

Introduction to Urine Drug Screening

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Disclosures

- None

Learning Objectives

- Understand the benefits and limitations of urine as a specimen type
- Understand the screen and confirm approach to urine drug screening
- Review the classes of drugs commonly tested

Urine drug screening: Laboratory process

Sample
collection

Screening

Confirmation
testing



Sample Collection

- Urine still preferred sample for most drug screening applications
 - » Non-invasive collection
 - » Large sample volume
 - » High concentration of analytes
 - » Longer detection windows than blood
 - » Simple matrix
 - » Many methods available



Image credit: Wikipedia.org

Sample Collection

- Disadvantages of urine
 - » Some patients unable or unwilling to give a urine sample
 - » Sample adulteration is a risk as most collections are not witnessed
 - Substitution
 - Addition
 - Dilution
 - Spiking
 - » Provides no information on drug dose, timing or impairment

Drug detection windows in urine

Drug	Urine detection window
Amphetamine/Methamphetamine	1-5 days
Delta9-THC	1-45 days
Cocaine metabolites	1-2 days
Opiates	1-3 days
Fentanyl	1-3 days
Methadone	3-12 days
Buprenorphine and metabolites	1-14 days
Phencyclidine	1-30 days
Benzodiazepines	1-21 days

Urine drug screening: Laboratory process

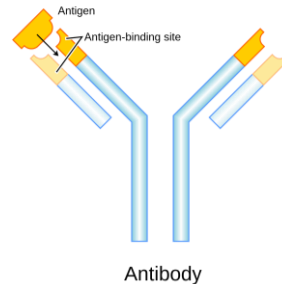
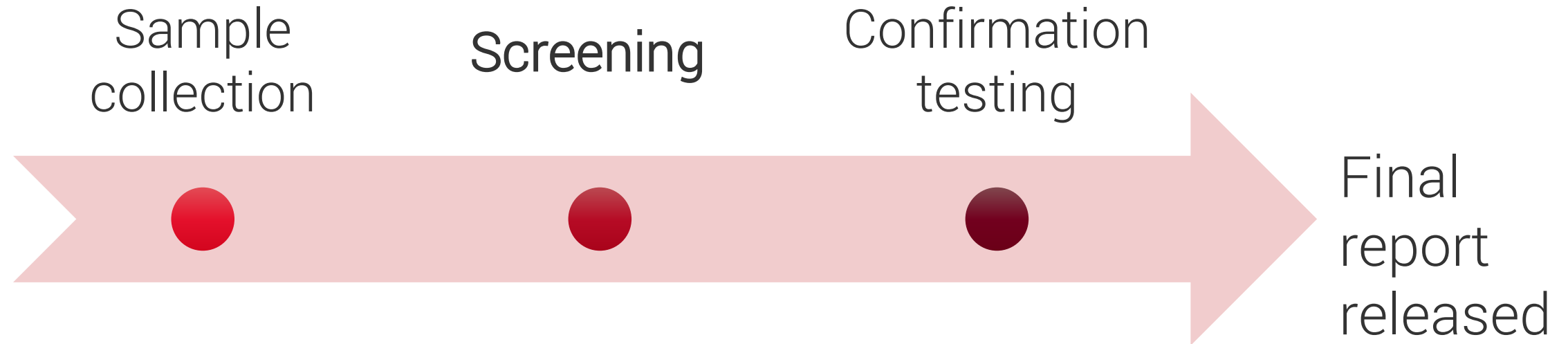
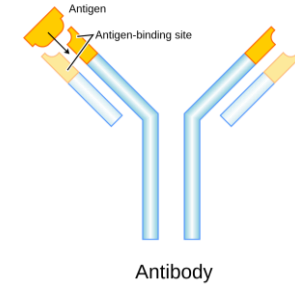


Image credit: Wikipedia.org

Screening



- Performed by immunoassay
 - » Fast
 - » Screen may be class based or for an individual analyte
 - » Many methods available (Lateral flow, CEDIA, EMIT, KIMS etc.)
 - Near patient and automated analyzer options
 - » Results should be considered presumptive only
- Typically positive screens are sent on for mass spectrometry based confirmation

Image credit: Wikipedia.org

Crossreactivity

- Class based urine drug screens do not react equally with all drugs within the class.
 - May not react as well
 - May not react at all
- } False Negatives e.g. Opiates, benzodiazepines

Opioids

- Diverse class of drugs used clinically for analgesia and opioid agonist therapy
- Refer to any molecule that can interact with endogenous opioid receptors
 - » Opiates are a subclass of opioids that refer to morphine, codeine

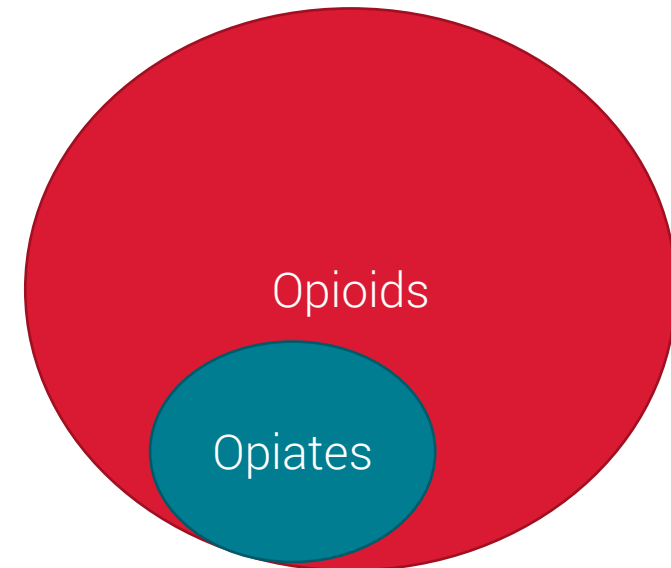
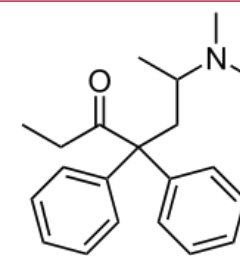
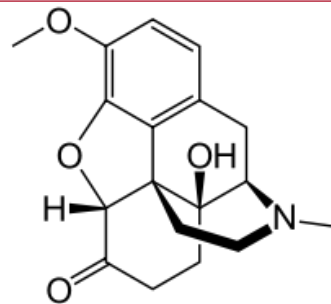
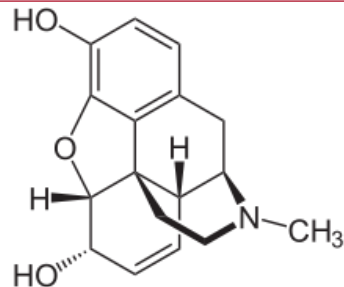


