Drug Analysis: Basics of the Process

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Presentation Overview

Part I: Exercise: Reading Drug Lab Reports

Motherisk Case Example

Key Legal Principles

Sarah Olson

Part II: Drug Analysis Basics

Dr. Korin Leffler

Part III: Questions

Exercise: Reading Drug Lab Reports

https://forms.gle/oPMKs46PUzfqq2o37

Motherisk Case Example

https://projects.thestar.com/motherisk/

Admissibility of Drug Testing Evidence

Rules of evidence are relaxed at non-adjudicatory hearings, but the evidence presented must still be **relevant and reliable**

- The courts have stated that in cases heard by a judge without a jury, it is presumed in the absence of some affirmative indication to the contrary that the trial judge, having knowledge of the law, is able to distinguish between competent and incompetent evidence (that is, admissible and inadmissible evidence) and base findings on competent evidence only. See *In re F.G.J.,* 200 N.C. App. 681, 686–87 (2009); *In re L.C.,* 181 N.C. App. 278, 284 (2007).
- This principle may relax the formality of bench trials, but it does not lessen the importance of correctly applying the rules of evidence. **The court's findings still must be based on competent, substantive evidence.** See *Little v. Little*, 226 N.C. App. 499 (2013) (holding that although appellate court generally presumes that trial court disregarded incompetent evidence, the only evidence supporting the trial court's finding in action for domestic violence protective order was inadmissible hearsay; therefore, admission of the inadmissible evidence was not harmless error).
- While the court may consider hearsay and other evidence that ordinarily would be inadmissible under the rules of evidence, the **court may consider only such evidence that it finds to be "relevant, reliable, and necessary.**" G.S. 7B-901; see also *In re K.G.W.,* 250 N.C. App. 62 (2016) (trial court had discretion to exclude respondent's expert testimony on ground that testimony would not assist trier of fact); *In re J.N.S.,* 207 N.C. App. 670, 679–80 (2010) (holding that unsworn testimony was not proper at disposition hearing); *In re P.O., 207* N.C. App. 35, 39–41 (2010) (holding that the trial court did not abuse its discretion in excluding certain hearsay evidence at a permanency planning hearing).

Abuse, Neglect, Dependency, and Termination of Parental Rights Proceedings in North Carolina, 11-6.

“Chemical analysis”/confirmatory testing required

- *State v. Ward,* 364 N.C. 133 (2010) – identification of controlled substances must be based on a scientifically valid chemical analysis
- *State v. Carter*, 237 N.C. App. 274 (2014) – it was error for the trial court to admit the investigator's testimony regarding field tests on the cocaine
- *State v. Pinnix*, 246 N.C. App. 190 (2016) (unpublished) – it was error to admit officer’s testimony regarding the identity of the pills without a scientifically valid chemical analysis of them
- *State v. Cobb*, 845 S.E.2d 870 (2020) - the testimony regarding the field test should have been excluded, not limited via judicial instruction
- *State v. Osborne*, 275 N.C. App. 323 (2020) – testimony about field drug test kits “might have been excluded had Osborne objected”

Different Testing Methods:

1. Hair follicle
2. Urine Dip Test
3. Blood
4. Oral Fluid

Hair Pros and Cons

Pros

- Ease of collection
- Noninvasive
- Can detect long term or chronic drug use
- Stability, hazard-free storage and transportability

Cons

- Cost (2x as much as urine)
- Hair grows at different rates
- No correlation between drug concentrations in hair and dose or time of administration
- More issues with environmental contamination
- Active drug use difficult to distinguish from passive exposure
- Potential for bias with race and hair color because drugs preferentially bind pigmented hair

Urine Pros and Cons

Pros

- Ease of collection
- Ease of testing
- Presence of higher concentrations of parent drug and/or metabolites
- Relatively inexpensive

Cons

- Drug concentrations can not be related to impairment
- Parent drug and/or metabolites can remain in urine for up to one week (only showing recent use)
- May be embarrassing if must be witnessed
- Specimens can be altered (ex: diluted with water, substitution, addition of other liquid to alter results)

Procedures for Testing for Drugs

Toxicology samples that are being tested for drugs are screened using a presumptive test, such as the ELISA test. If the screening yields a positive result, the sample must undergo an extraction and be tested using a confirmatory test to conclusively identify the substance that is present and potentially quantify the amount of the substance that is present.

