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PEARLS OF LABORATORY MEDICINE

Pain Management: Opioids

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Outlines

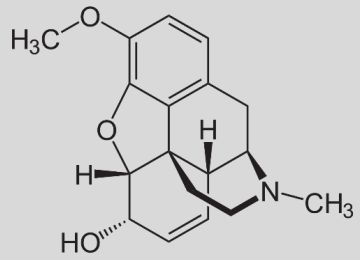
- The introduction of opioids
  - Opioid family
  - Biological function and medical application
  - Side effects and opioid crisis

- Laboratory detection of opioids
  - Specimens
  - Analytes
  - Methodologies

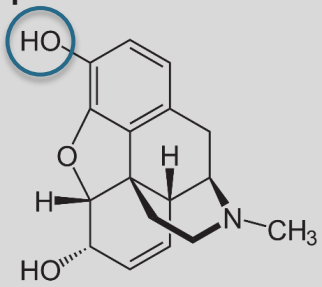
Opioid family

Natural opiates | Semisynthetic opiates | Synthetic opioids

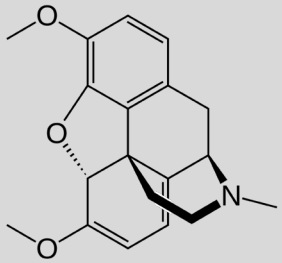
-Natural derivatives from opium



Codeine



Morphine



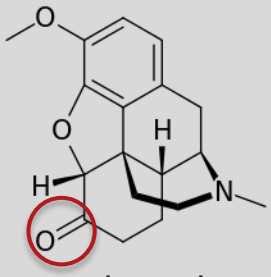
Thebaine

opium

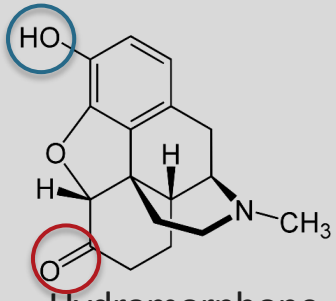
Opioids family

Natural opiates | **Semisynthetic opiates** | Synthetic opioids

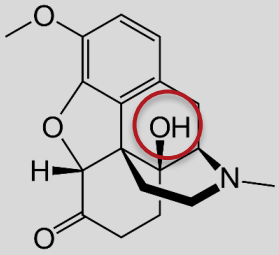
-Substituted derivatives of morphine or codeine, including