Image credit: Wikipedia.org

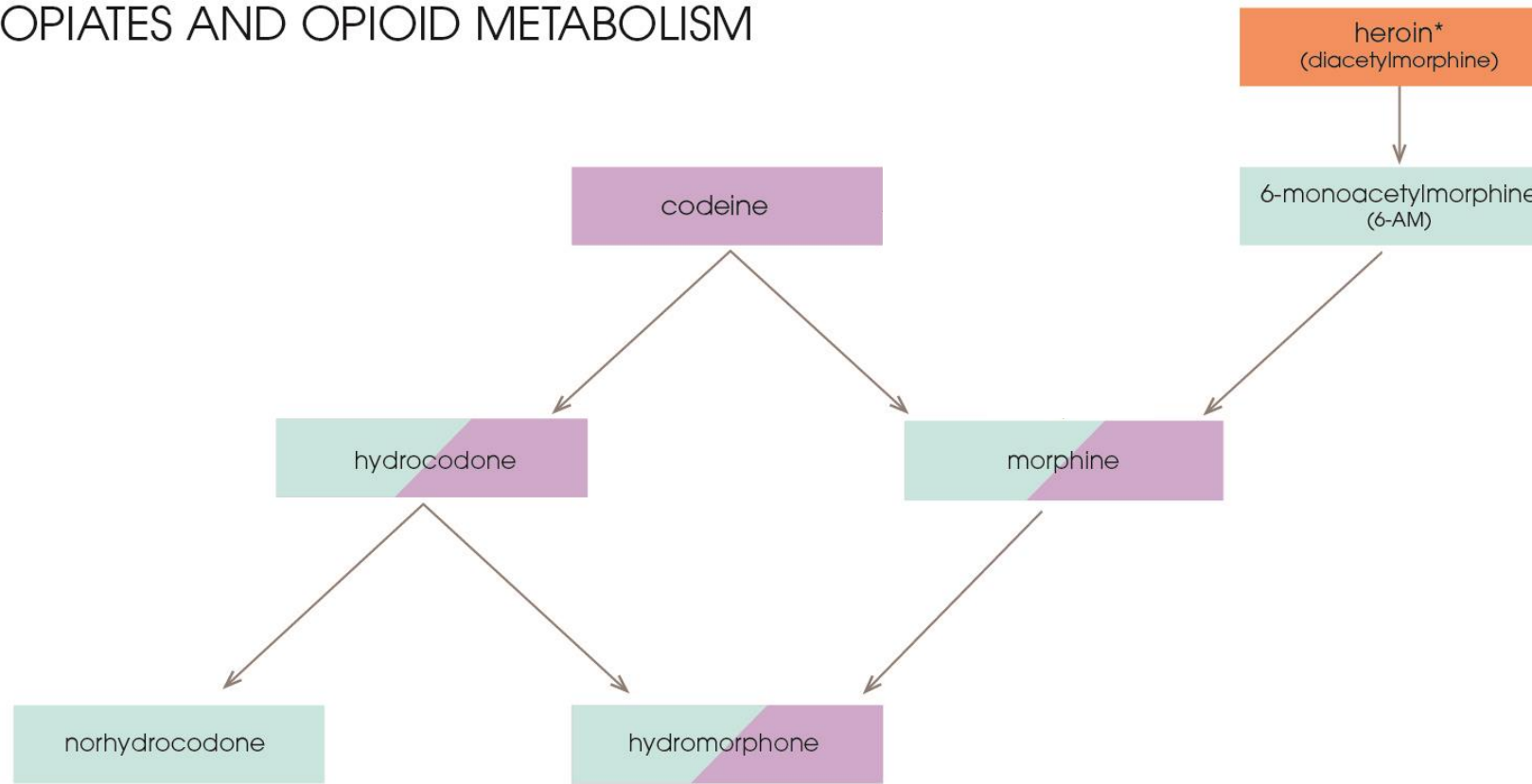
Opioids vs Opiates

- Opiate and opioid are often used interchangeably, but they are not the same thing

Opioids		
Opiates	Semi-synthetic Opioids	Synthetic Opioids
Morphine	Hydrocodone	Methadone
Codeine	Hydromorphone	Fentanyl
	Oxycodone	Buprenorphine
	Oxymorphone	Meperidine
		Naloxone