Presumptive Test

- ELISA Immunoassay

Confirmatory Test: Identification and Quantification

- Extraction
- Identification and Quantitation

Presumptive Test: Immunoassay (ELISA) or “Color Tests”

No extraction, minimal specimen handling, semiquantitative results

High sensitivity and moderate specificity

Basically, antibodies are produced to the specific drug and metabolites and if they bind, it produces a detectable result

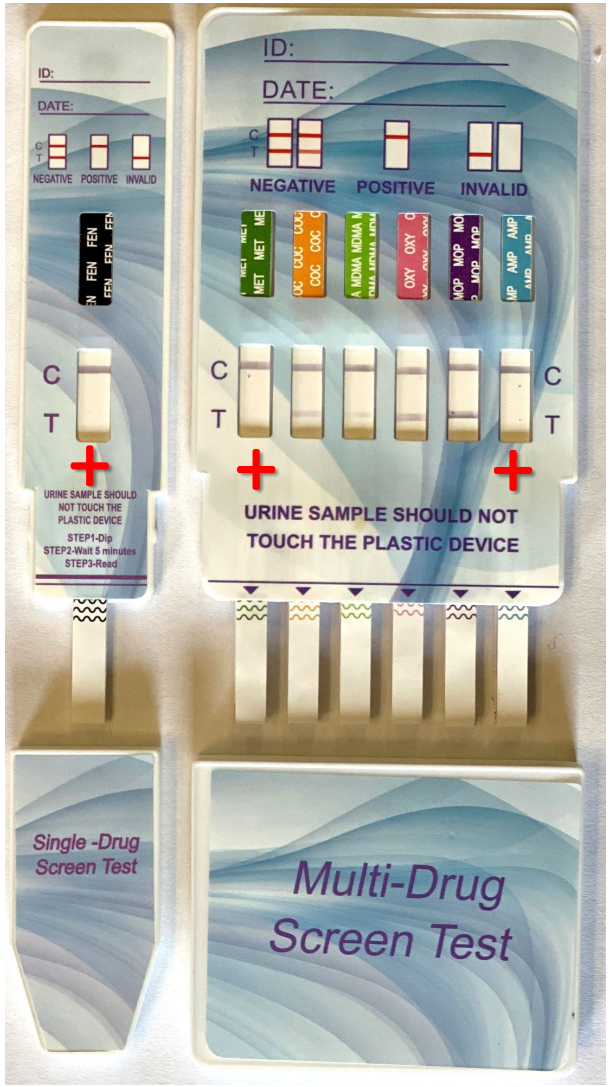
HIGH RATE OF FALSE POSITIVES

These are screening tests only: Thus, a ***Presumptive Result***

Need to continue to confirmation testing

Drug Identification

Point of Care Device ECU Forensic Pathology



Qualitative = +/-

13 Drugs of Abuse Evaluated

Device Dipped in Urine

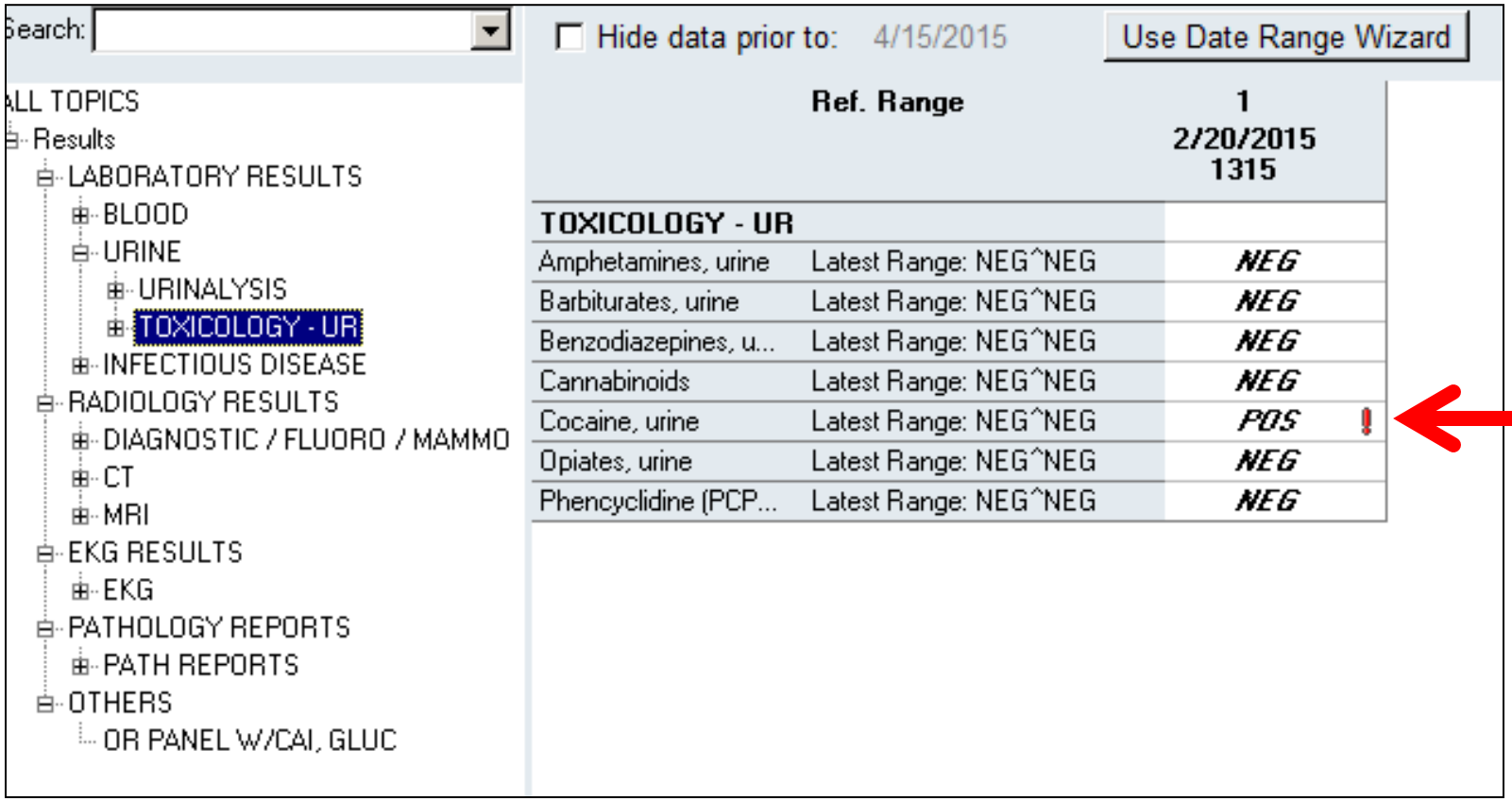
Positive (Exceeds Cutoff Level) in this urine sample:

- Fentanyl
- Methamphetamine
- Amphetamine

Drug Injury

Laboratory Testing

Urine Drug Screen: Qualitative = Yes/No



Blood, urine and hair contain a wide variety of compounds.

Confirmatory tests (GC/MS, LC-MS/MS) cannot be performed on whole blood or urine samples since there are too many compounds.

To solve this problem, prior to confirmatory analysis, the analyst must perform an extraction to isolate the testable portion of the sample.

Confirmatory Tests: Chromatography/Mass Spectrometry

Mass Spectrometry- basically, the sample is separated using chromatographic separation and enters the mass spectrometer. Once inside ion source, sample components are ionized and selectively monitored by a mass analyzer. (LC/MS/MS, GC/MS/MS, MS/MS)

More accurate and specific

Quantitative, with specific drug cutoffs

GC and MS provide distinct but complementary results; while GC separates components of a mixture, MS can analyze and identify these components.

Specific Guidelines for Validation

Limits of Detection, Quantification, Quality Control and Calibration Range
(Talk to an analytical chemist expert)

NOT the "end all, be all"

Table II. Contrast of Immunoassay and Chromatography Urine Drug Monitoring.

Immunoassay	Chromatography
Point-of-care testing or lab based	Lab-based testing
Initial test, presumptive	Confirmatory test, although can be used as initial test, definitive
Inexpensive	Costlier
Quick results	Delayed results
Sensitive, lacks specificity	Sensitive and specific
Higher cut-off limits	Lower cut-off limits (detect smaller amounts)
Qualitative, Semi-quantitative at best	Qualitative and quantitative
Tests for general chemical classes (ie, opiates, benzodiazepines)	Tests for specific drugs and their metabolites (ie, oxycodone, oxymorphone, noroxycodone, diazepam, oxazepam)

Drug Identification

Qualitative & Quantitative

Fentanyl
"Lethal Dose":
2 mg

https://www.dea.gov/galleries/drug-images/fentanyl

Fentanyl Recommended Serum Concentrations:

- **Analgesia: 1– 3 ng/mL**
- **Anaesthesia: 10–20 ng/mL**
- **Fatalities: ≥ 5-7 ng/mL ?**

6.0 ml Blood — CONDITION: Postmortem
SOURCE: Heart — OBTAINED: 16-sep-2021

Screen: Liquid Chromatography–Mass Spectrometry

Benzodiazepines	None Detected	11/21/2021
Cocaine metabolite	**Present**	11/21/2021
Ethanol	None Detected	11/21/2021
Gabapentin/Pregabalin	None Detected	11/21/2021
Naloxone	**Present**	11/21/2021
Opiates/Opioids	**Present**	11/21/2021

3.0 ml Blood — CONDITION: Postmortem
SOURCE: Femoral Vessel — OBTAINED: 16-sep-2021

Quantitation

Benzoylecgonine	**1.8 mg/L**	11/21/2021
Cocaethylene	None Detected	11/21/2021
Cocaine	**0.041 mg/L**	11/21/2021
Fentanyl	**21 ng/mL**	11/21/2021

You NEED two tests. *Why?*

Case Example:

JT is a 28-year-old male with chronic back pain prescribed oxycodone, which, for this patient, provides analgesic and functional benefit without adverse effects. The patient has a medical history significant for gastroesophageal reflux disease (GERD) and major depressive disorder (MDD). The other medications he is taking include ranitidine (GERD), bupropion for smoking cessation, and quetiapine for sleep. The individual takes a urine drug monitoring (UDM) test.

