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Area of Expertise
1 Chromatography & method development (LC & GC)
2 Forensic and drug-of-abuse analysis
3 Laboratory setup, validation, and data integrity
4 Regulatory & compliance-driven testing programs
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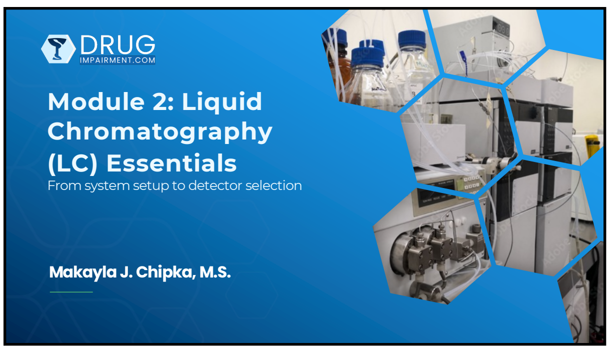
1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Module 2: Liquid Chromatography (LC) Essentials
From system setup to detector selection
Makayla J. Chipka, M.S.

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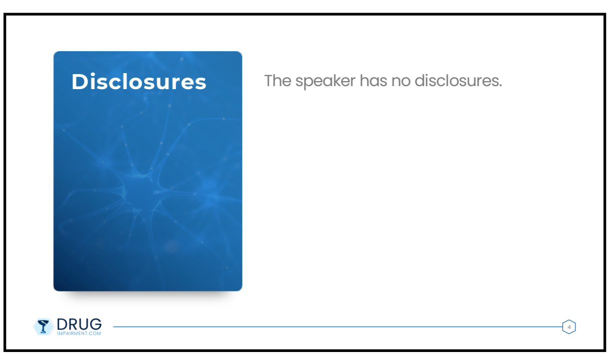
Disclosures
The speaker has no disclosures.
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Learning Objectives

1. Identify key LC system components and describe their functions
2. Set up, prime, and equilibrate an LC system correctly
3. Select appropriate columns and mobile phases based on analyte chemistry
4. Apply isocratic and gradient elution strategies effectively
5. Diagnose and troubleshoot common LC performance issues
6. Differentiate LC detector types, with emphasis on Triple Quadrupole and QTOF systems

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Lesson 2.1
Setting Up Your LC System

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7

LC System Flow Path

Solvent Reservoir to Detector

Mobile phase flows in a defined path: Solvent reservoir → degasser → pump → autosampler → injector → column → detector → waste

Why Flow Order Matters

Each component builds pressure, impacts retention, affects reproducibility, can introduce error if misconfigured

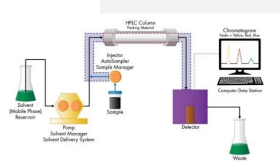

Image from Waters

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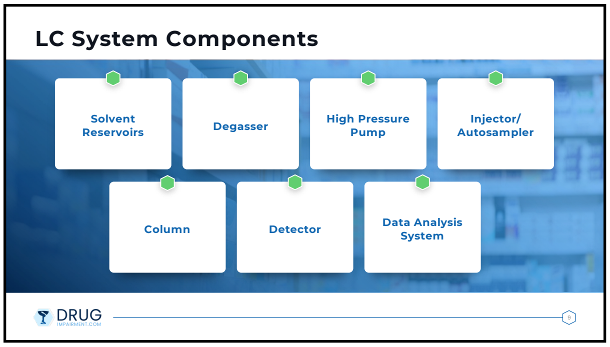
LC System Components
Solvent Reservoirs
Degasser
High Pressure Pump
Injector/ Autosampler
Column
Detector
Data Analysis System
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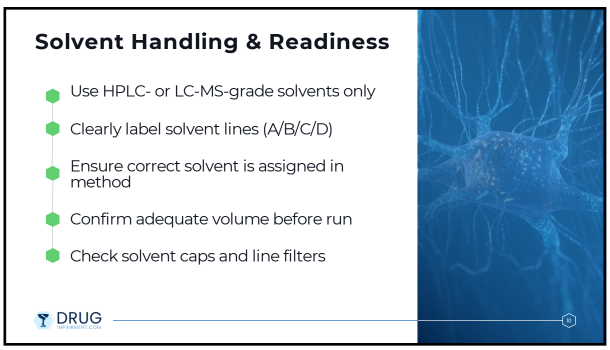
9


Solvent Handling & Readiness
Use HPLC- or LC-MS-grade solvents only
Clearly label solvent lines (A/B/C/D)
Ensure correct solvent is assigned in method
Confirm adequate volume before run
Check solvent caps and line filters
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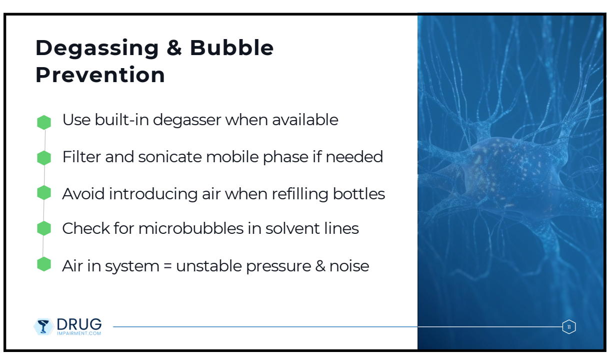
10


Degassing & Bubble Prevention
Use built-in degasser when available
Filter and sonicate mobile phase if needed
Avoid introducing air when refilling bottles
Check for microbubbles in solvent lines
Air in system = unstable pressure & noise
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11

Pump Startup & Line Priming

- Purge Each Solvent Line
- Prime Pump at Low Flow Rate
- Increase to Operating Flow Gradually
- Confirm Stable Baseline Before Injection

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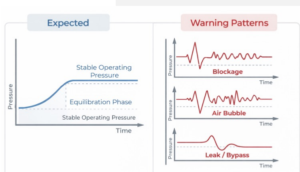
Pressure Monitoring at Startup

Normal Pressure Behavior

- Gradually increase when column is installed
- Stable once equilibrated
- Within expected method range

Warning Signs

- Rapid pressure spikes
- No pressure buildup
- Fluctuating pressure
- Unexpected pressure drop



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Leak Checks & Fittings Integrity

Inspect BEFORE you Inject

Before initiating any run, confirm the physical integrity of the LC system. Even minor leaks at solvent lines, pump heads, injector fittings, column connections, or the detector flow cell can cause pressure instability and poor reproducibility. Proper installation includes correct ferrules, appropriate tightening, and secure alignment of tubing. A consistent pre-run inspection routine prevents unnecessary troubleshooting and protects data quality.

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Column Installation

Proper Column Installation Matters

Proper column installation directly impacts retention stability and peak shape. Confirm flow direction, ensure system compatibility, and tighten fittings securely without overtightening. Tubing should align cleanly to minimize dead volume and prevent band broadening. If the column was stored in a different solvent, flush and equilibrate before analysis. Careful installation protects the column, reduces troubleshooting, and ensures reproducible results.

