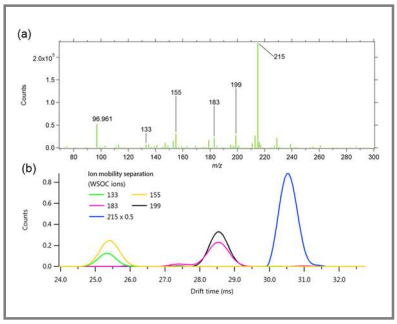
Environmental Chemical Analysis

Organic Environmental Analysis

Ricardo Bettencourt da Silva

2025/10/17



Environmental Chemical Analysis - Organic analysis

Content

- The relevance of Organic Environmental Analysis
- Analytical methods for organic environmental analysis
  - Sample preparation and clean-up
  - Analyte derivatization
  - GC analysis
    - Identification of the analyte
    - Quantification of the analyte
  - HPLC analysis
    - Identification of the analyte
    - Quantification of the analyte
  - Expressing results in different basis
  - Correction of results for poor recovery

Environmental Chemical Analysis – Organic analysis

The relevance of Organic Environmental Analysis

- Protecting Human Health & Ecosystems: Many organic compounds are toxic, persistent, bioaccumulative, or carcinogenic. These compounds are analysed to ensure drinking water safety, assess the health of aquatic and terrestrial ecosystems, and protect biodiversity.

- Regulatory Compliance: Environmental legislation (e.g., EU Water Framework Directive) sets strict limits for concentrations of priority pollutants. Analysis provides the data for compliance and enforcement.

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The relevance of Organic Environmental Analysis

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- Pollution Source Identification & Remediation: Chemical "fingerprinting" can help identify the source of a spill or contamination, and monitoring is essential for evaluating the effectiveness of cleanup efforts.

- Scientific Research: To understand the fate and transport of chemicals in the environment, their degradation pathways, and long-term ecological impacts.

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Analytical Methods | An Overview

- The analysis of organic environmental parameters is a multi-step process. The core instrumental analysis (GC or HPLC) is only one part.
- Sampling: The first critical step; an unrepresentative sample cannot be saved by the best laboratory.

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Analytical Methods | An Overview

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- Sample Preparation & Clean-up: Often the most time-consuming and error-prone part, but essential for removing interferences and concentrating the analytes.

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Environmental Chemical Analysis – Organic analysis

Analytical Methods | An Overview

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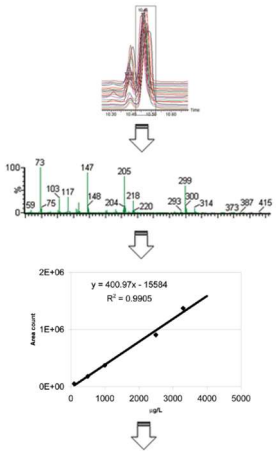
- Analysis: Using Gas Chromatography (GC) or High-Performance Liquid Chromatography (HPLC) to separate the complex mixture into individual components.
- Data Processing & Reporting: Converting instrument signals into meaningful, reliable concentrations.



(133 ± 50) µg/kg of pyrene
(95% conf. level)

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Analytical Methods | Sample Preparation and Clean-up

- Goals: Concentrate the analytes (to reach detection limits), remove interfering matrix components (e.g., humic acids, lipids), and convert the sample into a form suitable for the instrument.

- Common Techniques:
For Water: Solid-Phase Extraction (SPE) and Liquid-Liquid Extraction (LLE).

