

Hyphenated techniques

Introduction to Hyphenated Techniques

- Hyphenated techniques are analytical methods that combine two or more instrumental techniques to leverage the strengths of each.
- Typically, this involves chromatographic separation (like LC, GC, or HPTLC) followed by spectroscopic detection (like MS, UV, or IR).
- These techniques provide:
 - Separation (via chromatography)
 - Identification (via mass spectrometry or spectroscopy)
 - Quantification (based on peak area or intensity)

Purpose:

- Improve sensitivity, selectivity, structural elucidation, and quantification in complex sample matrices.

Examples of Hyphenated Techniques:

- LC-MS/MS** – Liquid Chromatography–Tandem Mass Spectrometry
- GC-MS/MS** – Gas Chromatography–Tandem Mass Spectrometry
- HPTLC-MS** – High Performance Thin Layer Chromatography–Mass Spectrometry

LC-MS/MS (Liquid Chromatography – Tandem Mass Spectrometry)

General Principle:

- LC separates analytes in a liquid mobile phase based on polarity, size, or charge.
- MS/MS (Tandem Mass Spectrometry) involves two stages of mass analysis:
 - The first MS isolates the parent ion (precursor).
 - The second MS analyzes the fragmented ions (daughter ions).
- Electrospray Ionization (ESI) or APCI is typically used to interface LC with MS.

Components

Component	Function
Liquid Chromatograph (LC)	Separates components in a liquid phase using a column and mobile phase.

Ionization Source	Converts eluted molecules into gas-phase ions (commonly ESI – Electrospray Ionization, or APCI – Atmospheric Pressure Chemical Ionization).
Mass Spectrometer (Triple Quadrupole)	Detects ions and fragments them to produce structural data. Consists of Q1 – Q2 – Q3.

Procedure / Workflow:

1. Sample Injection into LC

- Components are separated based on interaction with column.

2. Ionization (ESI/APCI)

- Converts eluted analytes into gas-phase ions.

3. MS1 (Precursor Ion Selection)

- Selects a specific ion of interest.

4. Collision Cell (CID)

- Precursor ion is fragmented using inert gas (e.g., nitrogen).

5. MS2 (Product Ion Analysis)

- Detects specific fragment ions.

6. Data Interpretation

- Quantification and identification using MRM (Multiple Reaction Monitoring).

Advantages:

- Extremely high sensitivity and specificity
- Can detect picogram to femtogram levels
- Excellent for complex biological matrices (plasma, serum, urine)
- Multiple analytes can be quantified simultaneously

Applications:

- **Pharmaceuticals:** Drug metabolism studies, bioequivalence.
- **Clinical:** Hormone quantification (e.g., testosterone, vitamin D).
- **Toxicology:** Drugs of abuse, forensic investigations.
- **Food safety:** Pesticide residue analysis.
- **Environmental:** Pollutants in water and soil.

GC-MS/MS (Gas Chromatography – Tandem Mass Spectrometry)



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