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Area of Expertise

1. Chromatography & method development (LC & GC)
2. Forensic and drugs-of-abuse analysis
3. Laboratory setup, validation, and data integrity
4. Regulatory & compliance-driven testing programs

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Chromatography Fundamentals

Foundations of LC & GC for Forensic and Analytical Applications

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Disclosures

The speaker has no disclosures.

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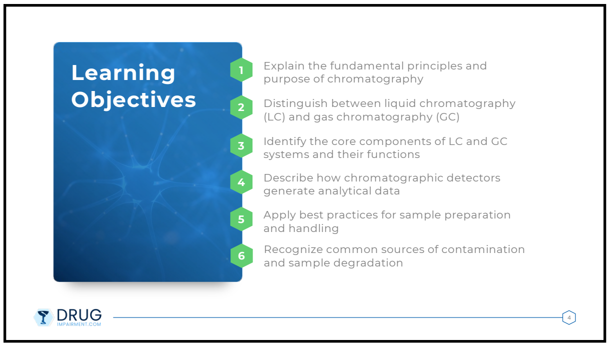
Learning Objectives
1 Explain the fundamental principles and purpose of chromatography
2 Distinguish between liquid chromatography (LC) and gas chromatography (GC)
3 Identify the core components of LC and GC systems and their functions
4 Describe how chromatographic detectors generate analytical data
5 Apply best practices for sample preparation and handling
6 Recognize common sources of contamination and sample degradation
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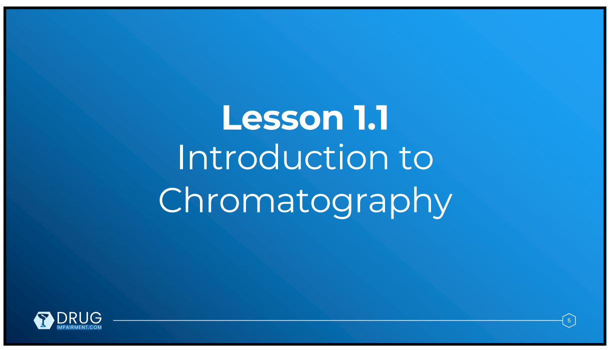
Lesson 1.1
Introduction to Chromatography
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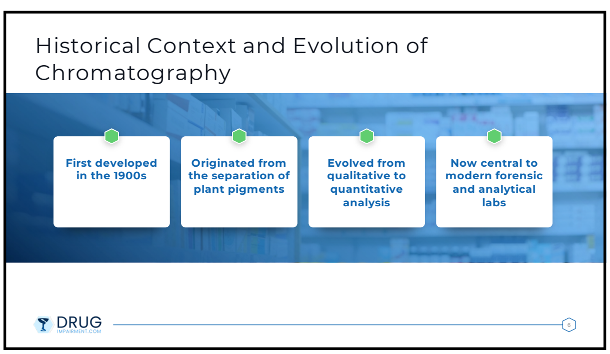
Historical Context and Evolution of Chromatography
First developed in the 1900s
Originated from the separation of plant pigments
Evolved from qualitative to quantitative analysis
Now central to modern forensic and analytical labs
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What is Chromatography?

- Analytical technique used to separate complex mixtures
- Separation occurs based on interactions with two phases
- Foundation of many forensic and drugs-of-abuse methods

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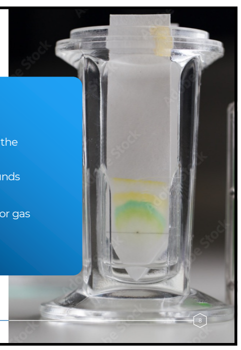
Stationary vs. Mobile Phase



Stationary Phase

- Does not move
- Provides separation surface
- Interacts differently with compounds

Mobile Phase

- Moves through the system
- Carries compounds forward
- Can be a liquid or gas

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Types of Chromatography

Liquid Chromatography (LC)

- Liquid Mobile Phase

Gas Chromatography (GC)