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Analytical Methods | **Sample Preparation and Clean-up**

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●● For Water: Solid-Phase Extraction (SPE):

SPE operates on chromatographic principles, separating analytes based on their different affinities for a solid stationary phase (sorbent) and a liquid mobile phase (the water sample and subsequent solvents). The general procedure involves four key steps:

- » Conditioning
- » Loading
- » Washing
- » Elution

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Analytical Methods | **Sample Preparation and Clean-up**

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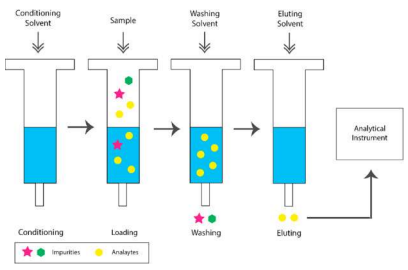
●● For Water: Solid-Phase Extraction (SPE):

Conditioning: The sorbent is activated and prepared by passing a solvent through the cartridge or disk to optimize the interaction with the analytes.

Loading: The water sample is passed through the conditioned sorbent. The target analytes are retained on the solid phase, while the water and unwanted matrix components pass through.

Washing: A rinsing solvent is used to remove any remaining impurities from the sorbent while leaving the target analytes bound.

Elution: A final, stronger solvent is passed through the sorbent to release and collect the concentrated analytes. The resulting extract is then ready for analysis by instruments like GC or HPLC.



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Analytical Methods | **Sample Preparation and Clean-up**

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●● For Water: Solid-Phase Extraction (SPE):

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Analytical Methods | **Sample Preparation and Clean-up**

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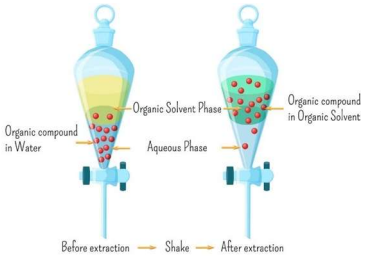
●● For Water: Liquid-Liquid Extraction (LLE): This technique involves using an immiscible solvent to separate compounds based on their solubility. By mixing the water with an organic solvent in a separatory funnel, a solute moves from the water phase to the organic phase, and the two layers are then separated, leaving the solute isolated in the extract. This is a common technique for sample cleanup or concentrating analytes from an aqueous sample.



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Analytical Methods | Sample Preparation and Clean-up

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●● For Water: Liquid-Liquid Extraction (LLE):

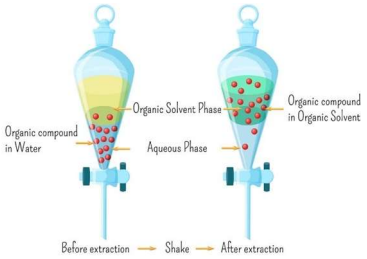
Separation stages:

Mixing: An aqueous water sample is mixed with an organic solvent in a separatory funnel.

Partitioning: Target compounds (analytes) migrate from the water into the organic solvent.

Separation: The mixture is left to settle, and the two liquid layers separate based on their densities. The denser liquid forms the bottom layer.

Collection: The layers are separated, and the organic layer containing the concentrated target compounds is collected for further analysis. The solvent can then be evaporated to further concentrate the analytes.



The process is repeated three times with three organic solvent aliquots to maximise extraction.

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Analytical Methods | Sample Preparation and Clean-up

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●● For Water: Liquid-Liquid Extraction (LLE):

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Analytical Methods | **Sample Preparation and Clean-up**

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●● For Solids (Soil/Sediment):

Pressurized Liquid Extraction (PLE): PLE is a technique that uses elevated temperature and pressure to quickly and efficiently extract compounds from solid or semi-solid samples using a liquid solvent.

QuEChERS: is a sample preparation technique for analysing compounds like pesticides in food and environmental samples. It stands for Quick, Easy, Cheap, Effective, Rugged, and Safe, and involves two main steps: an initial extraction followed by a cleanup using dispersive solid-phase extraction (d-SPE). This method uses less solvent and time compared to traditional techniques and it suitable for analysing a wide range of compounds.