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Column Equilibration

- Match Initial Mobile Phase Conditions
- Allow Sufficient Volume Through Column
- Monitor Baseline Stability
- Confirm Stable Pressure

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Autosampler Setup Checks

- Verify correct injection volume
- Confirm vial positions match sequence
- Ensure needle wash solvent is filled
- Check syringe integrity
- Inspect for carryover from previous runs

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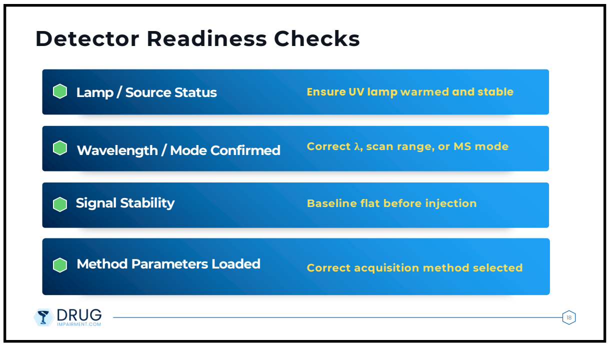
Detector Readiness Checks
Lamp / Source Status
Ensure UV lamp warmed and stable
Wavelength / Mode Confirmed
Correct λ, scan range, or MS mode
Signal Stability
Baseline flat before injection
Method Parameters Loaded
Correct acquisition method selected
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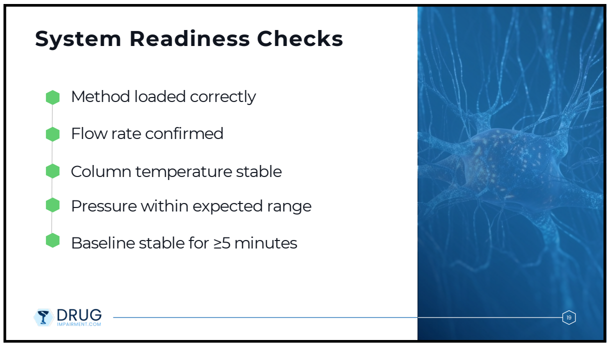
18


System Readiness Checks
Method loaded correctly
Flow rate confirmed
Column temperature stable
Pressure within expected range
Baseline stable for ≥5 minutes
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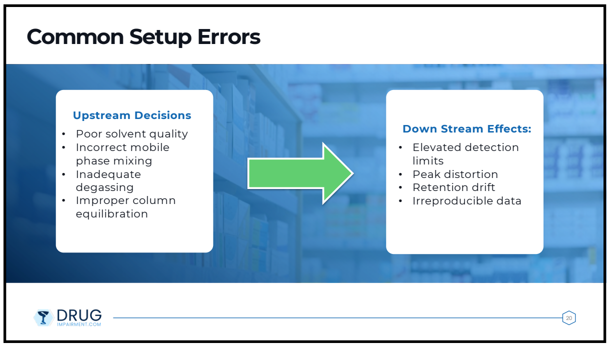
19


Common Setup Errors
Upstream Decisions
Poor solvent quality
Incorrect mobile phase mixing
Inadequate degassing
Improper column equilibration
Down Stream Effects:
Elevated detection limits
Peak distortion
Retention drift
Irreproducible data
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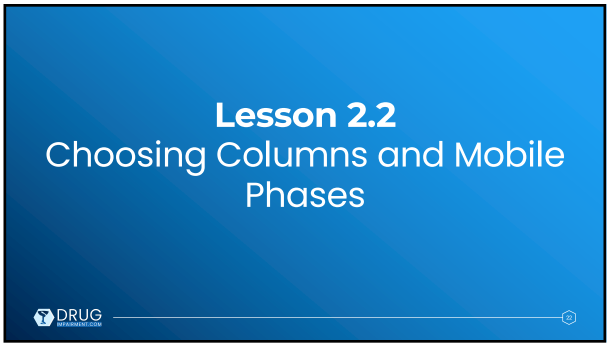
Lesson 2.2
Choosing Columns and Mobile Phases
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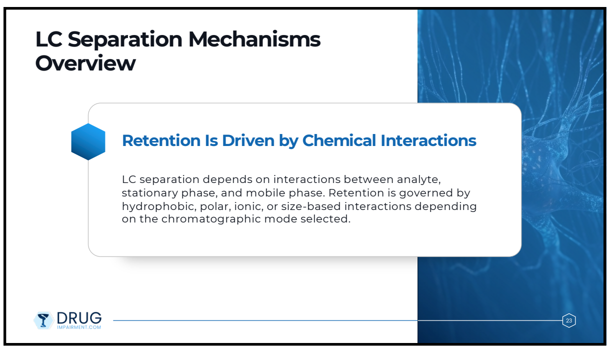
LC Separation Mechanisms Overview
Retention Is Driven by Chemical Interactions
LC separation depends on interactions between analyte, stationary phase, and mobile phase. Retention is governed by hydrophobic, polar, ionic, or size-based interactions depending on the chromatographic mode selected.
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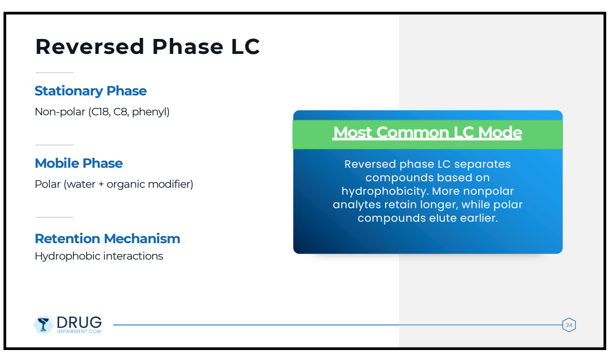
Reversed Phase LC
Stationary Phase
Non-polar (C18, C8, phenyl)
Mobile Phase
Polar (water + organic modifier)
Retention Mechanism
Hydrophobic interactions
Most Common LC Mode
Reversed phase LC separates compounds based on hydrophobicity. More nonpolar analytes retain longer, while polar compounds elute earlier.
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Column Chemistry Options

Column chemistry determines selectivity not just retention time.

- C18 (octadecyl) - general purpose
- C8 - less retention for hydrophobic compounds
- Phenyl - π–π interactions
- Polar embedded phases
- HILIC phases

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Column Physical Parameters

Particle Size

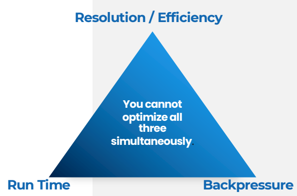
Smaller particles increase efficiency but increase backpressure.

Column Length

Longer columns improve resolution but increase run time.

Internal Diameter

Smaller ID improves sensitivity and reduces solvent consumption.



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Column Compatibility & Constraints

Chemical & Hardware Limits Matter

Columns have pH limits, pressure ratings, and solvent compatibility constraints. Operating outside manufacturer specifications shortens column life and compromises reproducibility.