- Gas Mobile Phase

Thin Layer Chromatography

- Planar, qualitative technique

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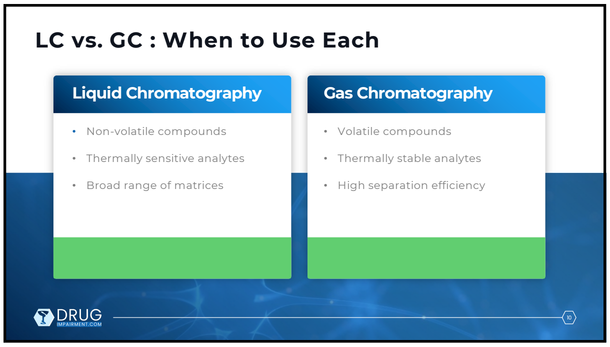
LC vs. GC : When to Use Each
Liquid Chromatography
Non-volatile compounds
Thermally sensitive analytes
Broad range of matrices
Gas Chromatography
Volatile compounds
Thermally stable analytes
High separation efficiency
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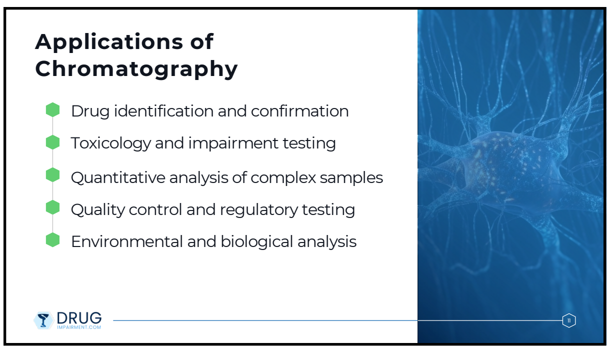
Applications of Chromatography
Drug identification and confirmation
Toxicology and impairment testing
Quantitative analysis of complex samples
Quality control and regulatory testing
Environmental and biological analysis
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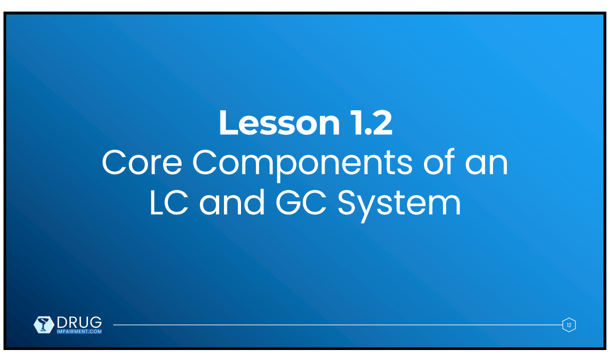
Lesson 1.2
Core Components of an LC and GC System
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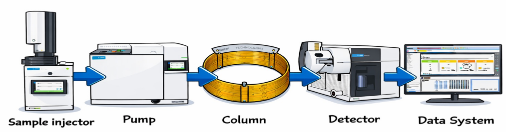
Core System Architecture



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Sample Injection Systems

Liquid Chromatography

- Liquid sample injected into a stream of mobile phase
- Consistency in injection volume and solvent compatibility is critical for reproducible results

Both LC / GC

- Autosamplers or manual injection

Gas Chromatography

- Heated injection port
- Samples are vaporized before entering the column
- Temperature and split conditions determine how much sample reaches the column

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Mobile Phase Delivery & Flow Control

Role of Pumps in Chromatography

- Deliver the mobile phase through the system
- Control flow rate and consistency
- Enable reproducible separation conditions

LC vs. GC

- Measure compounds as they elute from the column
- Convert chemical signals into measurable data

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Chromatographic Columns

Liquid Chromatography

- Packed columns
- Stationary phase particles
- Separation based on chemical interactions

Gas Chromatography

- Capillary columns
- Stationary phase coated on the inner wall
- Separation based on volatility and interactions

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What Influences Separation?

Stationary phase chemistry:
Determines how compounds interact with the column surface, such as whether polar or non-polar compounds are retained more strongly

Strength of analyte stationary phase interactions:
Stronger interactions lead to longer retention, while weaker interactions allow compounds to move through the column more quickly

Time spent in the column:
Influenced by factors such as column length and internal structure, which affect how long compounds remain in contact with the stationary phase

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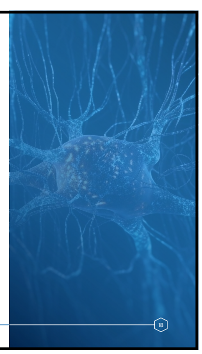
Detectors

- Measure compounds as they elute from the column
- Convert chemical signals into measurable data
- Response relates to compound concentration
- Detector choice impacts sensitivity and selectivity



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Detector Types : A High-Level Overview

Liquid Chromatography

- UV or optical detectors – responding to light absorption
- Mass Spectrometry – detecting by mass to charge ratios