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Analytical Methods | **Sample Preparation and Clean-up**

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●● For Solids (Soil/Sediment):

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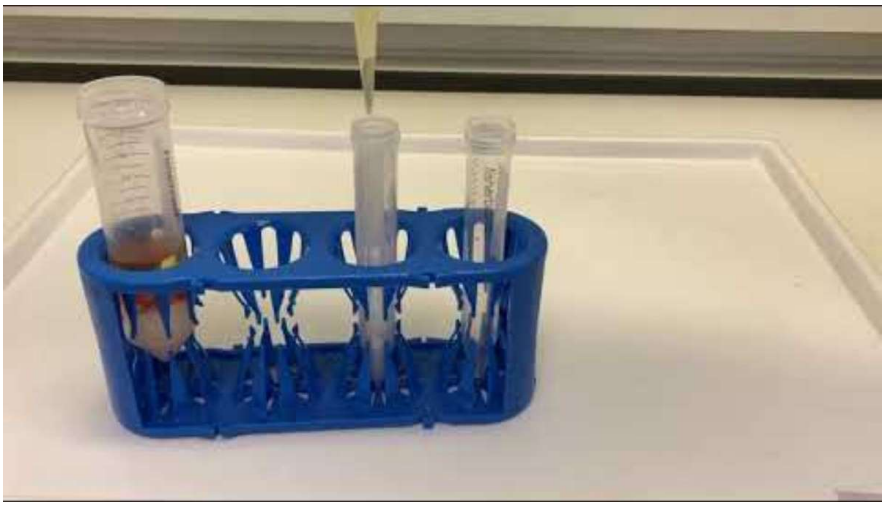
QuEChERS:



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Analytical Methods

Sample Preparation and Clean-up

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●● For Solids (Soil/Sediment):

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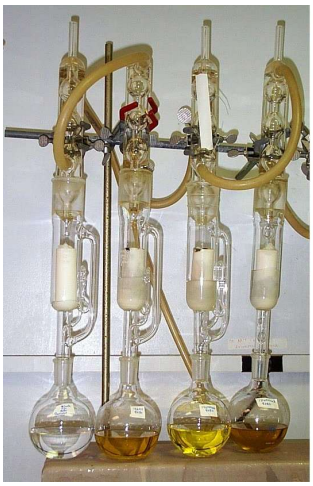
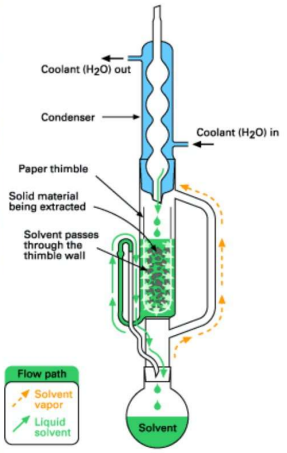
Soxhlet: is a continuous, automated solid-liquid extraction method used to isolate compounds from a solid sample using a solvent.





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Analytical Methods

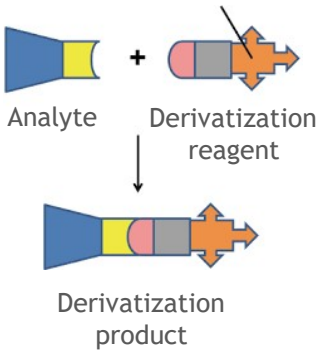
Analyte Derivatization

● Definition: A chemical reaction performed on the analyte to change its chemical structure.

●● Reasons for Derivatization:

1. Improve Chromatography: Make polar/thermolabile compounds volatile and stable for GC (e.g., silylation of phenols).
2. Enhance Detection: Add a chromophore or fluorophore for sensitive HPLC detection (e.g., derivatizing amines with dansyl chloride).
3. Increase Stability: Prevent degradation of the analyte during analysis.

●● Common Reactions: Silylation, acylation, alkylation (for GC); fluorescence tagging (for HPLC).



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Analytical Methods | **Analyte Derivatization**

- Definition: A chemical reaction performed on the analyte to change its chemical structure.