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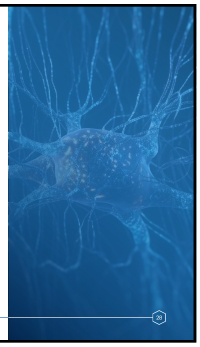
Mobile Phase Components

- Aqueous phase (water or buffer)
- Organic modifier (acetonitrile, methanol)
- Buffer systems
- pH modifiers
- Additives (i.e., formic acid for MS)



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28

Mobile Phase Strength & Elution Power

- **Organic Content**
  Higher organic = stronger elution in reversed phase.
- **Solvent Choice**
  ACN vs MeOH affects selectivity and viscosity.
- **Viscosity**
  Higher viscosity increases backpressure.
- **Temperature**
  Higher temperature reduces viscosity and alters retention.

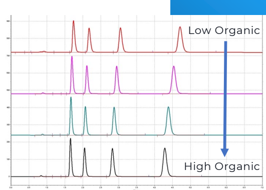

Image from Shimadzu

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pH Considerations

Low pH (Acidic Mobile Phase)

- Suppresses ionization of basic compounds
- Improves peak shape for amines
- Common for MS compatibility (formic acid)
- Often increases retention of weak bases
- Widely used in reversed-phase LC

Higher pH (Neutral to Basic)

- Suppresses ionization of acidic compounds
- Can improve retention of weak acids
- May change selectivity dramatically
- Requires pH-stable column chemistry
- Not always MS-friendly

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Isocratic vs. Gradient Elution

Isocratic

- Constant mobile phase composition
- Simple, reproducible
- Best for simple mixtures

Gradient

- Changing composition over time
- Better for complex mixtures
- Improves peak shape and runtime

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Column & Mobile Phase Paring

Hydrophobic analyte	→ C18 + water/ACN gradient
Highly polar analyte	→ HILIC + high organic start
Ionizable analyte	→ pH-controlled buffer system

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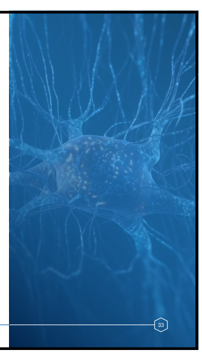
Common Selection Mistakes

- Ignoring analyte pKa
- Choosing column based on habit
- Using incompatible buffer with MS
- Not considering sample solvent strength
- Operating outside pH range



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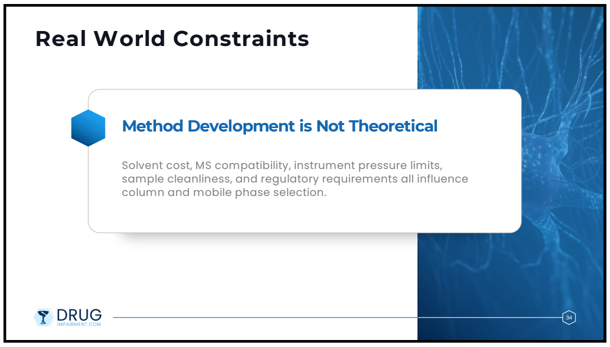
Real World Constraints
Method Development is Not Theoretical
Solvent cost, MS compatibility, instrument pressure limits, sample cleanliness, and regulatory requirements all influence column and mobile phase selection.
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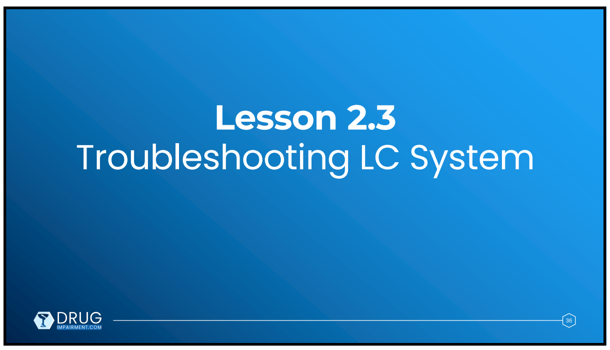
Lesson 2.3
Troubleshooting LC System
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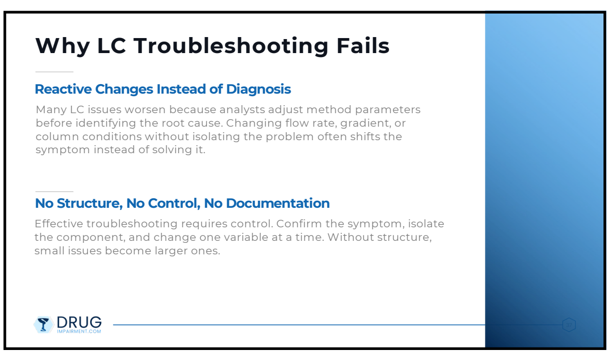
36


Why LC Troubleshooting Fails
Reactive Changes Instead of Diagnosis
Many LC issues worsen because analysts adjust method parameters before identifying the root cause. Changing flow rate, gradient, or column conditions without isolating the problem often shifts the symptom instead of solving it.
No Structure, No Control, No Documentation
Effective troubleshooting requires control. Confirm the symptom, isolate the component, and change one variable at a time. Without structure, small issues become larger ones.
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37

The Troubleshooting Hierarchy

- Confirm the Symptoms
- Check System Basics
- Isolate the Component
- Change ONE Variable at a Time
- Document What You Changed

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38

Start With The Simplest Explanation

Always Start Simple

Most LC problems originate from basic system conditions such as solvent quality, leaks, air in the lines, or column condition. Before modifying gradient profiles or changing column chemistry, verify that the system is physically stable and operating as expected.

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39

Normal Pressure Behavior

EXPECTED

- Gradual rise at startup
- Stable once equilibrated
- Within method's expected range
- Minor fluctuation during gradient changes
- Consistent between injections

ABNORMAL

- Rapid pressure spike
- Continuous drift upward
- Oscillating pressure
- Sudden pressure drop
- Pressure inconsistent between injections

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40

High Backpressure

Column Related

- Blocked inlet frit
- Column contamination
- Guard column saturation

Mobile Phase Issue

- High viscosity solvent
- Improper solvent mixing
- Particulate contamination

Sample Related

- Precipitate analyte
- Matrix buildup
- Injection of particulate

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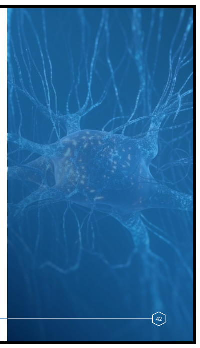
41

Sudden Pressure Increase

- Stop the run immediately
- Do not continue pumping at high pressure
- Remove column carefully
- Run system without column
- Inspect guard column and inlet frit



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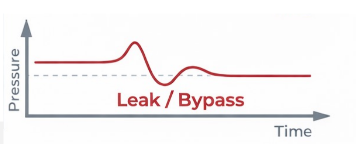
42

Low Or No Pressure



Flow Without Resistance

Low or absent system pressure indicates that mobile phase is not encountering normal column resistance. This typically suggests a leak, disconnected fitting, or pump delivery failure rather than a chromatographic issue.

