-butanol shows the full pattern: a modest M+ at 74, the m/z 31 oxonium ion, and 56 (M - 18) among its leading peaks. Benzylic alcohols cleave at the ring bond to give the hydroxytropylium ion at m/z 107, and phenols behave differently again, holding a strong molecular ion and losing CO (M - 28) and CHO (M - 29).

### 3.5 Fragmentation of amines

Alpha cleavage dominates amine spectra even more completely, and the charge bearing fragment is the iminium ion. A primary straight chain amine shows its base peak at **m/z 30**, CH2=NH2+, the nitrogen analogue of the alcohol's m/z 31: the pair 30 versus 31 is one of the neatest functional group discriminations in the whole subject. Secondary and tertiary amines give the substituted iminium ions (44, 58 and upward), the largest alpha substituent again being lost as radical. An odd molecular weight confirms the single nitrogen by the nitrogen rule, so the combination, odd M+, base peak 30 or its homologues, and no carbonyl in the IR, is effectively a proof of an aliphatic amine. Aromatic amines hold stronger molecular ions and fragment less, and amides split their behaviour between the carbonyl and nitrogen sides: primary amides show a characteristic McLafferty ion at m/z 59, which leads naturally to section 3.6.

### 3.6 Fragmentation of carbonyl compounds, and the McLafferty rearrangement

Carbonyl compounds fragment two ways, and a good interpretation looks for both.

**Alpha cleavage** breaks the bond beside the carbonyl. Ketones lose an alkyl radical to give the acylium ion R-C≡O+; for methyl ketones this is CH3CO+ at **m/z 43**. Aldehydes lose the aldehydic hydrogen or an alkyl group, giving M - 1 and the acylium ions; aromatic aldehydes hold on to a strong M+ and show M - 1 and the phenyl region peaks. Esters cleave on both sides of the oxygen: loss of the alkoxy radical gives the acylium ion, loss of the acyl radical gives the alkoxy cation, and protonated acid ions appear for larger esters. Carboxylic acids show M - 17 (loss of OH) and M - 45 (loss of COOH) alongside their rearrangement ion.

The **McLafferty rearrangement** is the second way, and the most examinable rearrangement in mass spectrometry. If the chain on one side of the carbonyl is long enough to present a **gamma hydrogen**, the molecular ion folds into a six membered cyclic transition state; the gamma hydrogen transfers to the carbonyl oxygen, the bond between the alpha and beta carbons breaks, and the products are an **enol radical cation** and a neutral alkene. The enol ion's mass is a fingerprint of the carbonyl class: **m/z 58** for methyl ketones with a gamma hydrogen (2-pentanone and upward), **m/z 74** for methyl esters, **m/z 60** for straight chain carboxylic acids, **m/z 59** for primary amides.