Gas Chromatography

- Flame based detectors – responding to an energy release as compounds are burned
- Mass Spectrometry – detecting by mass to charge ratio

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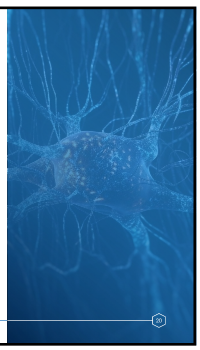
Data Systems & Software

- Convert detector signals into chromatograms
- Identify and integrate peaks
- Support quantitative analysis
- Enable data review, storage and traceability



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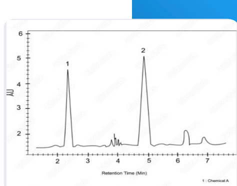
Understanding a Chromatogram

- **Peaks = detected compound**
- **Retention Time (RT) = time spent in column**
- **Peak size = amount of compound**
- **Full chromatogram = separation of a mixture**



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Chromatographic Workflow

- Sample is introduced into the system
- Compounds are separated in the column
- Separated compounds are detected
- Signals are processed into chromatograms
- Data are reviewed and interpreted

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Lesson 1.3
Sample Preparation and Handling

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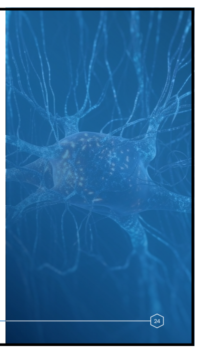
Importance of Sample Preparation

- Determines data quality before analysis begins
- Reduces matrix interference and contamination
- Protects analytical instruments
- Supports accurate and defensible results