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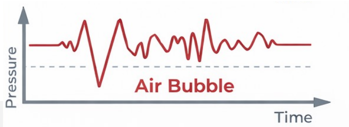
Fluctuating / Unstable Pressure



Pressure Instability

Oscillating pressure often indicates air in the system, degassing failure, or pump seal/check valve issues. The system is delivering flow inconsistently.

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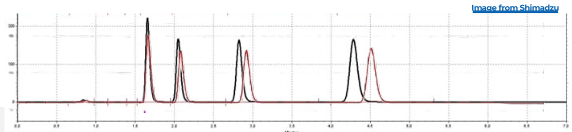
Retention Time Drift

Image from Shimadzu



Gradual Retention Shift

Peaks shift progressively earlier or later over multiple injections. This is commonly caused by temperature instability, mobile phase composition changes, or incomplete equilibration.

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Loss of Retention

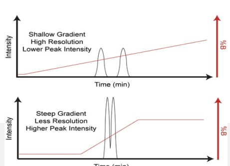

Image from Shimadzu

Sudden Loss of Retention

Analytes elute significantly earlier than expected, often indicating column degradation, incorrect mobile phase preparation, or pH shift.

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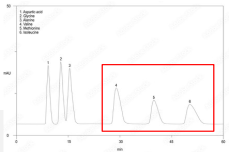
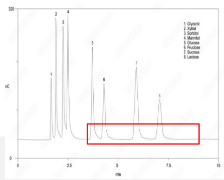
46

Peak Tailing





Peak Tails

A prolonged trailing edge typically indicates active sites on the column, contamination, overloading, or pH mismatch.

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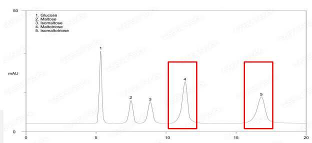
Peak Fronting



Peak Fronts

Distortion at the leading edge often results from column overloading or injection solvent mismatch.

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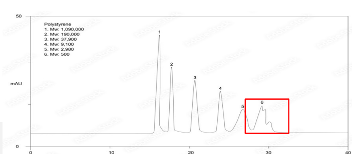
Peak Splitting



Peak Splitting

Split peaks may indicate injection solvent incompatibility, dead volume, or column void formation.

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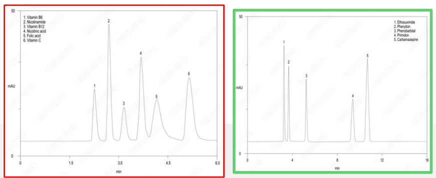
Broad Peaks



Loss of Efficiency

Broad peaks often reflect column aging, excessive system dispersion, or inappropriate flow conditions.

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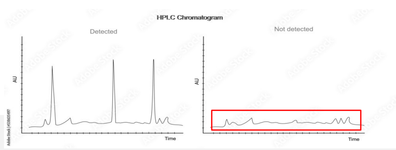
50

Noisy Baseline



Baseline Noise

High-frequency signal variation may result from air bubbles, contaminated solvent, or detector instability.

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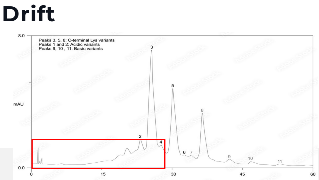
Baseline Drift



Baseline Drift

Gradual upward or downward movement of the baseline often indicates temperature instability or gradient inconsistencies.

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Ghost Peaks

- Carryover from previous injection
- Contaminated mobile phase
- Injector residue
- Column memory effects
- Impurities in solvent or glassware
- Incomplete column washing

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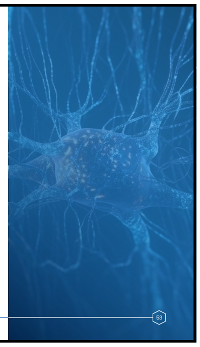

53

Carryover

- Inadequate needle wash solvent
- Strongly retained analytes
- Contaminated injector seat
- Insufficient column wash
- High-concentration previous sample
- Adsorption to system surfaces

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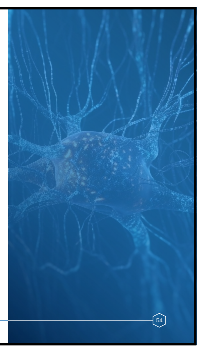

54

Missing or Reduced Peaks

- Incorrect wavelength or MS mode
- Ion suppression (LC-MS)
- Column degradation
- Sample precipitation
- Degraded analyte
- Incorrect mobile phase composition

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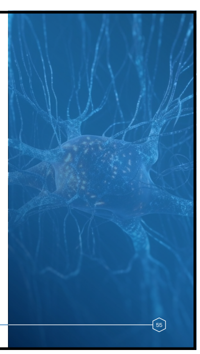

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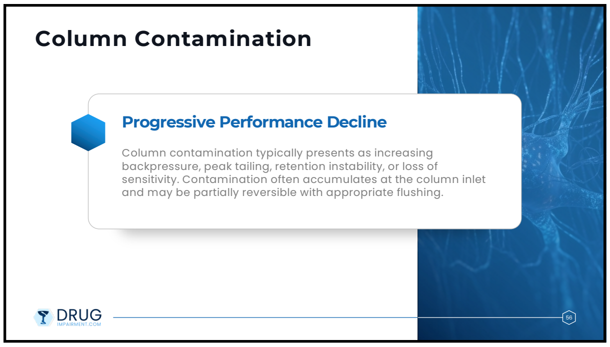
Column Contamination
Progressive Performance Decline
Column contamination typically presents as increasing backpressure, peak tailing, retention instability, or loss of sensitivity. Contamination often accumulates at the column inlet and may be partially reversible with appropriate flushing.
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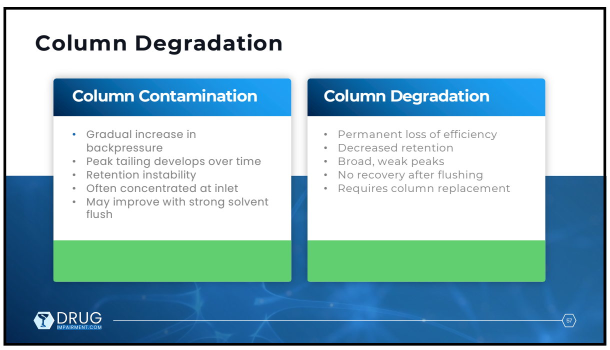
Column Degradation
Column Contamination
• Gradual increase in backpressure
• Peak tailing develops over time
• Retention instability
• Often concentrated at inlet
• May improve with strong solvent flush
Column Degradation
• Permanent loss of efficiency
• Decreased retention
• Broad, weak peaks
• No recovery after flushing
• Requires column replacement
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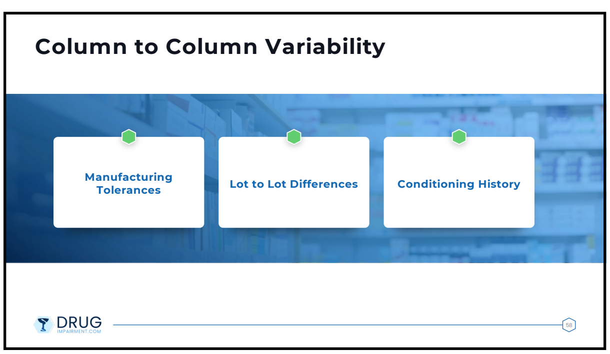
Column to Column Variability
Manufacturing Tolerances
Lot to Lot Differences
Conditioning History
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Symptom to Cause Mapping