OPIATES AND OPIOID METABOLISM

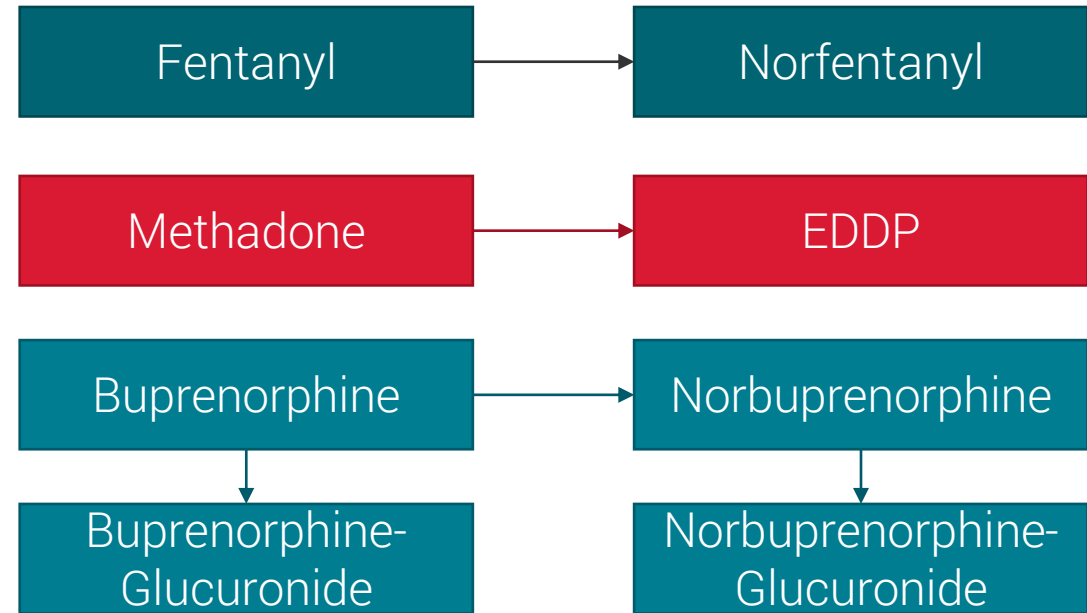


■ Non-drug ■ Drug (prescription) ■ Metabolites ■ Illicit drug

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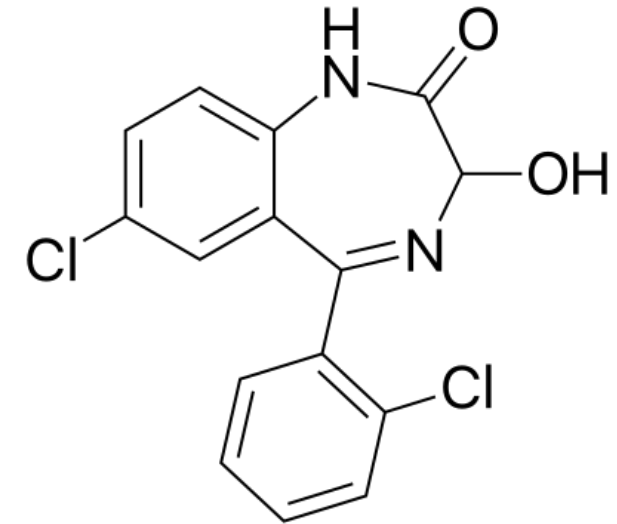
Opioid testing

- Immunoassays available for specific opioids
- Most mass spectrometry panels include opioids and their metabolites

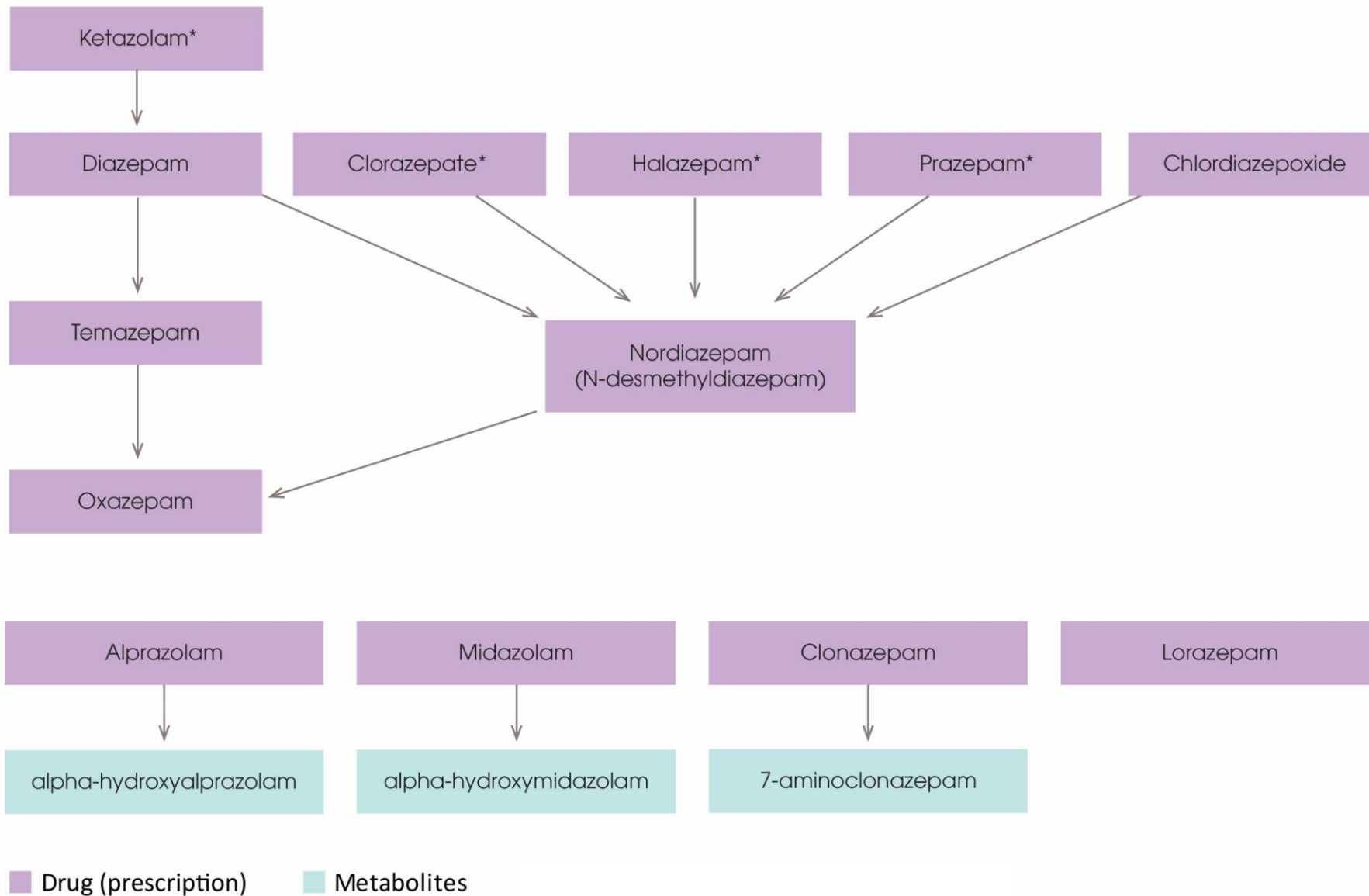


Benzodiazepines

- Sedative hypnotics that act at GABA_A receptors
- Commonly prescribed with multiple uses depending on pharmacokinetic properties
 - » Sedation
 - » Anti-anxiety
 - » Anti-seizure



BENZODIAZEPINE METABOLISM



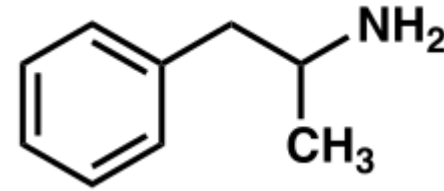
- Many immunoassays exploit the common metabolic path through nordiazepam
- Glucuronide metabolites may not react well in immunoassays

