

Unit 4: Hyphenated Chromatographic Techniques

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4.1 Why hyphenate: separation meets identification

A chromatograph separates a mixture and reports peaks in time; a spectrometer identifies compounds but struggles with mixtures. A **hyphenated technique** welds the two together in line: the chromatograph delivers pure components one at a time, and the spectrometer identifies each as it emerges. Every system in this unit is that idea in a different costume, and every one of them turns on the same engineering problem, the **interface**: the junction that must transfer analyte from the separation conditions (a pressurised liquid, a gas stream, an electrophoresis buffer) into the detector's conditions without losing resolution or analyte. Judging each technique means asking three questions: what does the separation side do well, what does the detector side add, and what does the interface cost?

4.2 GC-MS

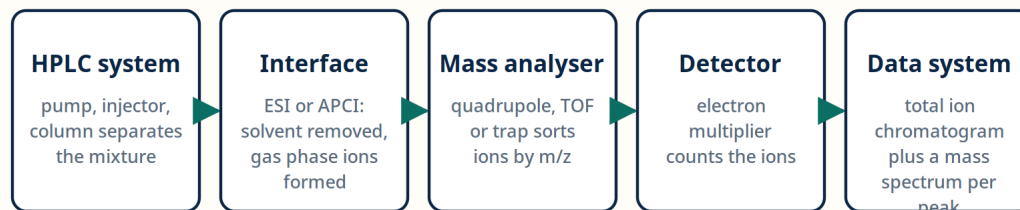
Gas chromatography separates volatile, thermally stable compounds on a capillary column in a helium or hydrogen stream; the mass spectrometer at the exit ionises each eluting component, usually by electron ionisation, and records a full mass spectrum per peak. The interface is the easiest in this unit because the GC effluent is already a dilute gas at low flow: a heated transfer line leads the column effluent straight into the ion source, sometimes through a jet separator that enriches analyte over carrier gas in older designs. The product of a run is a **total ion chromatogram** (peak per component) plus a searchable EI spectrum for each peak: library matching against collections of hundreds of thousands of reference spectra makes GC-MS the most confident routine identifier of volatile organics. Applications in pharmaceutical work: residual solvents, volatile impurities and degradation products, essential oil and flavour profiling, forensic and doping control samples, and environmental contaminants in raw materials. Its boundary is absolute: compounds that will not vaporise intact cannot be run, which is why the pharmaceutical mainstream lives in the next system instead.

4.3 LC-MS

Liquid chromatography handles the non-volatile, polar and fragile majority of drug molecules, and coupling it to MS was historically the hardest interface problem in analytical chemistry: about a millilitre per minute of liquid must become ions in a high vacuum. The solution is the atmospheric pressure interface. In **electrospray ionisation (ESI)** the eluent sprays through a charged needle, forming charged droplets that evaporate until gas phase ions, typically protonated molecules $[M+H]^+$, are released at atmospheric pressure and sampled through a small orifice into the vacuum. In **atmospheric pressure chemical ionisation (APCI)** the eluent is vaporised in a heated nebuliser and ionised by a corona discharge, suiting smaller, less polar molecules. Because these are soft ionisation methods, spectra are simple, often a single molecular ion species, which makes LC-MS superb for molecular weight confirmation and quantification but poorer than EI for library style structural matching; tandem MS (MS/MS) restores structural information by fragmenting selected ions in a collision cell.

LC-MS: coupling a chromatograph to a mass spectrometer

The interface is the hard part: it must pass analyte ions from liquid at atmospheric pressure into high vacuum



Result: retention time identifies the peak, the mass spectrum identifies the compound, one run gives both

The same pattern, separator plus detector, links GC-MS, LC-NMR, CE-MS and the other hyphenated systems of this unit

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Figure: The LC-MS chain. The interface, not the analyser, is the invention that made the technique routine.

Pharmaceutical applications dominate modern analysis: impurity identification in drug substance and product, metabolite profiling in discovery and development, bioanalysis of drugs in plasma for pharmacokinetic studies (usually triple quadrupole MS/MS in selected reaction monitoring mode), peptide and protein characterisation, and cleaning validation. LC-MS is the default hyphenation against which the others in this unit are the specialists.