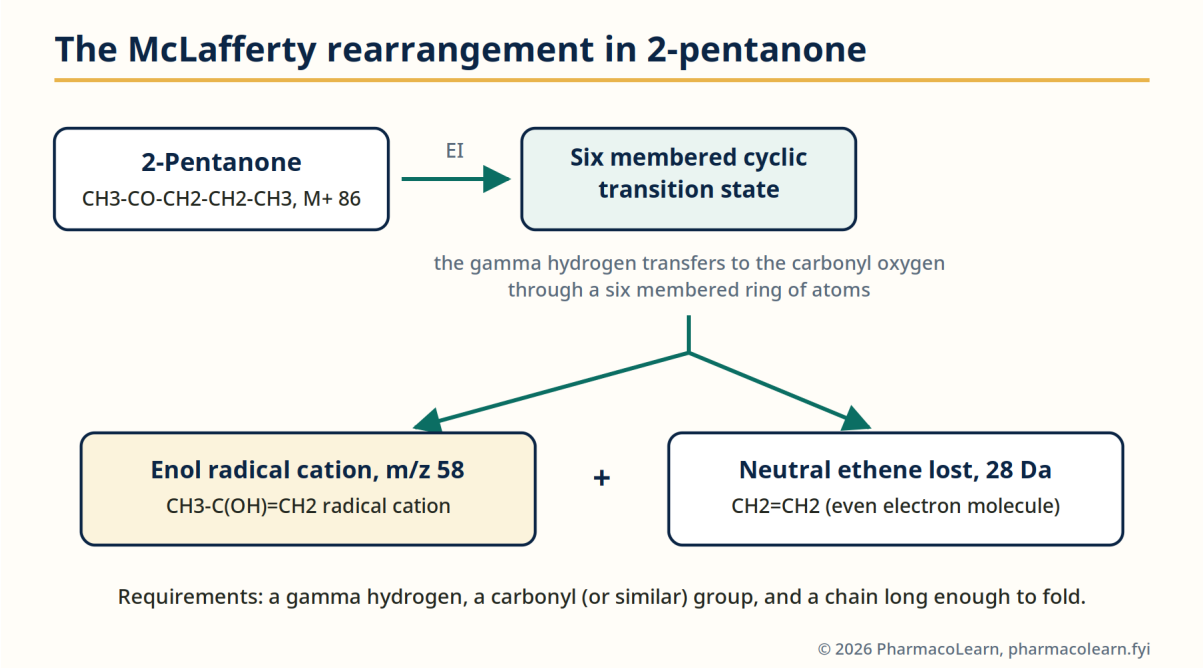


*Figure: The McLafferty rearrangement. Six membered transition state, gamma hydrogen transfer, alpha-beta bond cleavage: the three features every answer must name.*

Note the bookkeeping, because examiners probe it: the rearrangement ion is a radical cation (odd electron) formed from a radical cation by loss of an even electron neutral, which the even electron rule permits. A McLafferty peak therefore also proves a chain of at least three carbons beyond the carbonyl on one side, structural information from a single peak.

## 3.7 Metastable ions

Some ions survive the ion source but fall apart in the field free region between source and analyser. Their fragments appear not at the true fragment mass but as broad, low, diffuse peaks at an apparent mass m* given by:

**m* = (m2 squared) / m1**

where m1 is the decomposing parent and m2 the daughter. The classic example sits in the spectrum of toluene: the tropylium ion at 91 loses acetylene to give the cyclopentadienyl cation at 65, and the metastable peak appears at 65 x 65 / 91 = 46.4. A metastable peak is a certificate of parentage: it proves that a specific daughter came from a specific parent in one step, something intensities alone can never show. In interpretation, when two fragmentation routes to the same fragment compete, a metastable peak settles which one actually operates.

## 3.8 Isotopic peaks

The peaks one and two mass units above any ion are not noise; they are the isotope pattern, and they count atoms. Carbon-13 makes M+1 about **1.1 percent of M per carbon atom**, so the M+1 height estimates the carbon count of the ion (and of the molecule, applied to M+). The M+2 peak carries the halogen signatures: chlorine (chlorine-35 and chlorine-37 in a 75.8 to 24.2 natural ratio) gives M : M+2 about **3 : 1** for one chlorine, and about 9 : 6 : 1 across M, M+2, M+4 for two; bromine (79 and 81 in a 50.7 to 49.3 ratio) gives M : M+2 about **1 : 1**; two bromines give 1 : 2 : 1. Sulfur adds about 4.4 percent at M+2 per atom from sulfur-34, a quieter but useful signal.