Observed Symptom	Most Likely Source
Pressure Abnormalities	System hardware or column restriction
Retention Changes	Mobile phase composition, temperature, or column condition
Peak Shape Distortion	Column contamination, overload, or dead volume
Baseline Instability	Solvent quality, detector issues, or air in system

Start with the simplest explanation before changing chemistry.

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What Not to Change First

Avoid Major Adjustments First

Do not change column chemistry, gradient profile, or mobile phase composition before confirming mechanical stability. Large method changes should only follow structured diagnostics.

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Structured Troubleshooting Workflow

- Confirm the symptom
- Verify basic system stability
- Isolate the affected component
- Change one variable at a time
- Document every adjustment
- Re-Test With The Standard

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61

Lesson 2.4
Liquid Chromatography Detectors

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What an LC Detector Does

Converts Chemical Information into Signal

An LC detector measures a physical or chemical property of analytes as they elute from the column. That measurement is converted into an electronic signal, which becomes a chromatographic peak.

Determines What You Can/Cannot See

The detector does not separate compounds, it defines sensitivity, selectivity, and the type of analytical information you obtain.

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Where Detectors Sit in the LC Workflow

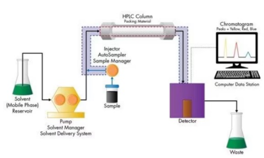

Image from Waters

After Separation

The detector sits downstream of the column. It measures compounds after chromatographic resolution has occurred.

Before Data Interpretation

Detector output feeds into acquisition software, where signal becomes peaks, integration, and quantitative results.

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Separation vs. Detection

Separation

- Occurs in the column
- Resolves compounds over time
- Affects retention and peak spacing
- Determines resolution
- Chemistry-driven

Detection

- Occurs after the column
- Converts analyte property into signal
- Determines sensitivity
- Determines selectivity
- Signal-driven

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What LC Data Represents

Understanding the Chromatogram

An LC chromatogram represents detector response versus time. Each peak reflects analyte presence and concentration, while peak shape and spacing reflect chromatographic performance. Detector type determines what chemical information is captured.

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Classes of LC Detectors

Optical Detectors

- UV Vis
- Diode Array (PDA/DAD)
- Fluorescence

Mass Spectrometric Detectors

- Triple Quad (QQQ)
- Quadrupole Time of Flight (QTOF)

Aerosol/Universal Detectors

- Evaporative Light Scattering Detector (ELSD)
- Charged Aerosol Detector (CAD)

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68

Sensitivity vs. Selectivity vs. Information

Not All Detectors Provide the Same Value

Sensitivity = how low you can detect.

Selectivity = how specifically you detect.

Information = how much chemical detail you obtain.

Detector choice determines analytical capability.

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What is a UV-Vis Detector

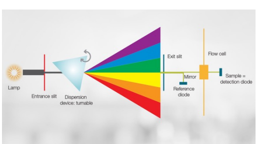

Image from Thermo Fisher Scientific

Absorbance Based Detection

Measures how much ultraviolet or visible light a compound absorbs as it passes through the flow cell.

Requires a Chromophore

Only compounds with light-absorbing functional groups produce signal.

Quantitative & Non-Destructive

Signal intensity is proportional to concentration within a linear range, and analyte is not destroyed.

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Beer-Lambert Law

Mathematical Relationship

A = ε b c

Where:

A = absorbance

ε = molar absorptivity

b = path length

c = concentration

Why it Matters in LC

As analyte concentration increases, detector response increases proportionally enabling quantitative analysis through calibration curves.

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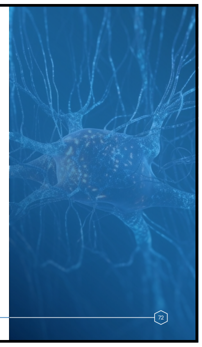
What UV-Vis Can Detect

- Aromatic compounds
- Conjugated systems
- Unsaturated compounds (double bonds)
- Many pharmaceuticals
- Organic acids and bases with chromophores
- Some pesticides and forensic analytes
- Colored compounds (visible range)



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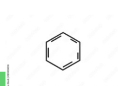
Chromophores

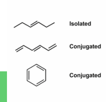
Aromatics

- Benzene rings
- Polycyclic aromatics
- Strong UV absorbance
- Common in pharmaceuticals



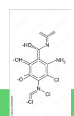
Conjugated Double Bonds

- Alternating single/double bonds
- Extended π systems
- Increased absorbance
- Can shift into visible range



Functional Groups

- Carbonyl (C=O)
- Nitro ($-NO_2$)
- Azo (–N=N–)
- Other π-electron groups



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Wavelength Selection Logic

Max Sensitivity (λmax)

- Select wavelength at maximum absorbance
- Highest signal intensity
- Best for quantitative accuracy
- Improves signal-to-noise ratio

Higher Selectivity

- Choose wavelength where fewer matrix components absorb
- Reduces interference
- Improves specificity
- May sacrifice some signal intensity

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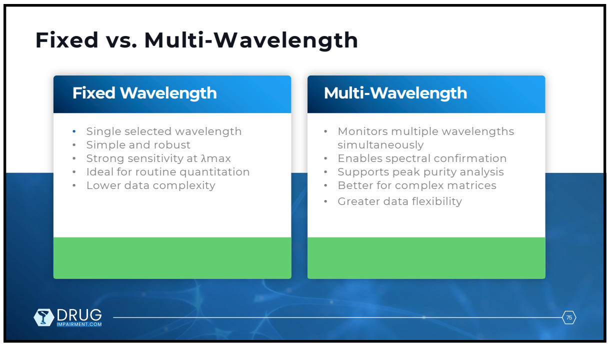