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●● Common Reactions:

Silylation: Chemical process that replaces an active hydrogen atom in a molecule with a silyl group, such as a trimethylsilyl (TMS) group. The most common methods involve reacting the molecule with a silylating agent, like N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA), often in the presence of a catalyst, to increase volatility and stability for analytical techniques like gas chromatography (GC).

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R-OH+Cl-Si(CH3)3→R-O-Si(CH3)3+HCl
R-OH+Cl-Si(CH3)3→R-O-Si(CH3)3+HCl

Analytical Methods | **Analyte Derivatization**

- Definition: A chemical reaction performed on the analyte to change its chemical structure.

(…)

●● Common Reactions:

Reaction example: Silylation of a hydroxyl group:

A compound with a hydroxyl group (like an alcohol or fatty acid) react with a a silylating agent, such as trimethylchlorosilane (TMSCl):

The active hydrogen on the OH group is replaced by a trimethylsilyl ($Si(CH_3)_3$ group.

$$R - OH + Cl - Si(CH_3)_3 \rightarrow R - O - Si(CH_3)_3 + HCl$$

The trimethylsilyl ether is more volatile and thermally stable than the original compound.

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Analytical Methods | **GC Analysis**

- Principle: Separation occurs in a heated oven where analytes are vaporized and carried by an inert gas (mobile phase) through a coated capillary column (stationary phase).

- Separation Mechanism: Analytes are separated primarily based on their volatility and their interaction with the stationary phase.

- Ideal For: Volatile and semi-volatile organic compounds (VOCs/SVOCs) that are thermally stable. Examples: solvents (BTEX), pesticides, PCBs, PAHs.

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Analytical Methods | **GC Analysis**

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Analytical Methods | **GC Analysis**

- Identification in GC:
- Retention Time (t_r): The primary identifier. The time it takes for a compound to travel through the column. We compare the t_r of an unknown peak to the t_r of a known standard analysed under identical conditions.
- Mass Spectrometry (MS) Detection:
- The mass spectrometer acts as a detector and breaks the eluting molecule into characteristic fragments.
- Identification is confirmed by: 1) Matching retention time with a standard, and 2) Matching the acquired mass spectrum to a reference spectra (provides a very high degree of confidence in identity)

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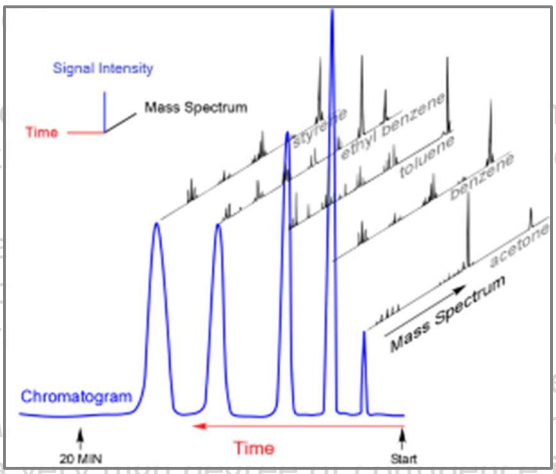
Analytical Methods | **GC Analysis**



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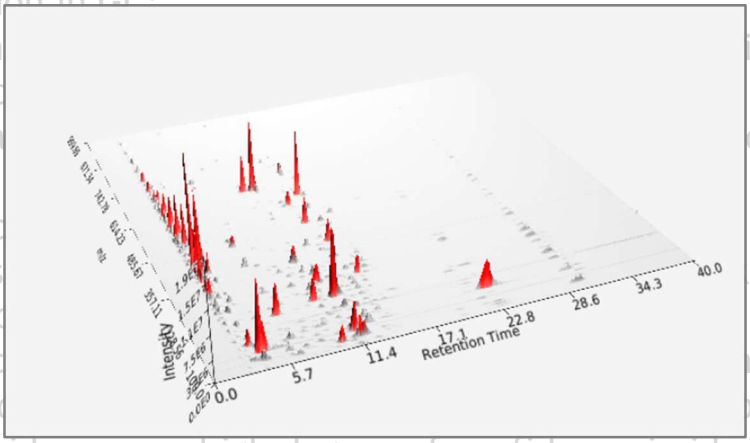
Analytical Methods | **GC Analysis**