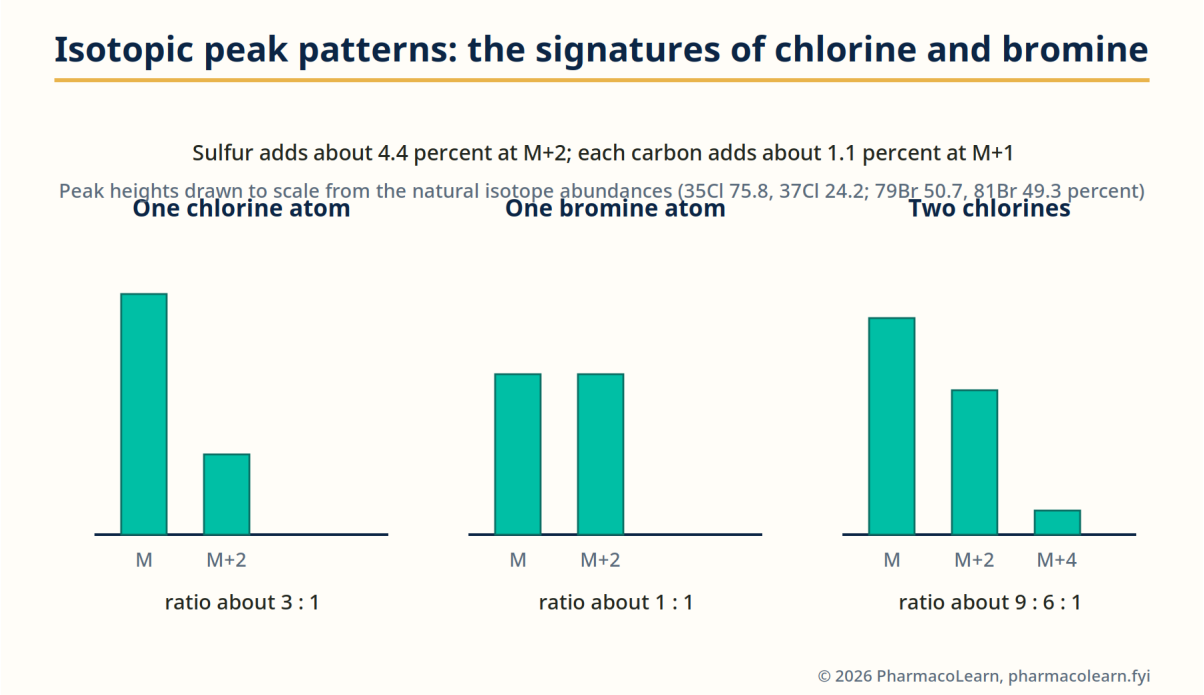


*Figure: Isotope signatures. These patterns are visible in every fragment ion that retains the halogen, not only in the molecular ion.*

The patterns apply to fragments as well as to M+, which multiplies their value: if the molecular ion shows one chlorine and a fragment at some lower mass still shows the 3 : 1 pair, that fragment retains the chlorine; if the pair vanishes below a certain fragment, the chlorine left with the neutral loss. Few single observations in mass spectrometry buy so much structural information so cheaply.

## 3.9 Interpretation workflow: a worked spectrum

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1. Find M+: apply the nitrogen rule; read DBE from it
2. Read M+1 (carbon count) and M+2 (Cl, Br, S)
3. List the major fragments; note neutral losses
   (15 CH3, 18 H2O, 28 CO/C2H4, 29 CHO/C2H5, 31 OCH3,
    43 C3H7/CH3CO, 45 COOH/OC2H5 ...)
4. Match fragments to the alpha cleavage and McLafferty
   fingerprints of the groups IR has already suggested
5. Use metastables, where present, to certify key steps
6. Assemble a structure; check EVERY major peak is explained
   and the DBE and nitrogen rule are satisfied
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**Worked example: acetophenone.** M+ at 120, strong; base peak 105; further peaks at 77 and 51; M+1 about 8.8 percent of M+. Nitrogen rule: even mass, zero or even nitrogens. M+1 suggests 8 carbons (8 x 1.1 = 8.8). A formula of C8H8O fits the mass (96 + 8 + 16 = 120) and gives DBE = 8 - 4 + 1 = 5. IR (given with the problem) shows a conjugated carbonyl near 1685 cm-1 and aromatic bands. Fragment 105 is M - 15, loss of a methyl radical: alpha cleavage of a methyl ketone, giving the benzoyl cation C6H5CO+, resonance stabilised and rightly the base peak. Fragment 77 is 105 - 28, loss of CO from the benzoyl ion, leaving the phenyl cation; 51 is 77 - 26, loss of acetylene from the phenyl cation, the standard aromatic cascade. The four DBE beyond the carbonyl are the benzene ring. Structure: C6H5COCH3, acetophenone, with every major peak assigned and every rule satisfied. That completeness, not the answer alone, is what full marks look like in this unit.

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