4.4 GC-AAS

Coupling gas chromatography to atomic absorption spectrometry answers a question neither instrument answers alone: not how much of an element a sample contains, but **in which chemical forms**. The GC separates volatile organometallic species; the effluent passes into a heated quartz furnace in the AAS light path, where the compounds are atomised and the element specific absorption is measured. The classic applications are speciation studies: methylmercury versus inorganic mercury in biological and environmental samples, alkyllead compounds, and organotin species. In pharmaceutical quality work its niche is elemental impurity speciation where the toxicology depends on the form, over and above the total element content that pharmacopoeial elemental impurity tests report. Sensitivity is high for the target element and the chromatogram contains only peaks of that element, a clean speciation fingerprint.

4.5 LC-FTIR

An infrared spectrum gives functional group proof that MS cannot, so LC-FTIR aims to record IR spectra of HPLC peaks. The obstacle is the solvent: water and the common mobile phases absorb IR radiation intensely and swamp the analyte. Two interface philosophies exist. **Flow cell** interfaces pass the eluent through a short path cell in the FTIR beam, workable only with IR transparent solvents and limited sensitivity. **Solvent elimination** interfaces deposit the eluent on a moving substrate (a rotating disc or a zinc selenide window), evaporate the solvent under vacuum and gentle heat, and measure the deposited analyte spots by reflectance or transmission, building a chromatogram from reconstructed IR responses (a Gram-Schmidt plot) with a full spectrum stored for every point. Solvent elimination wins on sensitivity and solvent freedom, including reversed phase gradients, and its applications are polymer additive analysis, drug-excipient compatibility studies and isomer discrimination, where IR distinguishes compounds whose mass spectra are nearly identical.

4.6 LC-NMR

NMR is the most informative and least sensitive of the spectroscopies, and LC-NMR exists to put that information onto HPLC peaks, typically for natural product isolation and metabolite identification, where an unknown's structure must be solved from micrograms. The interface is a flow probe: a small volume flow cell (typically 60 to 120 microlitres of active volume) sits in the magnet, connected to the column by narrow capillary tubing chosen to preserve chromatographic resolution. Operation modes trade time for spectrum quality: continuous flow for a first survey, **stopped flow**, in which the pump halts with the peak parked in the cell so long 1-D and even 2-D experiments can accumulate, and loop storage, in which peaks are parked in capillary loops for offline measurement. Two practical burdens define the technique: solvent signals must be suppressed (deuterated solvents are expensive at HPLC flow rates, so pulse sequence suppression of protonated solvent peaks is standard), and sensitivity demands concentrated peaks, often from repeated injections. Where it succeeds, the payoff is the complete Unit 2 argument, COSY, HSQC and HMBC included, performed on a component that was never isolated by hand.

4.7 CE-MS

Capillary electrophoresis separates by charge to size ratio in a narrow fused silica capillary under a high voltage, consuming nanolitres and giving exceptional plate counts for ionic species: peptides, oligonucleotides, charged metabolites and chiral separations with selector additives. Coupling to MS through an electrospray interface must respect the tiny CE flow (nanolitres per minute): a coaxial **sheath liquid** interface adds a make up flow around the capillary tip that both completes the electrical circuit and feeds the spray, at some cost in dilution; sheathless porous tip interfaces avoid the dilution for maximum sensitivity. Applications follow CE's strengths: charge variant profiling of therapeutic proteins, peptide mapping, metabolomics of ionic metabolites, and counter ion and small ion analysis. CE-MS is the specialist hyphenation for samples that are ionic, scarce and badly served by reversed phase LC.

4.8 HPTLC

High performance thin layer chromatography modernises the plate: finer, more uniform silica layers on glass or foil, instrumental sample application as narrow bands, controlled chamber development, and evaluation by scanning densitometry in absorbance or fluorescence. Identity is carried by the R_f value plus in situ spectra: the densitometer can record a UV spectrum of a spot on the plate, and plates can be eluted into a mass spectrometer through commercial elution interfaces for confirmation. Its pharmaceutical stronghold is herbal drug and Ayurvedic analysis, where pharmacopoeias prescribe HPTLC fingerprints for identity of crude drugs and extracts, alongside related substances testing and formulation assays where its virtues matter: many samples run side by side on one plate, minimal solvent use, the whole chromatogram permanently stored on the plate, and low cost per sample. As a screening and fingerprinting hyphenation (TLC with spectroscopic evaluation) it complements, rather than competes with, the column systems.