Fixed vs. Multi-Wavelength
Fixed Wavelength
Single selected wavelength
Simple and robust
Strong sensitivity at λmax
Ideal for routine quantitation
Lower data complexity
Multi-Wavelength
Monitors multiple wavelengths simultaneously
Enables spectral confirmation
Supports peak purity analysis
Better for complex matrices
Greater data flexibility
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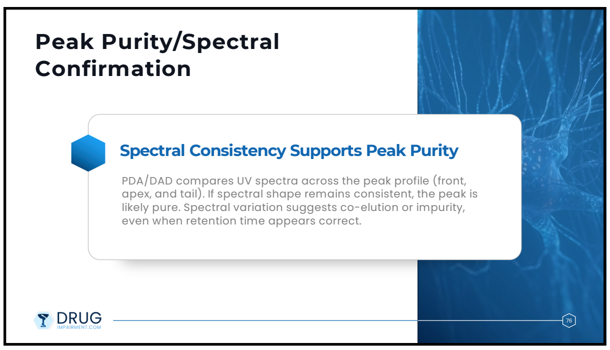

Peak Purity/Spectral Confirmation
Spectral Consistency Supports Peak Purity
PDA/DAD compares UV spectra across the peak profile (front, apex, and tail). If spectral shape remains consistent, the peak is likely pure. Spectral variation suggests co-elution or impurity, even when retention time appears correct.
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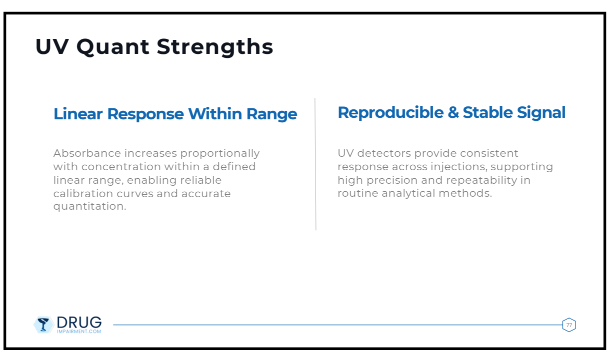

UV Quant Strengths
Linear Response Within Range
Absorbance increases proportionally with concentration within a defined linear range, enabling reliable calibration curves and accurate quantitation.
Reproducible & Stable Signal
UV detectors provide consistent response across injections, supporting high precision and repeatability in routine analytical methods.
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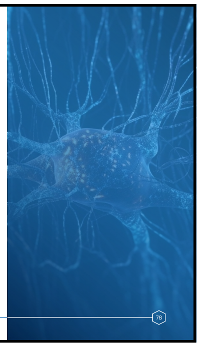
UV Limitations

- Requires a chromophore
- Limited selectivity in complex matrices
- Cannot confirm molecular structure
- Lower sensitivity compared to MS



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What is Fluorescence

Emission Based Detection

Fluorescence detection measures light emitted by a compound after it absorbs energy at a specific excitation wavelength. The emitted light is detected at a longer wavelength.

Selective & Highly Sensitive

Only compounds capable of fluorescence produce signal, resulting in lower background noise and significantly greater sensitivity compared to UV-Vis detection.

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UV vs. Fluorescence

UV-VIS Detection

- Measures light absorbance
- Requires a chromophore
- Moderate sensitivity
- Broader compound applicability
- Limited selectivity in complex matrices

Fluorescence

- Measures emitted light
- Requires fluorescent capability
- Much higher sensitivity
- Lower background noise
- Greater selectivity

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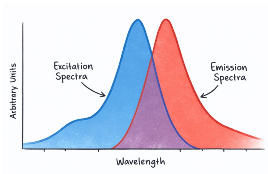
Excitation vs. Emission



Excitation Wavelength

The excitation wavelength is the specific wavelength of light absorbed by the analyte. This energy promotes electrons to a higher electronic state.

Emission Wavelength

After excitation, the molecule releases energy as light at a longer wavelength. This emitted light is measured by the fluorescence detector.

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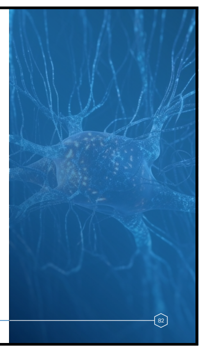
Native Fluorophores

- Polycyclic aromatic hydrocarbons (PAHs)
- Aflatoxins
- Aromatic amines
- Tryptophan (proteins)
- Riboflavin



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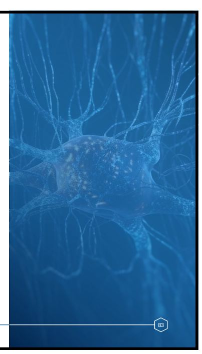
Non-Fluorescent Compounds

- Small aliphatic molecules
- Simple organic acids
- Many alcohols and ketones
- Highly flexible structures
- Compounds lacking conjugation



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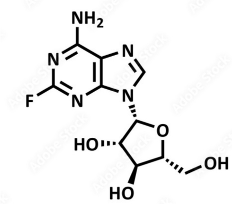
83

Derivatization Concept



Creating Fluorescent Capability

Derivatization involves chemically modifying a non-fluorescent compound by attaching a fluorescent tag. This allows compounds that do not naturally emit light to be detected by fluorescence.

Increasing Sensitivity & Selectivity

Fluorescent derivatives often provide significantly lower detection limits and reduced background interference, improving analytical sensitivity in complex matrices.

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Sensitivity Advantage

Fluorescence Detection Provides Significantly Lower Detection Limits

Because fluorescence measures emitted light rather than absorbed light, background signal is substantially reduced. This results in higher signal-to-noise ratios and detection limits that are often orders of magnitude lower than UV-Vis detection.

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Selectivity/Low Background

Fluorescence Detection Reduces Background Interference

Fluorescence measures emitted light at a specific wavelength, separate from the excitation source. Because most matrix components do not fluoresce, background signal is minimal, resulting in improved selectivity compared to UV-Vis detection.

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Quant Performance

Lower Limits of Detection

Fluorescence detection can achieve detection limits that are significantly lower than UV-Vis, often in the ng/mL range, due to improved signal-to-noise ratios and reduced background interference.

Strong Quantitative Linearity

Within an optimized concentration range, fluorescence provides reproducible and reliable quantitative response, supporting validated analytical methods when properly calibrated.

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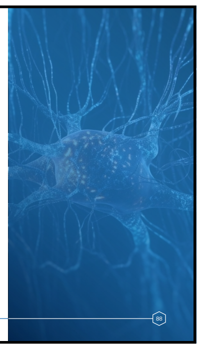
87

Fluorescence Limitations

- Only detects fluorescent compounds
- May require derivatization
- More complex method development (excitation/emission optimization)
- Limited universal applicability
- Instrument settings must be carefully controlled



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88

88

What is Refractive Index

Differential Optical Detection

RI detectors measure the difference in refractive index between:

- A reference cell (pure mobile phase)
- A sample cell (mobile phase + analyte)

When analyte passes through the flow cell, it changes how light bends (refracts), and that change is measured.

Detection Without a Chromophore

RI detects compounds that:

- Do not absorb UV
- Do not fluoresce
- Do not ionize well

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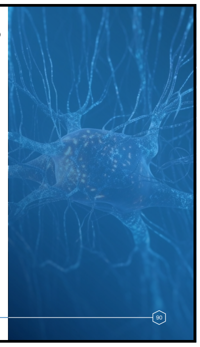
89

89

When is Refractive Index Used?