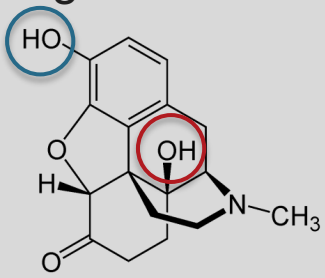
Hydrocodone



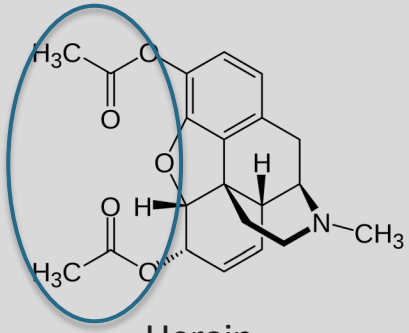
Hydromorphone



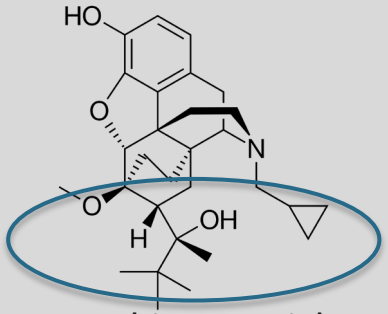
Oxycodone



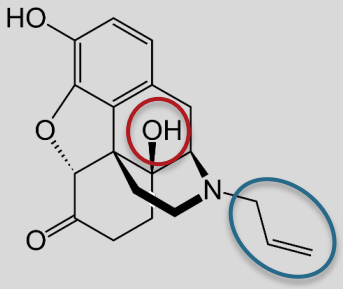
Oxymorphone



Heroin



Buprenorphine, partial agonist



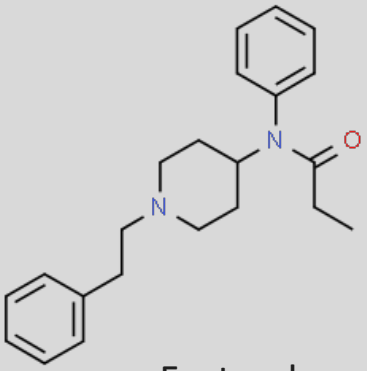
Naloxone, antagonist

Opioids family

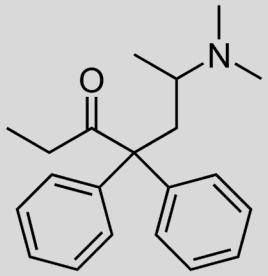
Natural opiates | Semisynthetic opiates | **Synthetic opioids**

-Structurally different from morphine

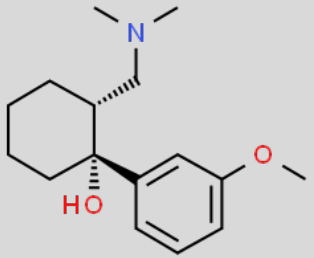
- Phenylpipiridines: fentanyl, alfentanil
- Diphenylpropylamines, *eg.* methadone
- Complex Analgesics, *eg.* tramadol, tapentadol



Fentanyl



Methadone, full agonist



Tramadol

Biological Function and Usage of Opioids

- Biological function