- Identification in GC:
  - Retention
  - Mass Spec
  - The mas
  - Identific



it takes for a compound to the t_r of an unknown pea der identical conditions.

s the eluting molecule into time with a standard, and a reference spectra (provides a very high degree of confidence in identity)

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Analytical Methods | **GC Analysis**

- Quantification in GC:
  - Principle: The instrument's response (e.g., peak area) tend to be proportional to the amount of analyte.
  - The Calibration Curve:

1. Analyse a series of standards or calibrators with known concentrations.
2. Plot the peak area (or height) against the concentration.
3. Fit a line (linear regression) to the data points.

  - Quantifying the Unknown: The sample is injected, the peak area is measured, and the concentration is calculated using the equation of the calibration curve.
  - Internal Standards: Are often used to correct for variations in injection volume and sample preparation losses.

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Analytical Methods | **GC Analysis**

- Quantification in GC:

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●● Internal Standards (IS):

An IS is a chemical compound added to calibrators and sample at the same concentration. It is used to correct for variations in injection volume and other errors by comparing its chromatographic response (peak area or height) to that of the target analyte. The ratio between Analyte and IS signals is determined.

The ideal internal standard should be (1) chemically similar to the analyte, (2) well-separated in the chromatogram, (3) stable under the analytical conditions, and (4) absent from the original sample.

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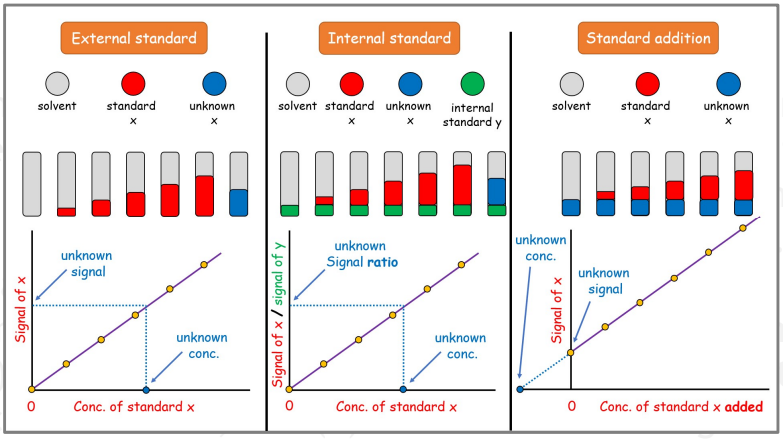
Analytical Methods | **GC Analysis**

- Quantification in GC [Different calibration stategies]:



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Analytical Methods | GC Analysis

Exercise (1/2)

You are given GC–MS peak areas for pyrene and a constant concentration internal standard (deuterated pyrene, d-pyrene). Determine the pyrene concentration in the unknown water sample A. Finally convert the concentration measured in the final extract back to the original water sample concentration using the sample preparation details below §.

Std ID	[Pyrene] (μg L^{-1})	Area (pyrene)	Area (d-pyrene)
S1	0.50	60,500	240,100
S2	1.00	119,700	239,850
S3	2.00	240,200	240,050
S4	5.00	599,200	240,200

Unknown sample A (Prepared sample analysis):
Peak area (pyrene) = 216,150
Peak area (d-pyrene) = 239,880

§ Sample preparation details:
Sample volume extracted = 0.50 L
Final extract volume = 1.00 mL

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Analytical Methods | HPLC Analysis

- Principle: Separation occurs at room temperature using a liquid mobile phase (e.g., water, methanol) pumped at high pressure through a tightly packed column.