- The analyte has no UV absorbance
- The method is isocratic
- The sample concentration is moderate to high
- Analytes are sugars, polyols, or polymers



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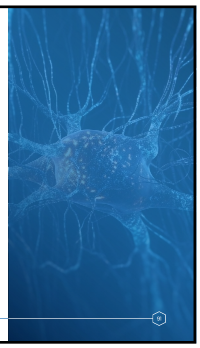
Limitations of Refractive Index

- Low Sensitivity
- Isocratic Only
- Temperature Sensitive
- Limited Selectivity



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91

Why Aerosol Detectors Exist?

The Limitation of Optical Detectors

- UV requires chromophores
- FLD requires fluorescence
- RI lacks sensitivity and gradient compatibility

The Solution: Measure Particles, Not Light

Aerosol detectors (ELSD, CAD) work by:

- Nebulizing the mobile phase
- Evaporating solvent
- Detecting remaining non-volatile particles

They respond to mass, not optical properties.

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What is Evaporative Light Scattering Detection (ELSD)

What Is ELSD?

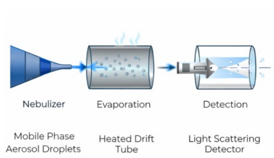
- Universal detector for non-volatile compounds
- Does not rely on chromophores
- Measures light scattered by particles after solvent removal

When Is It Used?

- Used when UV fails
- Ideal for sugars, lipids, polymers, surfactants
- Compatible with gradient methods (unlike RI)

93

How ELSD Works



Evaporative Light Scattering Detection

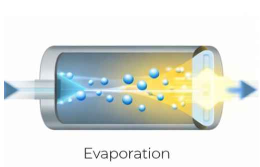
ELSD works by converting liquid effluent into measurable particles.

Three Steps:

1. Nebulization: Mobile phase forms aerosol droplets
2. Evaporation: Solvent evaporates in heated tube
3. Detection: Remaining non-volatile particles scatter light

94

What is a Charged Aerosol Detection (CAD)?



CAD

- Universal mass-sensitive detector
- Detects non-volatile and semi-volatile analytes
- Does not require UV chromophores
- Compatible with gradient elution

Measures charged particles after solvent evaporation

Why it is Powerful

- Near-uniform response across compound classes
- Better sensitivity than ELSD (typically)
- Wide dynamic range
- More reproducible than ELSD

95

ELSD vs. CAD

Feature	ELSD	CAD
Detection Principle	Light Scattering	Charged Particle Current
Response	Non-Linear	More Uniform
Sensitivity	Moderate	Higher
Reproducibility	Moderate	Improved
Gradient Compatible	Yes	Yes

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Limitations of ELSD/CAD

ELSD

- Lower sensitivity compared to UV/FLD (for some analytes)
- Non-linear response (requires log-log calibration)
- Sensitive to mobile phase volatility
- Baseline drift during gradient elution
- Cannot detect volatile analytes

CAD

- Response still semi-nonlinear (though more uniform than ELSD)
- Sensitive to mobile phase composition changes
- Requires volatile buffers only
- More expensive/higher maintenance
- Not compatible with highly volatile compounds

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What is a triple-quad (QQQ)?

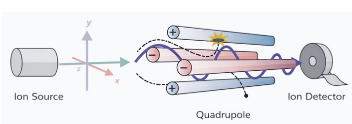
Quadrupole Mass Filtering

- Q1: Selects a specific precursor ion (m/z)
- Q2 (collision cell): Fragments the ion via collision-induced dissociation (CID)
- Q3: Filters specific product ions

Targeted Quantitation (MRM)

Triple-quads operate primarily in:

- MRM (Multiple Reaction Monitoring)
- Extremely high sensitivity
- Exceptional selectivity in complex matrices
- Gold standard for regulated quantitation



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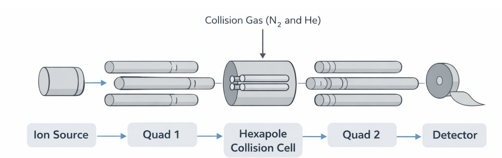
98

Q1 – Q2 – Q3 Architecture



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Targeted Analysis Concept

Multiple Reaction Monitoring (MRM)

Triple-quad mass spectrometers operate in targeted mode.

Instead of scanning all ions, the system:

- Selects one precursor ion (Q1)
- Fragments it in Q2 (collision cell)
- Filters for one specific product ion (Q3)

This defined m/z → m/z transition creates exceptional selectivity and sensitivity.

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SIM vs. MRM

Single Ion Monitoring	Multiple Reaction Monitoring
• Monitors a single m/z value • No fragmentation step • Moderate selectivity • Higher background in complex matrices • Often used in single-quad MS	• Monitors a precursor → product ion transition • Requires fragmentation (QQQ) • Exceptional selectivity • Dramatically reduced background noise • Gold standard for quantitative LC-MS/MS
Good sensitivity – limited specificity	High sensitivity + high specificity

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Quant Robustness

Matrix Tolerance

- MRM monitors specific precursor → product ion transitions
- Reduces interference from co-eluting compounds
- Improves accuracy in complex biological or environmental samples

Reproducible Quantitation

- High selectivity lowers false positives
- Stable ion transitions improve precision
- Supports regulatory-grade quantitative workflows

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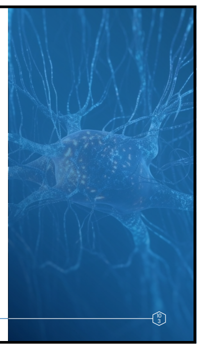
Triple Quad Limitations

- Targeted analysis only
- Requires known transitions
- Limited structural information
- Unit mass resolution
- Not ideal for unknown discovery



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What is Quadrupole Time of Flight (QTOF)?

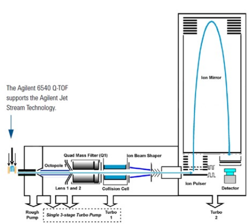

Image from Creative Protemics

Hybrid Mass Analyzer

Combines a quadrupole mass filter (Q1) with a time-of-flight (TOF) analyzer.

Q1 can isolate precursor ions, while TOF measures exact mass with high resolution and accuracy.

High-Resolution Accurate Mass (HRAM) Detection

Measures ions based on flight time after acceleration in a field-free region.

Provides accurate mass, isotopic pattern data, and structural information for unknown identification.

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Q + TOF Architecture

Mass Selection: Select the ion of interest

Collision Cell: Fragment the selected ion

Time of Flight: Measure exact mass with high resolution

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Full Scan Acquisition

Comprehensive Detection

Full scan records all ions within a defined mass range, not just targeted transitions. This allows broad screening capability in complex samples.

Retrospective Data Mining

The complete spectral dataset enables retrospective analysis, supporting unknown identification and expanded investigations without reinjection.