  Bind to opioid receptors and elicit analgesia and euphoria.

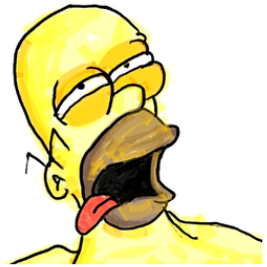
- Medical application
  - Pain management, common prescribed opioids: codeine, morphine, hydrocodone, hydromorphone, oxycodone, and oxymorphone
  - Antidote for overdose: naloxone
  - Addiction treatment: buprenorphine and methadone

Biological Function and Usage of Opioids

- Adverse effects:
  - Toxidrome: central nervous system and respiratory depression, bradycardia, hypotension, hypothermia, coma, and miosis
  - Tolerance, physical dependence, and addiction

Narcotic (Opioid) Toxidrome

Mnemonic : "CPR-3H"



C : Coma
P : Pinpoint pupils
R : Respiratory depression
H : Hypotension
H : Hypothermia
H : Hyporeflexia

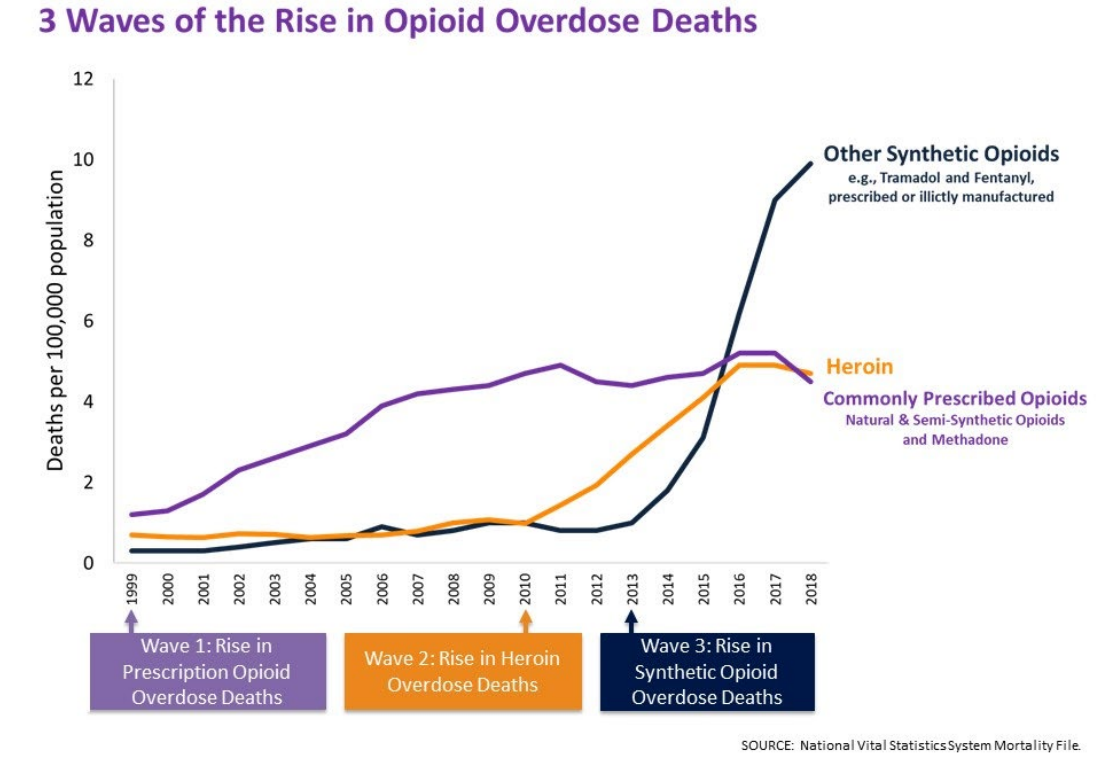
NOTE: Meperidine (*Demerol*) will not cause miosis