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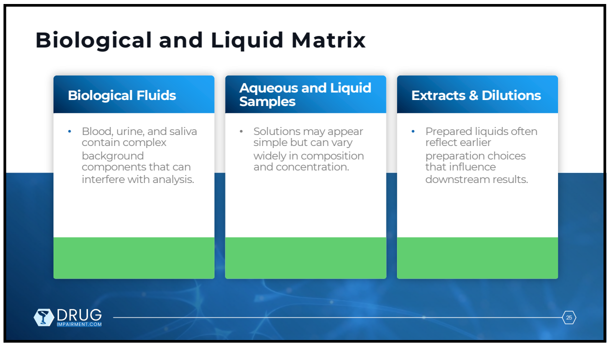
Biological and Liquid Matrix
Biological Fluids
• Blood, urine, and saliva contain complex background components that can interfere with analysis.
Aqueous and Liquid Samples
• Solutions may appear simple but can vary widely in composition and concentration.
Extracts & Dilutions
• Prepared liquids often reflect earlier preparation choices that influence downstream results.
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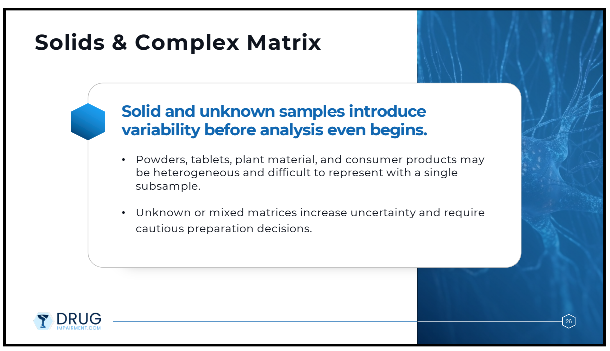
Solids & Complex Matrix
Solid and unknown samples introduce variability before analysis even begins.
• Powders, tablets, plant material, and consumer products may be heterogeneous and difficult to represent with a single subsample.
• Unknown or mixed matrices increase uncertainty and require cautious preparation decisions.
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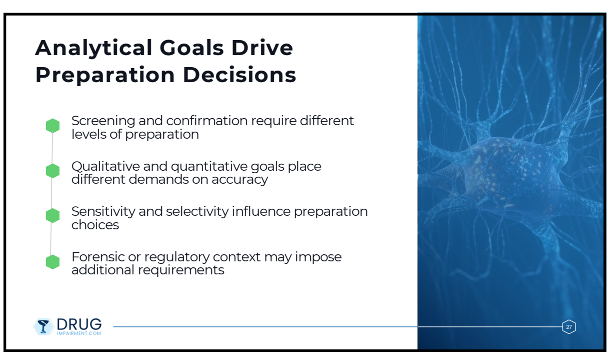
Analytical Goals Drive Preparation Decisions
Screening and confirmation require different levels of preparation
Qualitative and quantitative goals place different demands on accuracy
Sensitivity and selectivity influence preparation choices
Forensic or regulatory context may impose additional requirements
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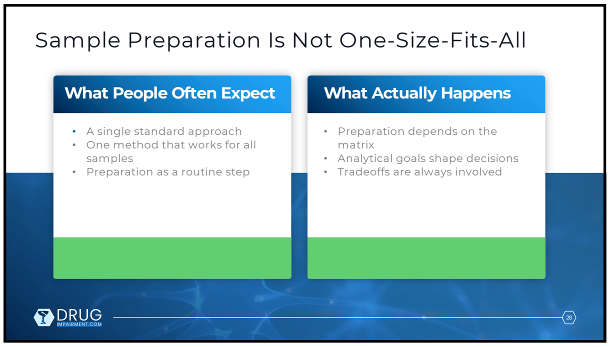
Sample Preparation Is Not One-Size-Fits-All
What People Often Expect
A single standard approach
One method that works for all samples
Preparation as a routine step
What Actually Happens
Preparation depends on the matrix
Analytical goals shape decisions
Tradeoffs are always involved
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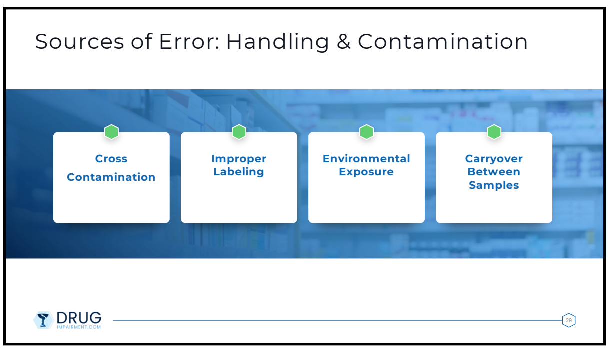
Sources of Error: Handling & Contamination
Cross Contamination
Improper Labeling
Environmental Exposure
Carryover Between Samples