Crossreactivity

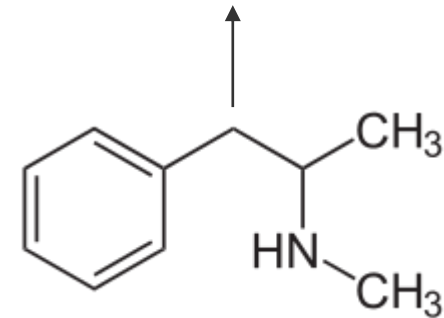
- Class based urine drug screens do not react equally with all drugs within the class.
 - May not react as well
 - May not react at all } False Negatives e.g. Opiates and Benzodiazepines
- All immunoassays may react with something that is not the drug of interest
 - » False Positives e.g. Amphetamines

Amphetamines

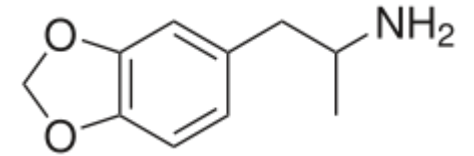
- Stimulants used clinically for treatment of ADHD and narcolepsy
 - » Common medications containing or metabolizing to amphetamine are Adderall and Vyvanse
- Poisoning can cause the sympathomimetic toxidrome
 - » Agitation
 - » Sweating
 - » Tachycardia
 - » Hyperthermia
 - » Paranoia/delusions



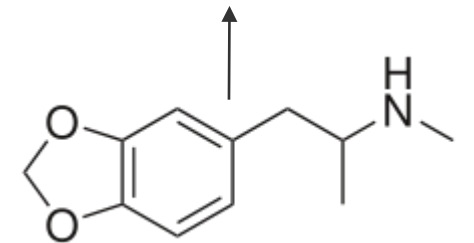
Amphetamine



Methamphetamine



Methyleneedioxyamphetamine
(MDA)



MDMA "Ecstasy"

Amphetamines immunoassays

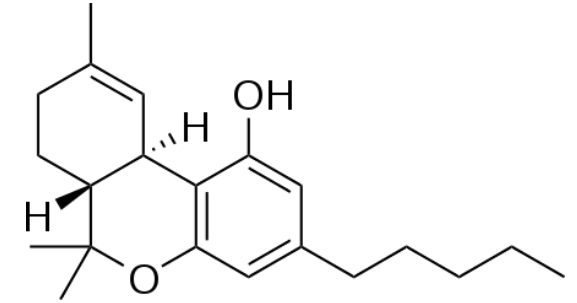
- False positive rates for amphetamines screens reported up to 40%.
 - » Labetalol
 - » mCPP (Trazodone metabolite)
 - » Ranitidine
 - » Bupropion
- Positive screens should be confirmed

Immunoassay urine drug screen panels

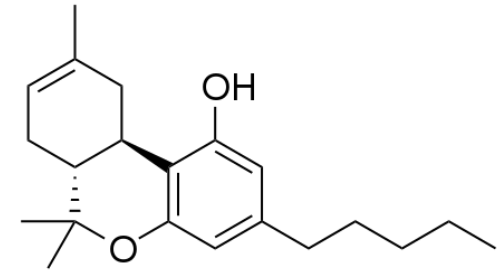
- Amphetamines
- Opiates
- Benzodiazepines
- THC Metabolite
- Cocaine metabolite

Cannabinoids

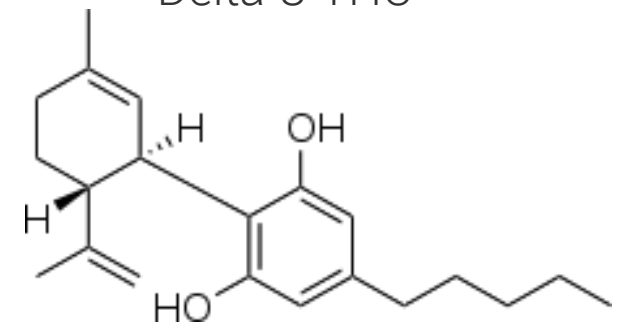
- Refer to any chemical that can interact with the Cannabinoid receptors
- Naturally are derived from the *Cannabis sativa*, *ruderalis* or *indica* plants
 - » Hundreds of cannabinoids are present in plant matrix
 - Historically most interest has been in Delta-9-tetrahydrocannabinol (THC) for drug testing
 - Cannabidiol (CBD) and Delta-8-THC now also of interest