Antidote: Naloxone
Start with **0.04 mg** and titrate up q 2-3 min as need for ventilation to 0.5 mg, 2 mg, 5 mg, up to max 10-15 mg

Opioids Crisis

From 1999–2018, almost 450,000 people died from an overdose involving any opioid, including prescription and illicit opioids.

Two out of three drug overdose deaths in 2018 involved an opioid.



https://www.cdc.gov/drugoverdose/epidemic/index.html

Laboratory Detection of Opioids

- Clinical questions
  - Check patient compliance to prescribed opioids
  - Identify undisclosed recreational drug use
- Specimens, urine, serum/plasma, saliva, others (hair, sweat)

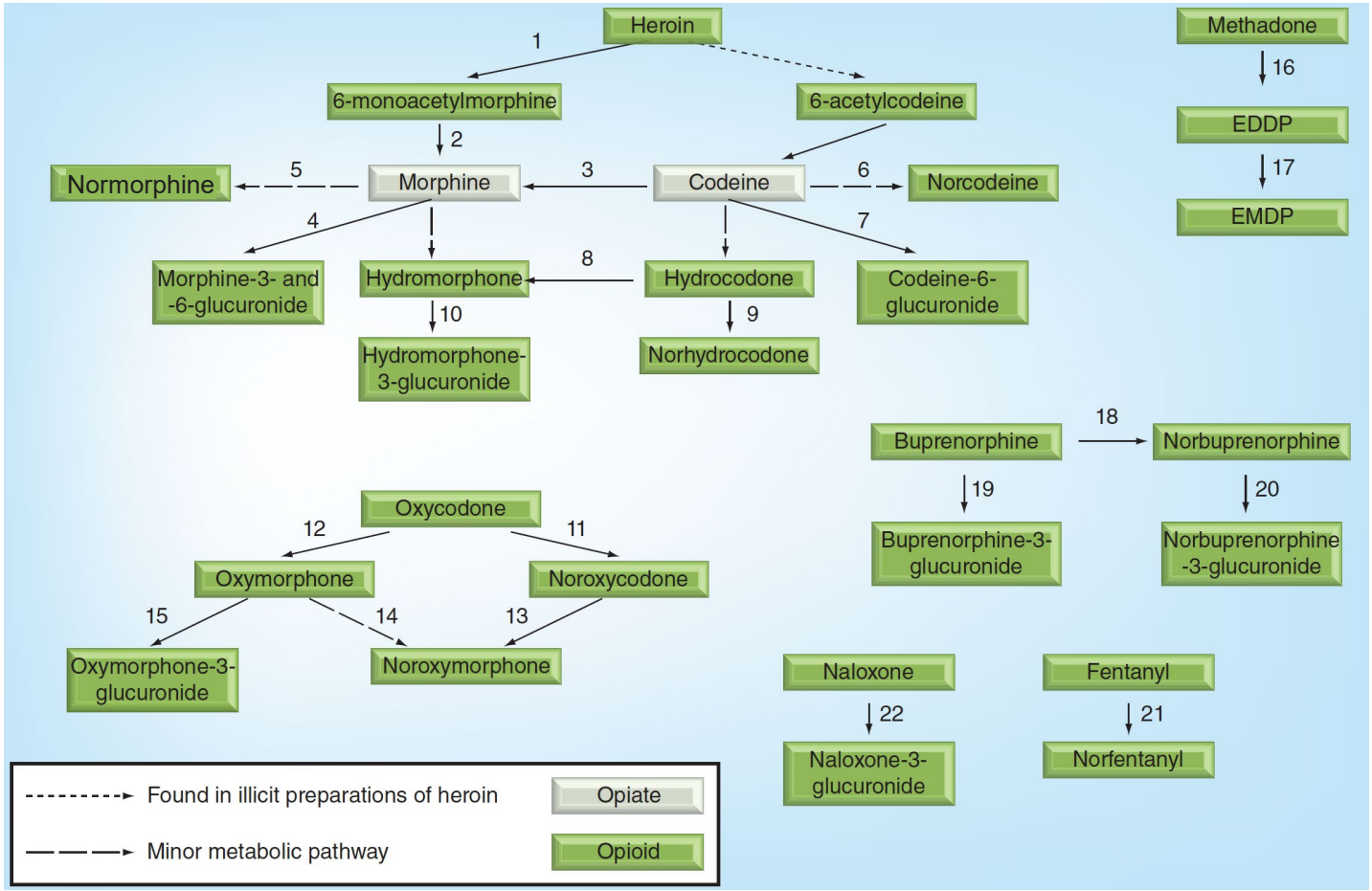
  * Check the earliest specimens if possible
- Analytes
  - Parent drugs
  - Metabolites
- Methods
  - Immunoassays
  - Mass spectrometry

To Detect Opioids: Specimens

- Urine, the most common specimen type for drug screening
  - Pros: longer detection window, usually higher concentration, less interference or sample processing
  - Cons: can be tampered intentionally and unintentionally
- Specimen integrity
  - Expected temperature: 90 –100°F/ 32 –38°C

    *Read the temperature strip within 5 minutes after the void
  - Expected pH: 4.5 –9.0
- Specimen validity testing
  - Creatinine: ≥ 20.0 mg/dL
  - Specific gravity: ≥ 1.003
  - General oxidants: *e.g.*, < 200 μg/mL for nitrites

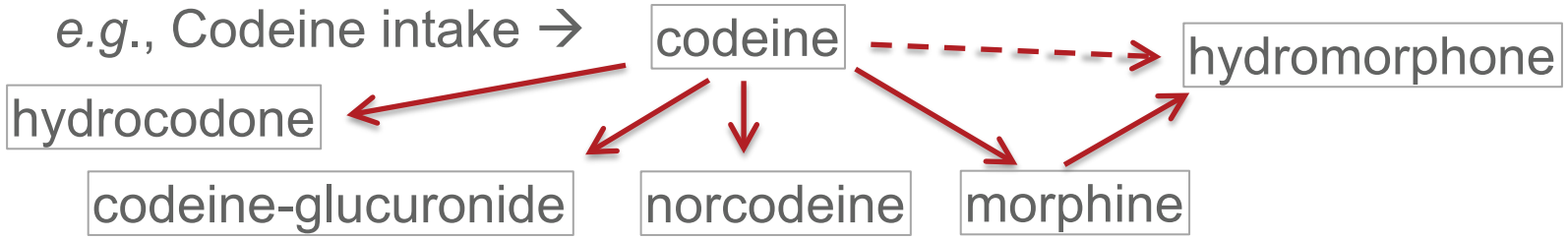
To Detect Opioids: Metabolism of Opioids