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DDA vs. DIA

Data Dependent Acquisition

- Selects top N most intense precursor ions
- Triggers MS/MS based on real-time signal intensity
- Uses dynamic exclusion to avoid repeat scans
- High-quality MS/MS spectra for selected ions
- May miss low-abundance or transient species

Data-Independent Acquisition

- Instead of selecting top N ions, DIA fragments everything within the defined mass window
- The instruments steps across the m/z range in sequential windows and fragments all ions in each window.
- Nothing is intensity dependent
- Comprehensive fragmentation coverage
- Multiple precursors are fragmented together

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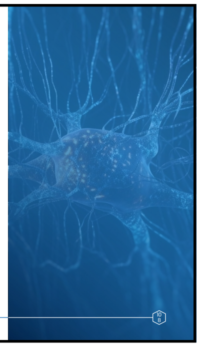
QTOF Strengths

- Combines QQQ quantitation with ion trap scanning
- Enhanced product ion (EPI) spectra for structural confirmation
- Sensitive MRM quantitation
- Targeted + triggered MS/MS capability
- Faster and more robust than full HRMS systems



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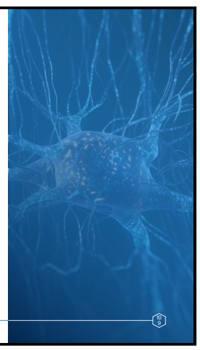
QTOF Limitations

- Not as sensitive as triple quadrupole for trace-level quantitation
- Quant performance may not match dedicated MRM systems
- Generates large, complex datasets
- Higher cost and operational complexity
- Advanced data processing required for DIA workflows



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The Detector Spectrum

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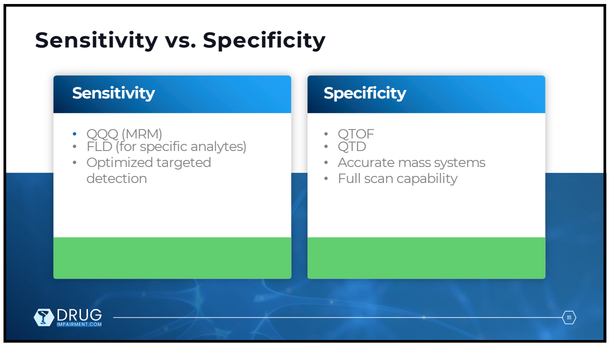
110


Sensitivity vs. Specificity
Sensitivity
QQQ (MRM)
FLD (for specific analytes)
Optimized targeted detection
Specificity
QTOF
QTD
Accurate mass systems
Full scan capability
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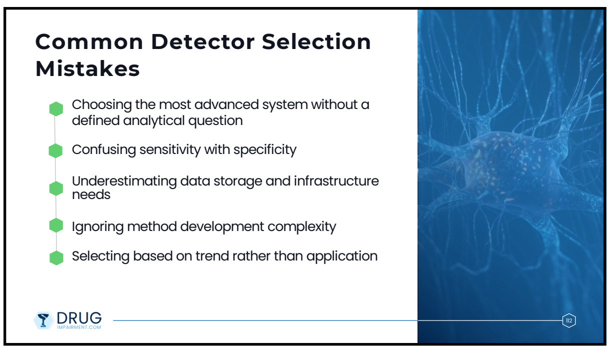
111


Common Detector Selection Mistakes
Choosing the most advanced system without a defined analytical question
Confusing sensitivity with specificity
Underestimating data storage and infrastructure needs
Ignoring method development complexity
Selecting based on trend rather than application
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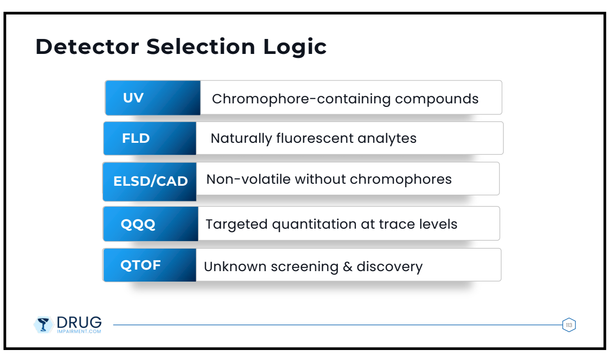
Detector Selection Logic
UV
Chromophore-containing compounds
FLD
Naturally fluorescent analytes
ELSD/CAD
Non-volatile without chromophores
QQQ
Targeted quantitation at trace levels
QTOF
Unknown screening & discovery
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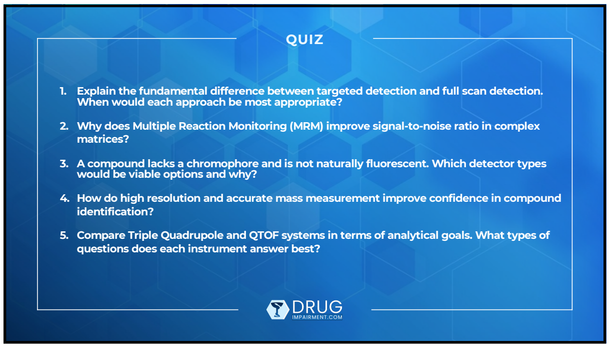
113


QUIZ
1. Explain the fundamental difference between targeted detection and full scan detection. When would each approach be most appropriate?
2. Why does Multiple Reaction Monitoring (MRM) improve signal-to-noise ratio in complex matrices?
3. A compound lacks a chromophore and is not naturally fluorescent. Which detector types would be viable options and why?
4. How do high resolution and accurate mass measurement improve confidence in compound identification?
5. Compare Triple Quadrupole and QTOF systems in terms of analytical goals. What types of questions does each instrument answer best?
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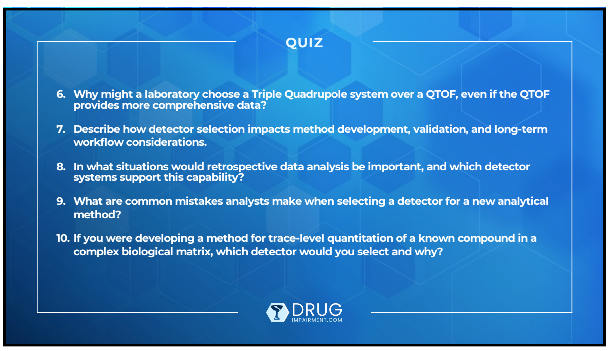
114


QUIZ
6. Why might a laboratory choose a Triple Quadrupole system over a QTOF, even if the QTOF provides more comprehensive data?
7. Describe how detector selection impacts method development, validation, and long-term workflow considerations.
8. In what situations would retrospective data analysis be important, and which detector systems support this capability?
9. What are common mistakes analysts make when selecting a detector for a new analytical method?
10. If you were developing a method for trace-level quantitation of a known compound in a complex biological matrix, which detector would you select and why?
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115


CONTACT ME
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116