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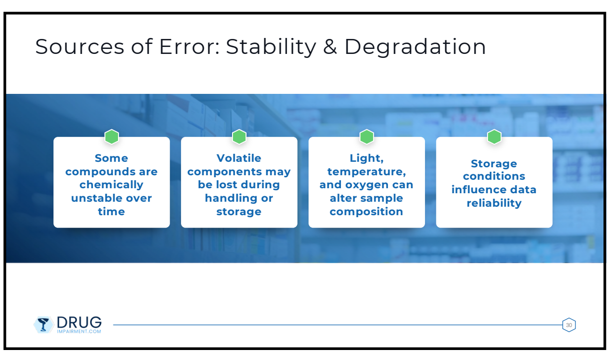
Sources of Error: Stability & Degradation
Some compounds are chemically unstable over time
Volatile components may be lost during handling or storage
Light, temperature, and oxygen can alter sample composition
Storage conditions influence data reliability
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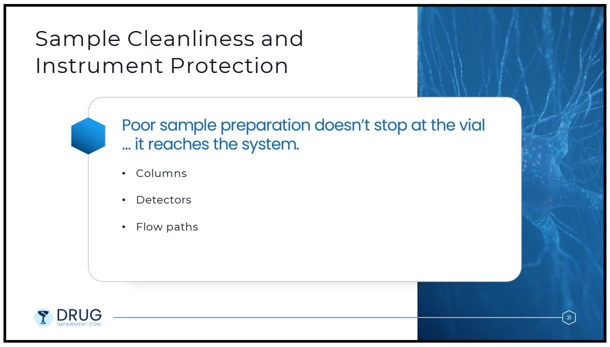
Sample Cleanliness and Instrument Protection
Poor sample preparation doesn't stop at the vial
... it reaches the system.
• Columns
• Detectors
• Flow paths
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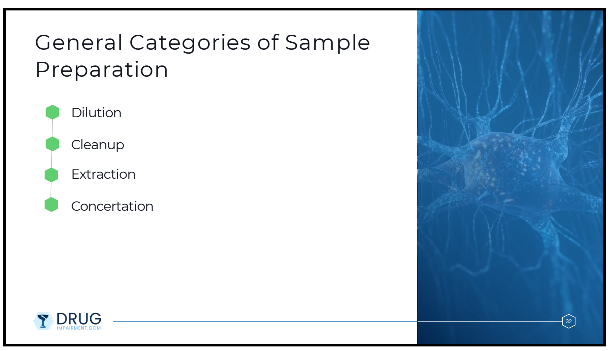
General Categories of Sample Preparation
Dilution
Cleanup
Extraction
Concertation
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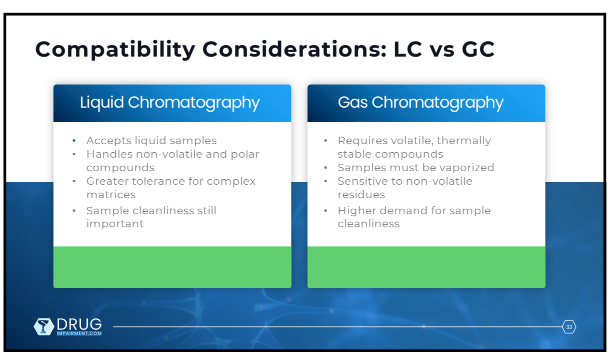
Compatibility Considerations: LC vs GC
Liquid Chromatography
• Accepts liquid samples
• Handles non-volatile and polar compounds
• Greater tolerance for complex matrices
• Sample cleanliness still important
Gas Chromatography
• Requires volatile, thermally stable compounds
• Samples must be vaporized
• Sensitive to non-volatile residues
• Higher demand for sample cleanliness
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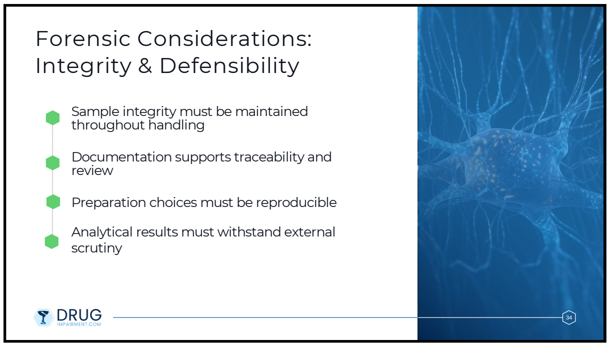
Forensic Considerations: Integrity & Defensibility
Sample integrity must be maintained throughout handling
Documentation supports traceability and review
Preparation choices must be reproducible
Analytical results must withstand external scrutiny
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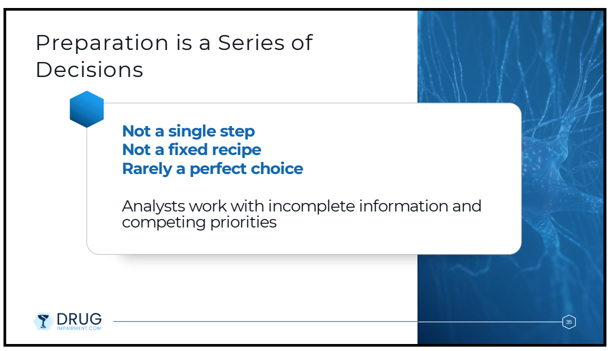
Preparation is a Series of Decisions
Not a single step
Not a fixed recipe
Rarely a perfect choice
Analysts work with incomplete information and competing priorities
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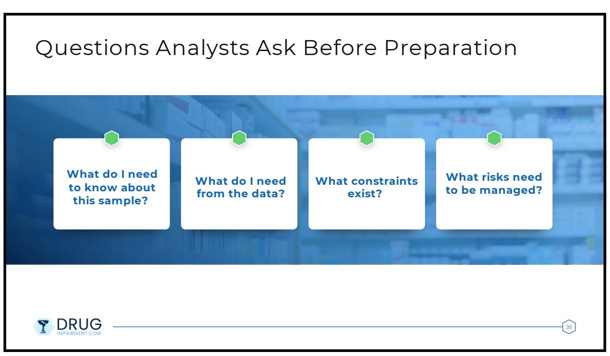