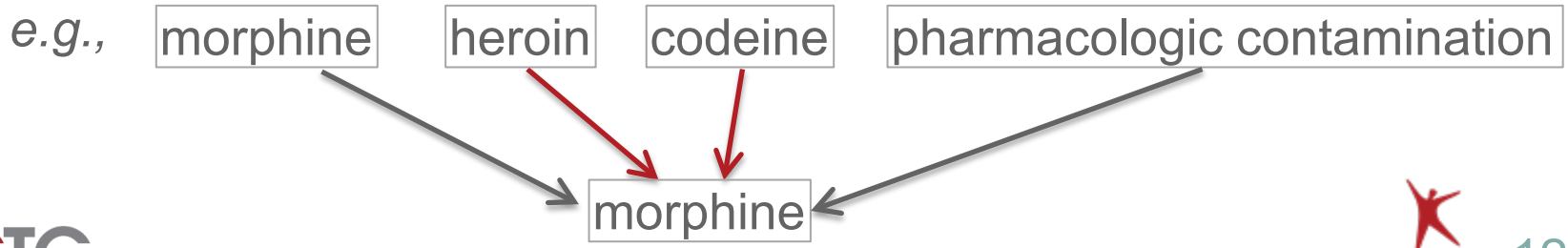
French D. The challenges of LC-MS/MS analysis of opiates and opioids in urine. Bioanalysis. 2013 Nov;5(22):2803-20. doi: 10.4155/bio.13.244. PMID: 24256360.

To Detect Opioids: Analyte Selection

- When a patient takes one opioid, both parent drug and its metabolite(s) may be detected.



- When one opioid is detected in urine, it may indicate
  - intake of this opioid
  - intake of other parent drugs
  - sometimes contamination of other drugs

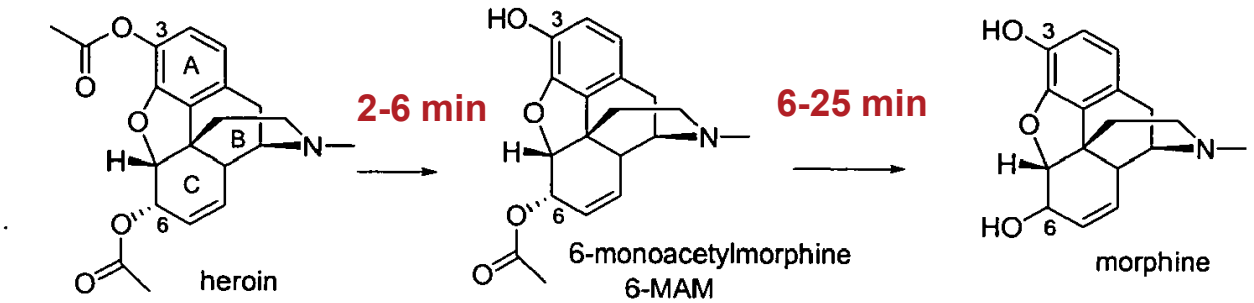


To Detect Opioids: Analyte Selection

- Metabolites, rather than parent drugs
  - To monitor patient compliance with prescribed opioids, *e.g.,*
    - Methadone → EDDP
    - Buprenorphine → norbuprenorphine

  Exclude purposeful drug diversion, adding drug to urine after collection.

  - For rapid-metabolized drugs, *e.g.,* heroin → 6-MAM



To Detect Opioids: Analytes and Detection Window

Drugs	Analytes	Detection window
Morphine	Morphine, morphine-glucuronide	1-3 days
Codeine	Codeine, codeine-glucuronide, morphine, hydrocodone	1 to 2 days
Heroin	6-MAM	12-24 hrs
Methadone	EDDP	3 to 4 days
Buprenorphine	Norbuprenorphine	Up to 11 days
Oxycodone	Noroxycodone, noroxymorphone, oxycodone, oxymorphone	1 to 3 days
Oxymorphone	Noroxymorphone, oxymorphone	1 to 4 days
Fentanyl	Fentanyl, norfentanyl	1 to 3 days