Delta-9-THC



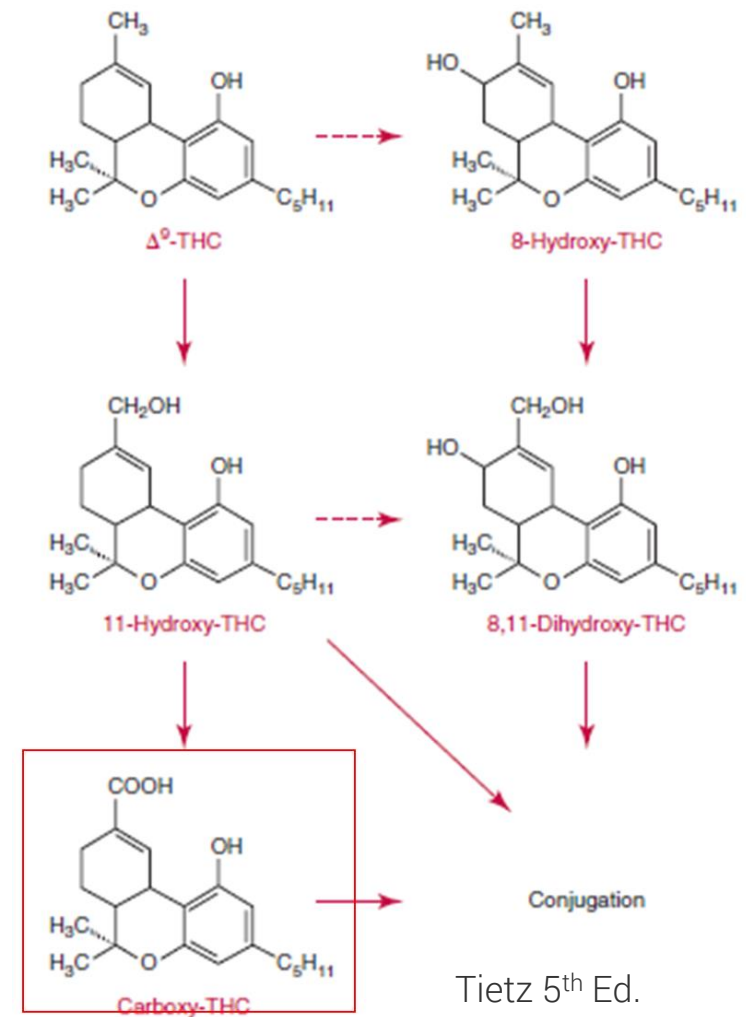
Delta-8-THC



Cannabidiol

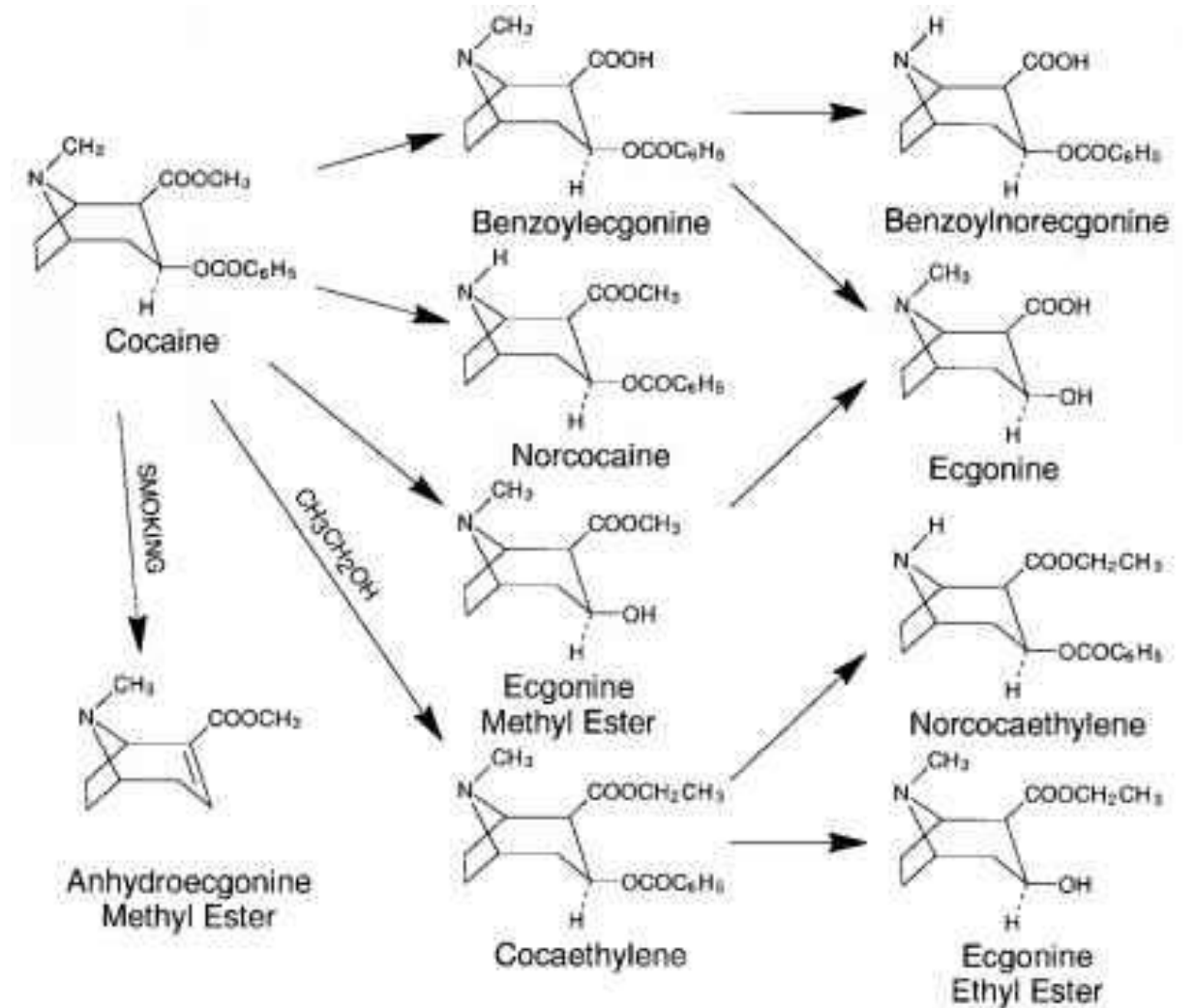
Cannabinoids – Drug Screens

- Most immunoassays are targeted towards carboxy-delta9-THC the major urinary metabolite of delta-9-THC
 - » Crossreactivity with delta-8-THC, dronabinol
 - » Variable or no crossreactivity with CBD
 - » No crossreactivity with synthetic cannabinoids