- Separation Mechanism: Analytes are separated based on their polarity and their relative affinity for the mobile phase vs. the stationary phase.

- Ideal For: Non-volatile, thermally labile, or highly polar compounds. Examples: many pesticides, pharmaceuticals, dyes, and polar degradation products.

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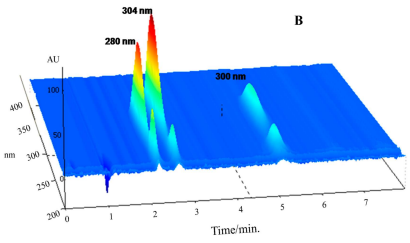
Analytical Methods | **HPLC Analysis**

Environmental Chemical Analysis – Organic analysis

Analytical Methods | **HPLC Analysis**

- Identification & Quantification in HPLC:
- Identification:
- Retention Time (t_r): The primary tool, just like in GC.
- Diode Array Detector (DAD): Provides UV/Vis spectra for each peak. The spectrum acts as a fingerprint, adding confidence to the identification based on t_r alone.



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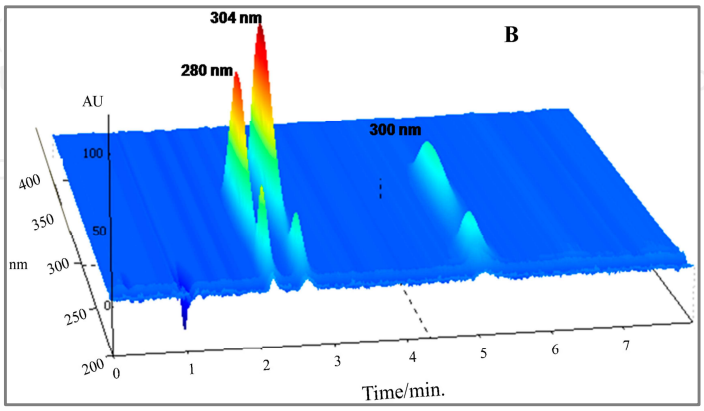
Analytical Methods | **HPLC Analysis**

- Identification & Quantification in HPLC:
- Identification:
- Retention Time (
- Diode Array Det
- peak. The spectrum
- identification based



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Analytical Methods | **HPLC Analysis**

- Identification & Quantification in HPLC:
- Identification:

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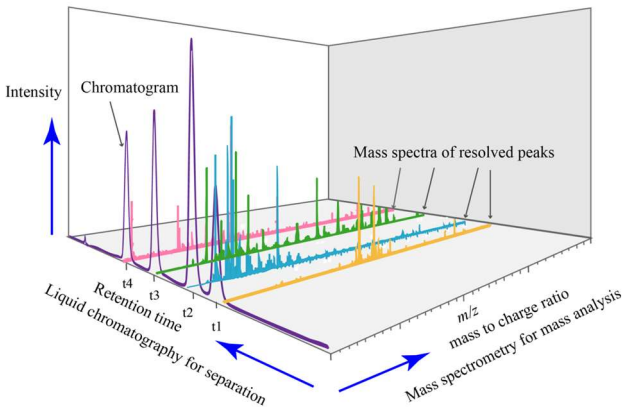
- HPLC-MS: Coupling to a mass spectrometer provides definitive identification based on molecular weight and fragmentation pattern, similar to GC-MS.