To Detect Opioids: Methods

	Immunoassay	Mass spectrometry
Pros	rapid, easy for automation, less labor and cost	high specificity, multiplex capacity, rapid assay development
Cons	prone to false positive and false negative, detect only class of drugs and not specific drugs, need to develop Abs	high requirement of instrument and techniques, not automated yet
Applications	screening	confirmation

To Detect Opioids with Immunoassays

- Competitive immunoassays
  - **E**nzyme-**M**ultiplied **I**mmunoassay **T**echnique (EMIT)
  - **C**loned **E**nzyme **D**onor **I**mmuno**a**ssay (CEDIA)
  - **F**lorescence **P**olarization **I**mmuno**a**ssay (FPIA)
  - Lateral flow immunoassays
- Lab detection of opioids by immunoassays
  - Opiate immunoassays：
    - ✓ detect natural opiates or some semisynthetic opioids but not fully-synthetic opioids
    - ✓ fail to distinguish between various opioids.
  - Specific immunoassays for individual opioids: methadone, buprenorphine, oxycodone/oxymorphone, 6-MAM, fentanyl, *etc.*

To Detect Opioids with Immunoassays

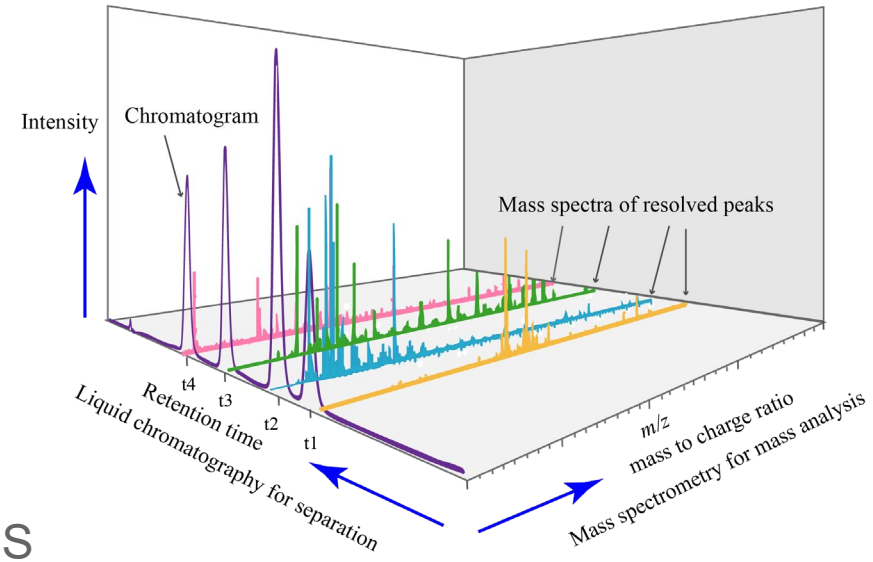
- Immunoassays are prone to false positive and false negative.
  - Presumptive positive
  - Unexplainable negative

- Moreover, opioid immunoassays are unable to distinguish between various opioids.