Questions Analysts Ask Before Preparation
What do I need to know about this sample?
What do I need from the data?
What constraints exist?
What risks need to be managed?
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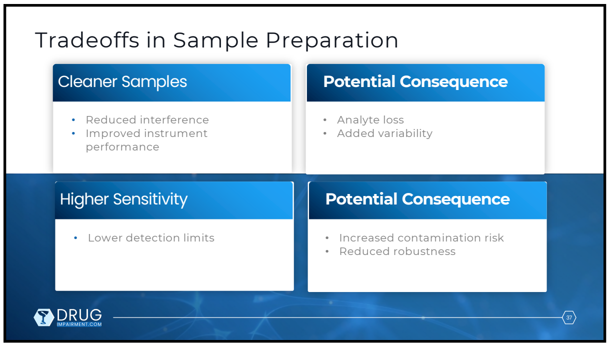
Tradeoffs in Sample Preparation
Cleaner Samples
Reduced interference
Improved instrument performance
Potential Consequence
Analyte loss
Added variability
Higher Sensitivity
Lower detection limits
Potential Consequence
Increased contamination risk
Reduced robustness
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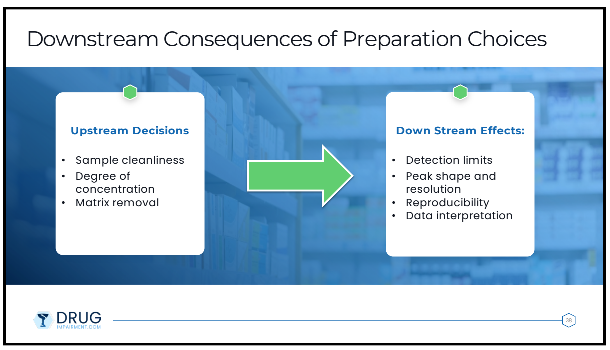
Downstream Consequences of Preparation Choices
Upstream Decisions
Sample cleanliness
Degree of concentration
Matrix removal
Down Stream Effects:
Detection limits
Peak shape and resolution
Reproducibility
Data interpretation
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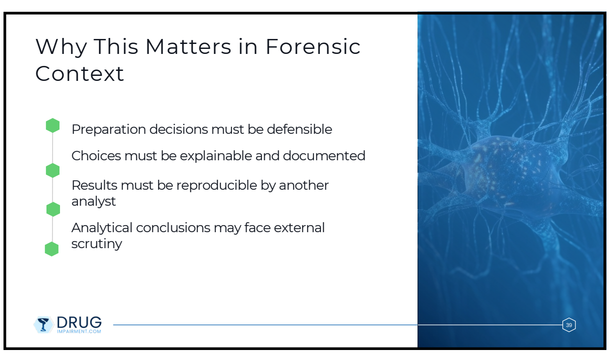
Why This Matters in Forensic Context
Preparation decisions must be defensible
Choices must be explainable and documented
Results must be reproducible by another analyst
Analytical conclusions may face external scrutiny
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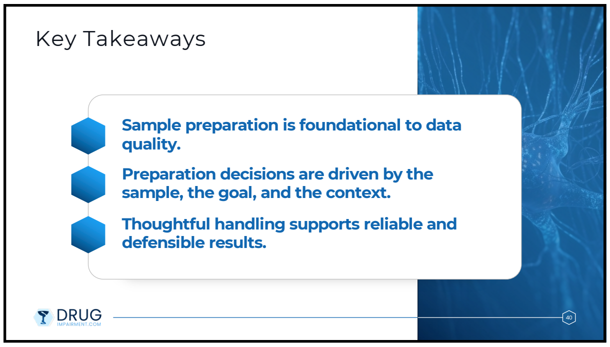
Key Takeaways
Sample preparation is foundational to data quality.
Preparation decisions are driven by the sample, the goal, and the context.
Thoughtful handling supports reliable and defensible results.
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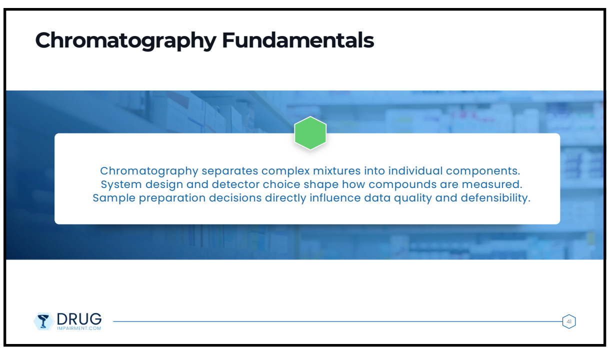
Chromatography Fundamentals
Chromatography separates complex mixtures into individual components.
System design and detector choice shape how compounds are measured.
Sample preparation decisions directly influence data quality and defensibility.
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