4.9 Supercritical fluid chromatography

SFC uses a supercritical fluid, in practice carbon dioxide above its critical point (31.1 degrees Celsius, 73.8 bar), as the main mobile phase component, usually with a polar modifier such as methanol and sometimes an additive. The fluid's low viscosity and high diffusivity give fast, high efficiency separations at several times HPLC speeds, and decompression returns the CO₂ to a gas that simply leaves, simplifying fraction collection. Detection is by UV in routine work, with MS coupling straightforward because the decompressed stream suits atmospheric pressure ionisation. Its flagship pharmaceutical application is **chiral separation**: SFC on chiral stationary phases is the first choice for enantiomer analysis and preparative enantiomer purification in discovery chemistry, where its speed and easy solvent removal save days. It also serves lipophilic compound classes poorly retained in reversed phase, such as fat soluble vitamins, steroids and triglyceride type lipids.

4.10 Ion chromatography and ion exclusion chromatography

Ion chromatography is ion exchange chromatography engineered for sensitive ion measurement: low capacity exchangers, an eluent of dilute hydroxide or carbonate, and, in the standard suppressed mode, a suppressor device that converts the eluent to a low conductivity form (water or carbonic acid) while converting analyte ions to their more conducting acid or base forms, read by a conductivity detector. It quantifies anions (fluoride, chloride, nitrite, nitrate, phosphate, sulfate) and cations (sodium, potassium, ammonium, calcium, magnesium) at trace levels, serving water for pharmaceutical purposes testing, counter ion assay of drug salts, and ionic impurities. **Ion exclusion chromatography** separates by a different mechanism on a fully sulfonated cation exchanger: strong acids are excluded from the resin pores by Donnan exclusion and elute early, while weak acids partition into the pore liquid according to their ionisation and elute later, separated essentially by pKa. Its home ground is the profiling of weak organic acids, in fermentation broths, foods and pharmaceutical formulations, with conductivity or UV detection.

4.11 Flash chromatography

Flash chromatography is the preparative member of the list: column chromatography accelerated by moderate pressure (typically a few bar of air or nitrogen) pushing solvent through a cartridge of silica gel of defined particle size, following the method popularised by Still. Modern systems automate it: prepacked disposable cartridges, gradient pumps, UV (and often evaporative light scattering) detection, and fraction collection triggered by the detector trace. It is not an identification technique; its place in this unit is as the isolation engine that feeds every other technique. Reaction mixtures from medicinal chemistry are purified in minutes, natural product extracts are fractionated into testable components, and the purified outputs go forward to NMR and MS for the structural arguments of Units 2 and 3. Scale up is by cartridge size, from milligrams to hundreds of grams.

4.12 Choosing the hyphenated system

Analytical problem	First choice	Why
Volatile, thermally stable unknowns	GC-MS	EI library spectra identify directly
Polar, fragile or high mass analytes; bioanalysis	LC-MS (ESI, MS/MS)	Soft ionisation, sensitivity, quantification
Elemental speciation (which form of the metal)	GC-AAS	Element specific detection after separation
Functional group proof for an LC peak; isomer cases	LC-FTIR	IR evidence MS cannot give
Full structure of an unisolated natural product	LC-NMR (stopped flow)	Complete NMR without manual isolation
Ionic metabolites, protein charge variants	CE-MS	Charge based separation, minute samples
Herbal identity fingerprints, many samples cheaply	HPTLC	Parallel samples, pharmacopoeial fingerprints
Enantiomers, fast purification of isomers	SFC	Chiral phases, speed, easy recovery
Trace anions and cations; drug salt counter ions	Ion chromatography	Suppressed conductivity sensitivity
Weak organic acid profiles	Ion exclusion chromatography	Separation by pKa
Purification before identification	Flash chromatography	Fast, automated, scalable isolation

The pattern to carry into examinations: name the analyte class and the question first, and the technique follows. Hyphenation questions are almost never asking for instrument trivia; they are asking whether the candidate can match a separation mechanism and a detector to a stated problem.

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