MS-based confirmation tests

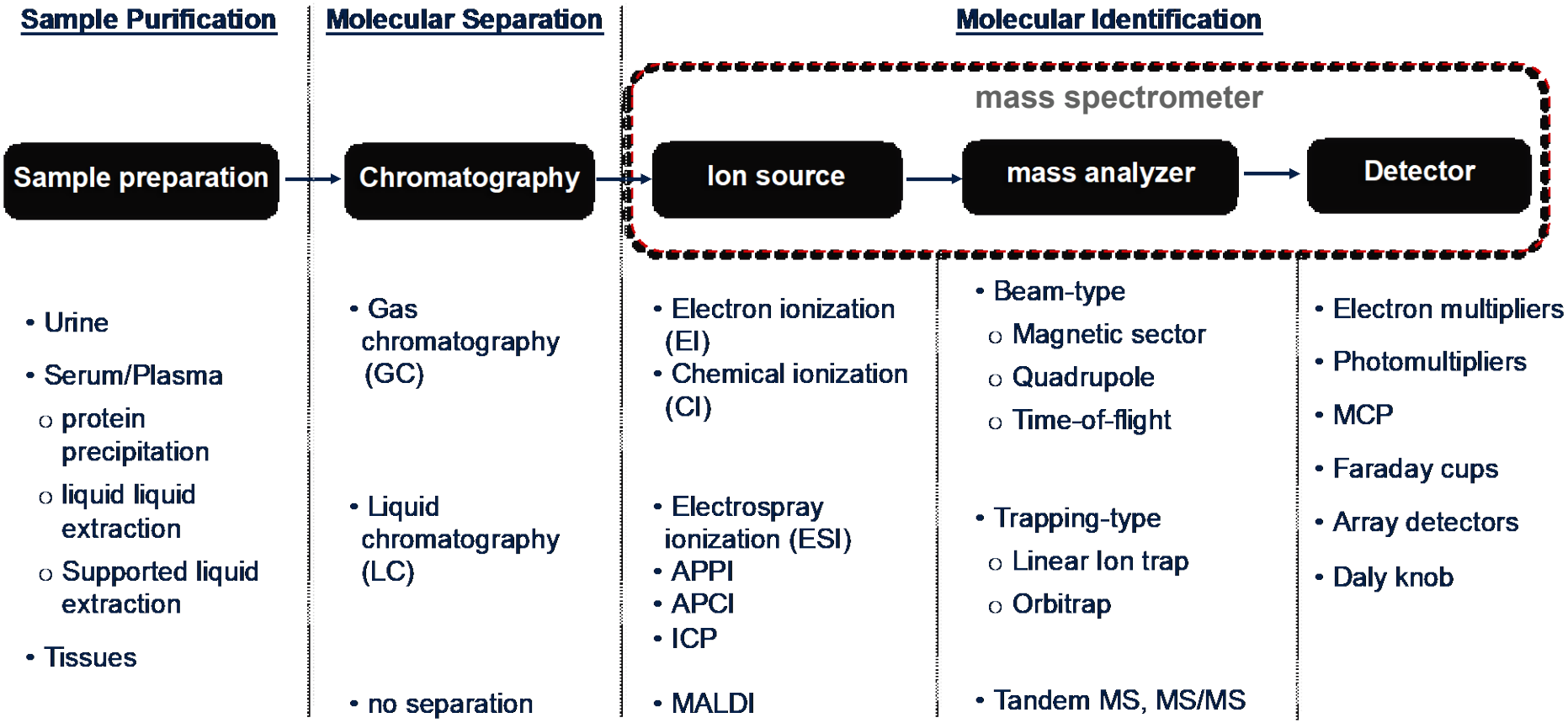
To Detect Opioids with Mass Spectrometry

- Individual drugs and metabolites are identified by retention time, mass (*m/z*), isotope, MS/MS or transitions, *etc.*



- MS methods
  - Gas chromatography (GC)-MS
  - Liquid chromatography tandem MS (LC-MS/MS)
  - Liquid chromatography high-resolution MS (LC-HRMS)

To Detect Opioids with Mass Spectrometry



To Detect Opioids with Mass Spectrometry

- Lab developed tests

  For example,

  - Opioid confirmation test (LC-MS/MS), common opioids
  - Fentanyl analog screening (LC-HRMS)

- Method validation- CLSI guidelines
  - C50-A: Mass Spectrometry in the Clinical Laboratory
  - C43-A2: 50 Mass Spectrometry in the Clinical Laboratory
  - C62: Liquid Chromatography-Mass Spectrometry Methods

Summary

- Opioid family includes natural, semi-synthetic opiates, and fully-synthetic opioids. They have similar biological function, but fully synthetic opioids are structurally different.
- To detect opioids, it is important to select the right specimen at the right time, right analytes, and right methods.
- Usually, immunoassays are used for screening and MS methods for confirmation of presumptive positive or unexplainable negative results.
- Knowledge of the analytical techniques (immunoassays and MS) and how drugs are metabolized are essential to test selection and result interpretation.

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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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- **Patents:** No disclosures

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