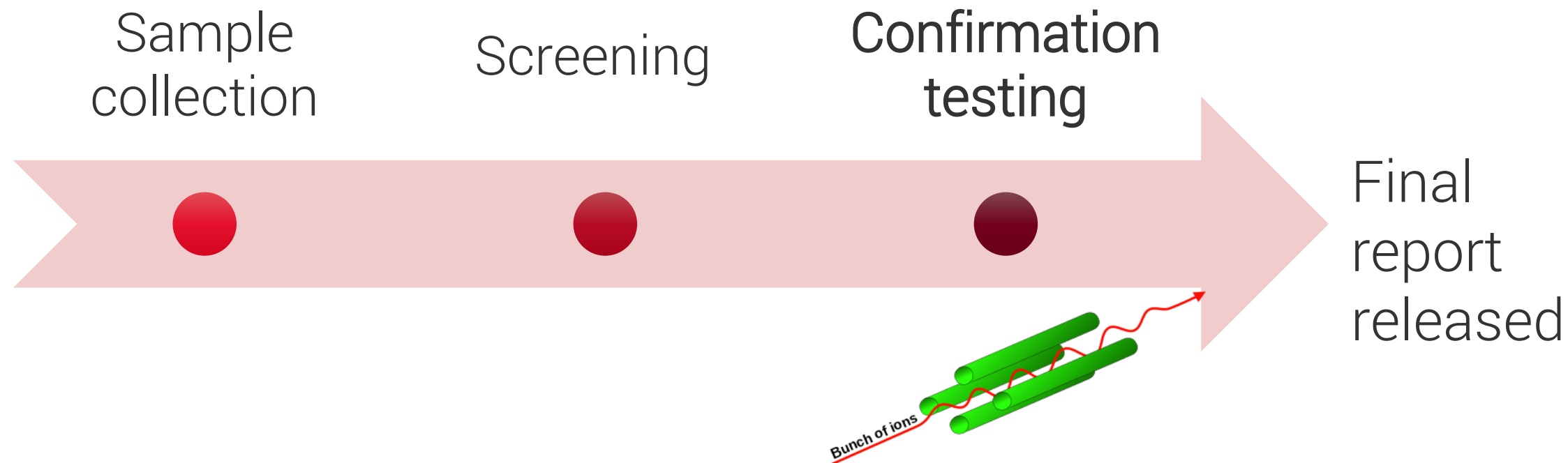
Cocaine

- Poisoning also causes sympathomimetic toxidrome
- Benzoyllecgonine (BEG) is the major metabolite monitored in urine
 - » Cocaethylene may be monitored for evidence of concurrent alcohol use



<https://www.mussenhealth.us/analytical-toxicology/cocaine.html>

Urine Drug Screening: Laboratory process

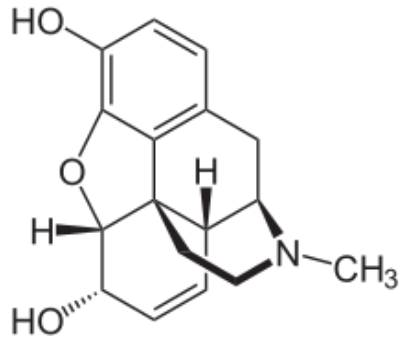


Drug screen confirmation

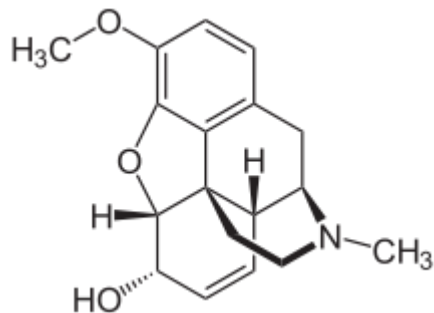
- Performed by mass spectrometry (gold standard)
 - » Liquid chromatography-mass spectrometry (LC-MS) has become popular
 - » Looks at the mass to charge ratio of an analyte (increased specificity)
 - » Many methods use tandem mass spectrometry which further increases specificity
- Major advantages
 - » Monitor parent and metabolite(s) separately
 - » Monitor multiple analytes simultaneously within a sample

Mass Spectrometry Principles

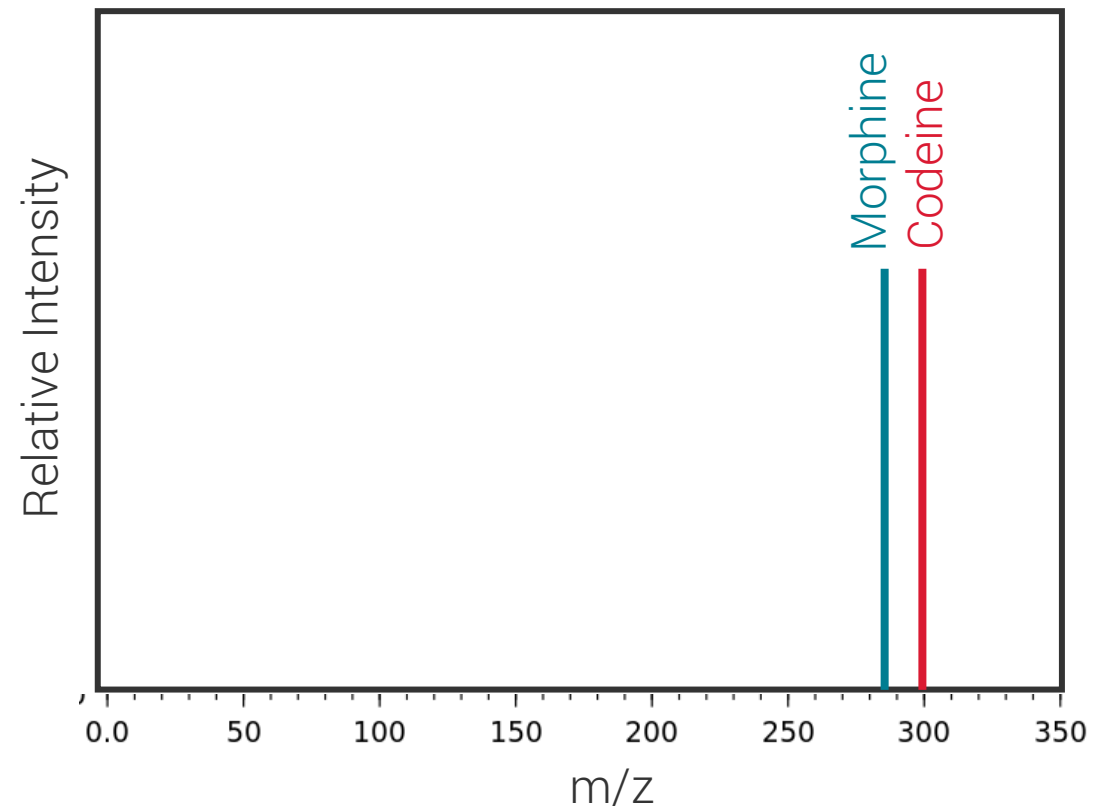
- MS differentiates analytes based on their mass to charge (m/z) ratio



