Split Specimens, Chromatography/Mass Spectrometry and Assay Interference

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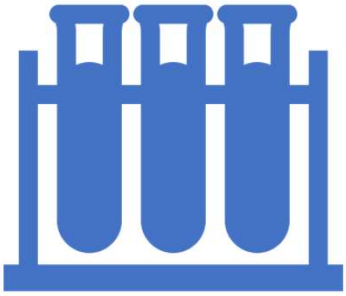


Split Specimens: What happens at Lab B?

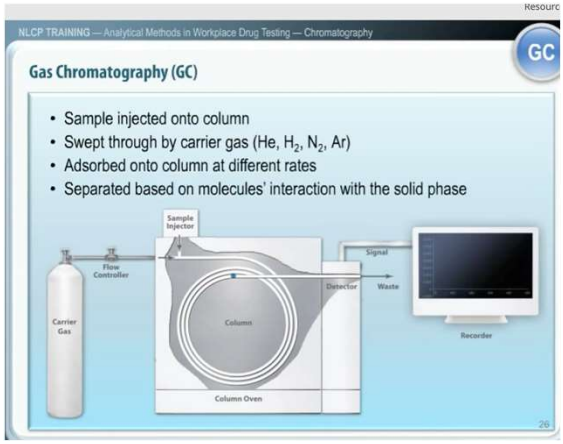
- Lab B is chosen based on capability: (analyte, sample type, Limit of Detection aka LOD)
- Lab B receives sample: Confirms ID, seal integrity and chain of custody
- Lab B Continues and maintains COC
- Aliquot for designated test
- Performed at Lab B's LOD
- Report results: Confirmed for (name analyte) of Failed to Reconfirm

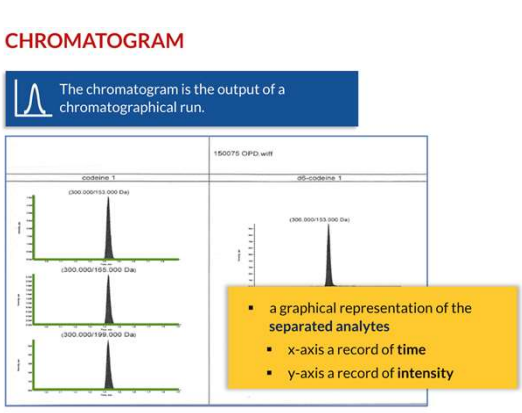
What is Chromatography/Mass Spectrometry?

- **Chromatography and mass spectrometry** are two powerful analytical techniques widely used in chemistry, biochemistry, and various scientific fields. They are often employed together to separate, identify, and quantify components of complex mixtures
- **Chromatography** is a technique used to separate and analyze the individual components of a mixture
- **Mass spectrometry** is an analytical technique used to determine the composition, structure, and abundance of molecules in a sample.

Split Specimens: Failed to Reconfirm

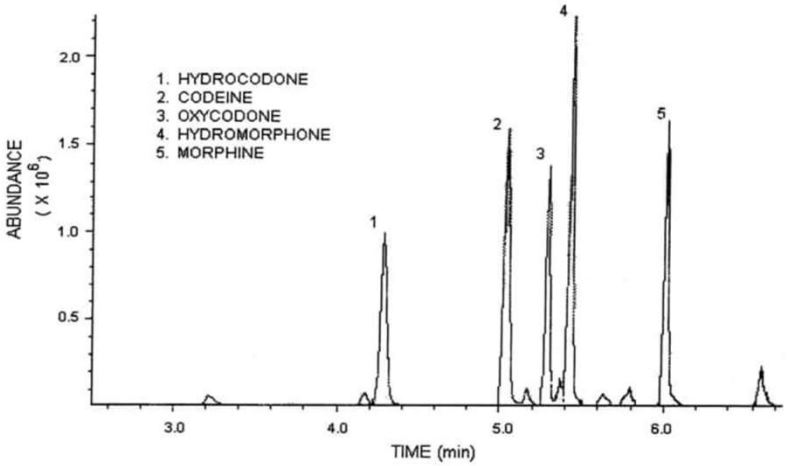
- Before reporting Lab B repeats the test on a new aliquot
- If not reconfirmed: perform SVT (urine)
- Contact MRO to discuss
- If Lab B identifies an interfering substance unique to their testing method then a Lab C may be chosen by the MRO.