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Analytical Methods | **HPLC Analysis**

- Identification & Quantification in HPLC:
  - Identification:

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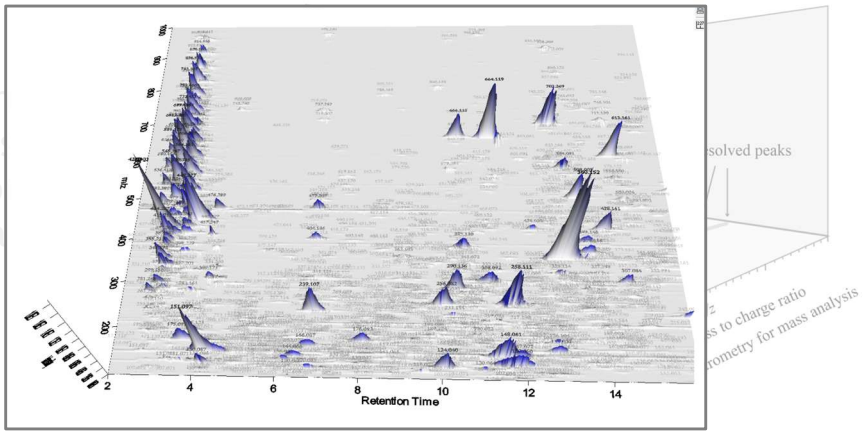
●●● HPLC-MS: Couplin
spectrometer provide
identification based o
weight and fragmenta
similar to GC-MS.



Environmental Chemical Analysis – Organic analysis

Analytical Methods | **HPLC Analysis**

- Identification & Quantification in HPLC:
  - Quantification:
    - The process is identical to GC.
    - A calibration curve is constructed using standards.
    - The peak area (or height) of the unknown is used to calculate its concentration from the calibration curve.
    - Internal standards are highly recommended, especially for complex techniques like LC-MS/MS (relevant due to matrix effects).
    - The standard addition method must be used when matrix effects are more relevant.

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Expressing Results on Different Bases

- The basis for reporting is critical for interpreting and comparing data.
- Liquid Samples (Water): Typically reported as mass/volume (e.g., µg/L, ng/L). Reporting in parts-per-billion/billion is ambiguous.
- Solid Samples (Soil, Sediment, Sludge):
- Wet Weight Basis: Concentration per mass of the sample "as is." This is how you analyse it. Can be variable due to moisture content.
- Dry Weight Basis: Concentration per mass of the dried sample. This removes the variability of water content and is often required for regulatory comparisons.

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Expressing Results on Different Bases

- The basis for reporting is critical for interpreting and comparing data.
- Lipid-Normalized: For biota tissue, results may be normalized to lipid content to account for the fact that many organics accumulate in fats.

Ex. DDT in blood levels in Greek children normalised for lipid content
(Costopoulou et al., Environ. Int. 187 (2024) 108686)

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Correction of Results for Poor Recovery

● Recovery, *R*: Measures the efficiency of the entire method (extraction + analysis). It is determined using spiked samples or Reference Materials:

$$R = c/C$$

where *c* and *C* are the estimated and reference concentrations.

●● The Dilemma: Should we correct the sample result if recovery is poor (e.g., 60%)?

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Environmental Chemical Analysis – Organic analysis

Correction of Results for Poor Recovery

●● General Rule: If recovery is within conventional control limits (e.g., 80-120%), do not correct. The result is reported as is.

●● When correction may be applied: If recovery is outside limits but is consistent, understood, and allowed by the lab's procedure. The result is then calculated as:

$$c_c = c/R$$

where *c* and c_c are the original and corrected concentrations for observed recovery (In some cases it is reported *c* and *R* separately).

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Analytical Methods

GC Analysis

Exercise (2/2)

Take the previous example of the determination of pyrene in sample A, the Table below with results from the analysis of samples without native pyrene spiked at 4 ng/L, and the minimum admissible mean analyte recovery of 80%, revise the determination performed previously.

Spiked samples results (ng/L)						
2.67	2.99	3.31	2.26	3.19	2.35	2.95
2.95	3.02	3.23	2.49	2.59	2.68	3.2
3.04	2.73	2.87	2.69	3.09	3.05	3.07
2.4	2.99	2.81	3.27	2.96	2.94	2.47
	Mean:		2.87			
	Standard deviation:		0.29			

Discuss the impact of your options.

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