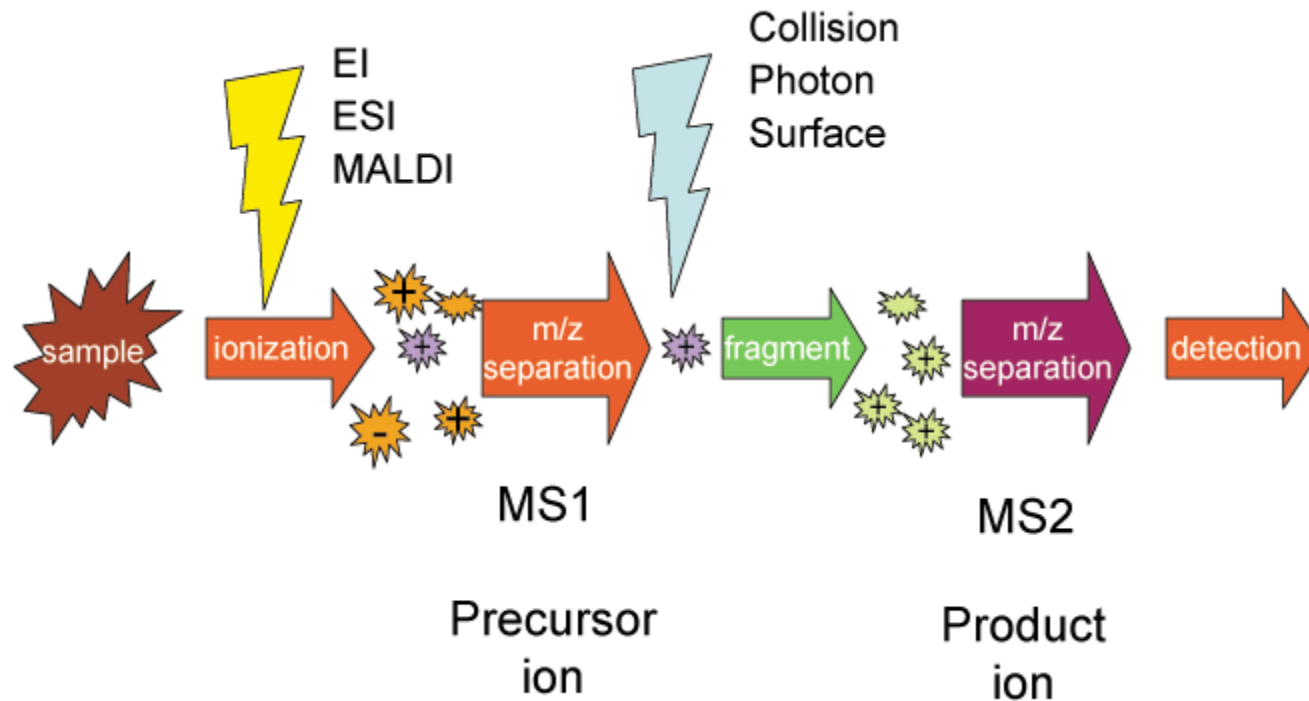
Morphine
 $m/z = 286$



Codeine
 $m/z = 300$



Tandem mass spectrometry

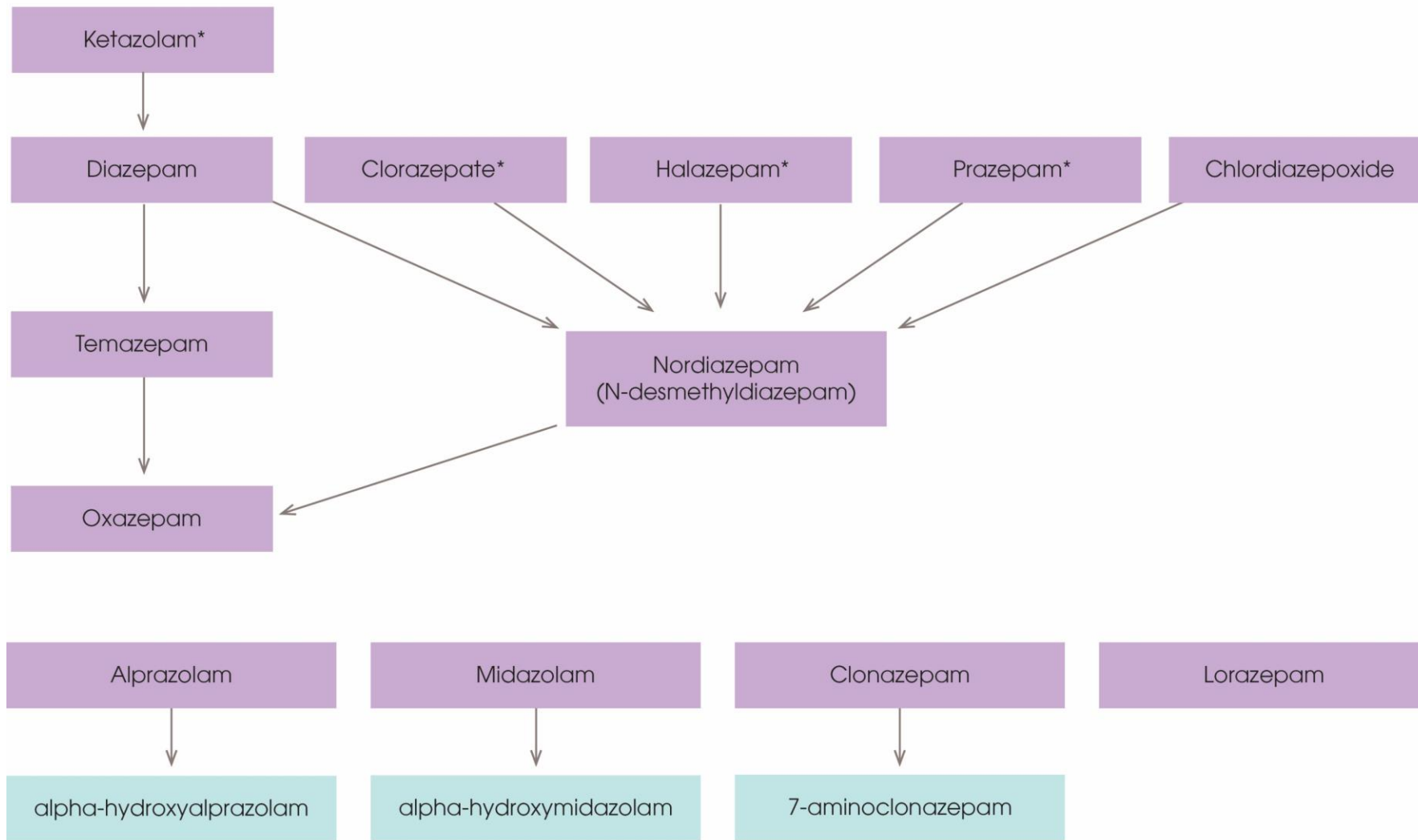


- Select for the m/z ratios of the parent and product ions
 - » Commonly performed using multiple reaction monitoring (MRM) on LC-MS/MS systems
 - » 2+ MRM transitions should be monitored per analyte