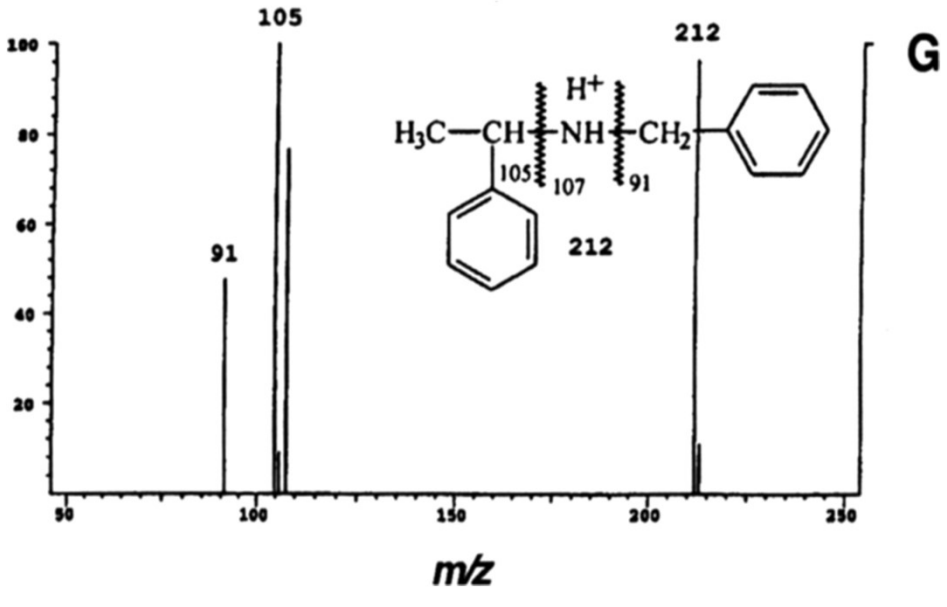


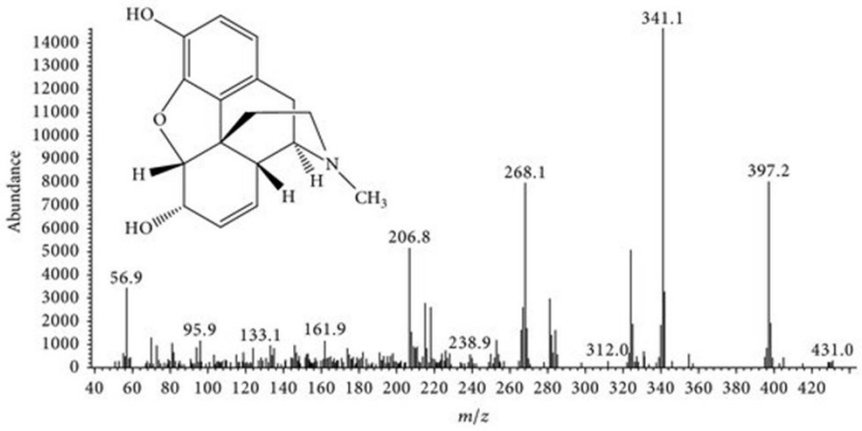


Chromatography/Mass Spectrometry

- Two different techniques to make a positive identification
  - 1) **Retention Time** time (min. to sec.) that is takes for the drug molecule to travel from the front of the chromatograph instrument to the detector (Mass Spectrometer). This is called (time retained by the chromatography system)
  - 2) **Mass Spectra** Molecular structure elucidated in the Mass Spectrometer
  - **Positive identification of a molecule**: The retention time and mass spectra are constants for a given molecule in a given analytical system when operated under specific parameters.

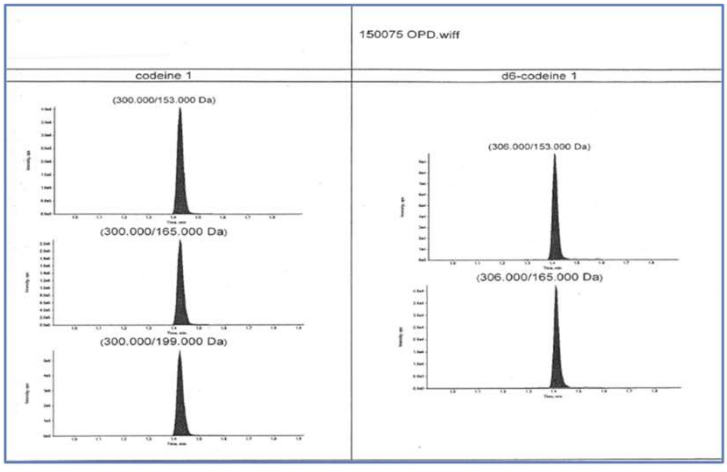

1. HYDROCODONE
2. CODEINE
3. OXYCODONE
4. HYDROMORPHONE
5. MORPHINE
ABUNDANCE
(X 10^6)
2.0
1.5
1.0
0.5
3.0
4.0
5.0
6.0
TIME (min)
1
2
3
4
5


105
212
91
100
80
60
40
20
50
100
150
200
250
m/z
G
H+
H3C—CH—NH—CH2
105
107
91
212



CHROMATOGRAM

The chromatogram is the output of a chromatographical run.



Maximum Permitted Tolerances for Ion Ratios

Relative Intensity (% of base peak or target ion)	Tolerance for EI (relative)	Tolerance for all Other Ionization Techniques (relative)
>50%	± 20%	± 20%
20-50%		± 25%
10-20%		± 30%
<10%	± 50%	± 50%

From Commission of the European Communities (2002). Official Journal of the European Communities; Guidance Document for Laboratories and Inspectors, National Laboratory Certification Program, Research Triangle Park, NC, 2002; and U.S. Department of Health and Human Services Food and Drug Administration, Center for Veterinary Medicine (2001).

Positive result requirements

- Acceptable calibration and quality control for the analyte
- Acceptable chromatography (Symmetrical)
- Correct retention time
- Mass Spectra ion ratios meets criteria
- Quantitation is ≥ applicable cutoff/reporting limit

Assay Interference

- Immunoassay screening (mostly urine)
  - Abnormally LOW absorbance readings
    - May apply to one or more analytes
  - Spectral interference flagged by testing equipment
    - May apply to one or more analytes
  - Possible causes
    - Sample condition (bloody or highly flocculent urine)
    - Medication
    - Adulterant
  - Laboratory reporting
    - Spectral Interference Flag: Invalid
    - Individual analytes affected: May report Invalid for entire sample or specific analytes
    - SAMHSA protocol: If low absorbance for only some analytes report the entire sample as Invalid.

THANK YOU!

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