Mass Spectrometry Drug Screen Interpretation

1. Understanding metabolic pathways
 - e.g. Diazepam and metabolites

BENZODIAZEPINE METABOLISM



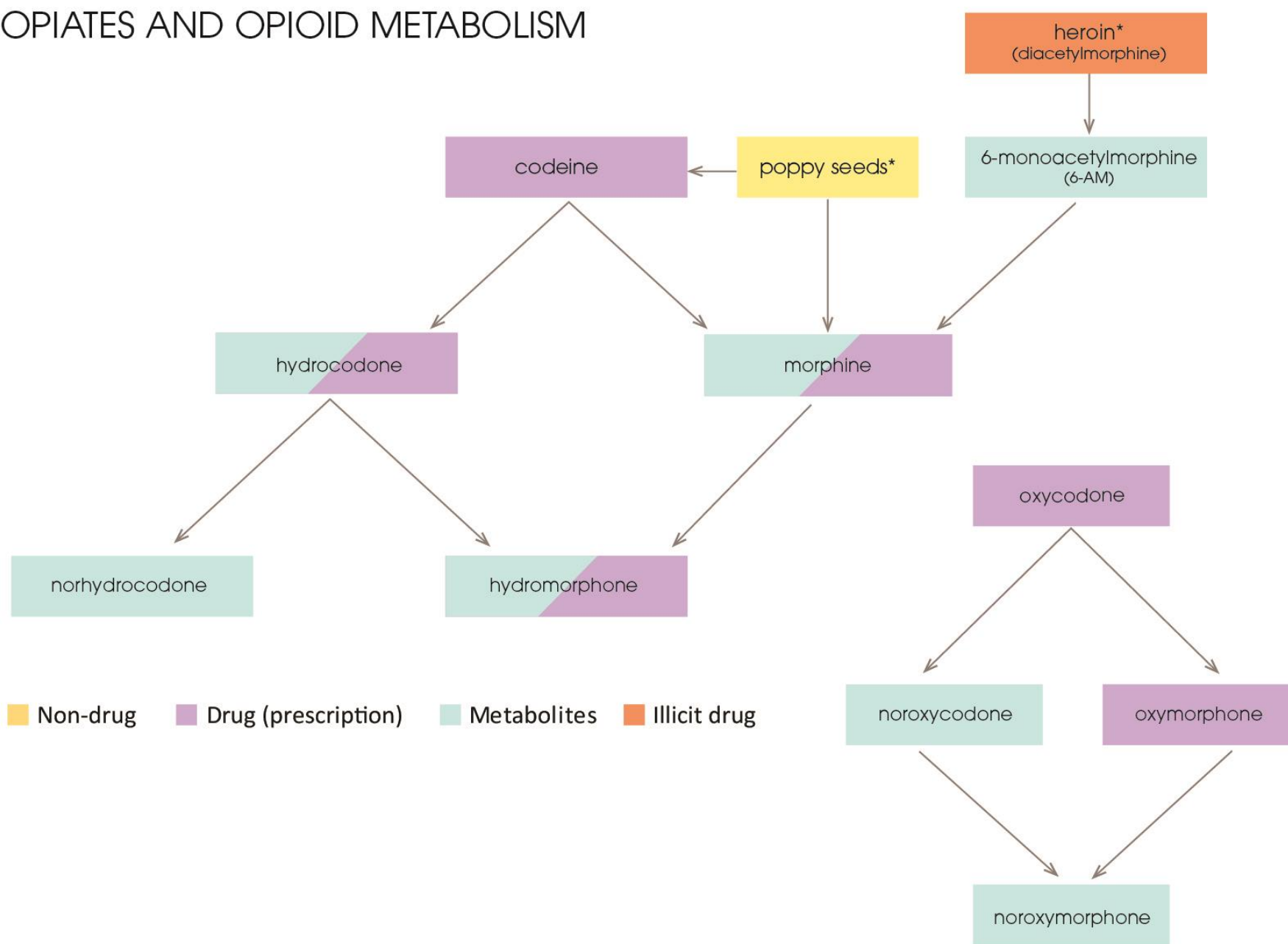
■ Drug (prescription)

■ Metabolites

Mass Spectrometry Drug Screen Interpretation

1. Understanding metabolic pathways
 - e.g. Diazepam and metabolites
2. Unexpected positives from drug impurities or foods
 - » Opiates and poppy seeds
 - » Oxycodone and drug impurities

OPIATES AND OPIOID METABOLISM



- Poppy Seed consumption usually shows up as low concentrations of codeine and/or morphine

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Pharmaceutical impurities

Formulation	Process Impurities	Allowable Limit (%)	Typically Observed (%)
Codeine	Morphine	0.15	0.01–0.1
Hydrocodone	Codeine	0.15	0–0.1
Hydromorphone	Morphine	0.15	0–0.025
	Hydrocodone	0.1	0–0.025
Morphine	Codeine	0.5	0.01–0.05
Oxycodone	Hydrocodone	1.0	0.02–0.12
Oxymorphone	Hydromorphone	0.15	0.03–0.1
	Oxycodone	0.5	0.05–0.4

Pesce et al 2012 Pain Medicine 13:868-885

Mass Spectrometry Drug Screen Interpretation

1. Understanding metabolic pathways
 - e.g. Diazepam and metabolites
2. Unexpected positives from drug impurities or foods
 - » Opiates and poppy seeds
 - » Oxycodone and drug impurities
3. Targeted methods only see what they are programmed to see

Mass spectrometer specificity

- Many MS confirmation methods use a targeted approach
 - » ie. The instrument only scans the m/z ratios that are preprogrammed during method development
 - » Other analytes might be present, but the instrument will not look at them
 - Novel Psychoactive Substances (e.g. Synthetic cannabinoids)
 - Analogues (e.g. Fentanyl Analogues)
 - Cutting agents (e.g Xylazine)

Conclusions

- Urine remains the specimen of choice for clinical drug screening
- Immunoassay screens are common but should be considered presumptive results
- Mass spectrometry provides higher specificity and can speciate parent and metabolites together



ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.