The patient's immunoassay results return as shown:

Substance	Result
Amphetamine	Positive
Opiates	Negative
Oxycodone	Positive
Methadone	Positive
Cocaine	Negative
Tetrahydrocannabinol	Negative
Phencyclidine	Negative
Benzodiazepines	Negative

Kominek C. Cases in Urine Drug Monitoring Interpretation: How to Stay in Control (Part 1). Pract Pain Manag. 2019;19(2).
https://www.practicalpainmanagement.com/treatments/addiction-medicine/drug-monitoring-screening/cases-urine-drug-monitoring-interpretation

Let’s talk about it…..

The patient’s UDM is positive for oxycodone and amphetamine. The positive oxycodone is expected. The methadone may be a false positive from several sources, two of which include kratom or quetiapine.

The positive amphetamine is not expected.

However, amphetamine immunoassay is highly cross-reactive and needs definitive testing; so the urine sample is sent for GC-MS testing.

Example of some false positives

Table V: False Positives on Amphetamine Immunoassay.

Amantadine	Ephedrine	Pseudoephedrine
Benzphetamine	Fluoxetine	Promethazine
Brompheniramine	Isometheptene	Ranitidine
Bupropion	MDMA	Selegiline
Carbidopa/Levodopa	Phentermine	Thioridazine
Chlorpromazine	Phenylephrine	Trazodone
Desipramine	Phenylpropanolamine	Trimethobenzamide
Doxepin		Trimipramine

MDMA is methylenedioxymethamphetamine.

Store brand cough med

Sudafed

Weight loss

Prozac

Phenergan

Dimetapp

Midrin

Zantac

Wellbutrin

Weight loss

Sudafed

Kominek C. Cases in Urine Drug Monitoring Interpretation: How to Stay in Control (Part 1). Pract Pain Manag. 2019;19(2).
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Of note, the patient is currently prescribed ranitidine and bupropion that are listed as possible false positives on the amphetamine immunoassay. It is important to order additional testing to make sure it is a false positive and not due to the presence of actual methamphetamine/amphetamine.

Results of GC-MS testing were shown as follows: Bupropion, Oxycodone, *Noroxycodone*, and *Oxymorphone*. Based on the definitive testing, he is in the clear, as the results show expected prescribed medications and their *metabolites* and no unexpected substances are present.

Window of Detection

The length of time a substance or metabolite can be detected is found as the window of detection or detection time.

Numerous factors determine the window of detection—including chemical properties of the substances being tested, individual metabolism rates and excretion routes, route of administration, frequency of use, and amount of substance used, sensitivity/specificity of the test, cut-off concentrations, individual patient factors (eg, health, diet, weight, gender, fluid intake, pharmacogenomic profile), and the biological specimen tested.

What does all that mean? *May be wise to consult an expert*

Table IV: Federal Workplace Cutoff Values.

Substance	Initial drug test level (immunoassay ng/mL)	Confirmatory drug test level (GC-MS, ng/mL)
Marijuana metabolites	50	15
Cocaine metabolites	150	100
Opiate metabolites	2000	2000
Phencyclidine	25	25
Amphetamines	500	250

Kominek C. Cases in Urine Drug Monitoring Interpretation: How to Stay in Control (Part 1). Pract Pain Manag. 2019;19(2).
https://www.practicalpainmanagement.com/treatments/addiction-medicine/drug-monitoring-screening/cases-urine-drug-monitoring-interpretation

Drug Identification

Laboratory (or Field) Testing

- **Specimens**
  - Blood
  - Urine
  - Breath (ethanol)
  - Hair
- **Options**
  - **Drug Screen** → Confirmation / Quantitation
    - Quick + / - determination → Confirmation +/- Quantitation [ ]
    - Usually used for drugs of abuse; Can be done for all drugs
  - **Focused Assay**
    - Evaluation for level of specific medication taken by patient
      - Antibiotics: peak and trough levels
      - Antiseizure medication: Dilantin, carbamazepine
      - Anti-Rejection Drugs: Tacrolimus, Cyclosporin
      - Other

1

2

Slide from Medical School

Major take away: All controlled substance should have two tests at minimum

In 2008 the language was that presumptive tests are “used to evaluate evidence in determining the possible presence of controlled substances into general categories.”

Therefore, confirmatory tests that are substance-specific must be performed in order to positively identify the substance.

https://forensicresources.org

Consult an Expert or IDS Forensic Resources!

Specific Drug Profiles

THC/Cannabinoids/Marijuana

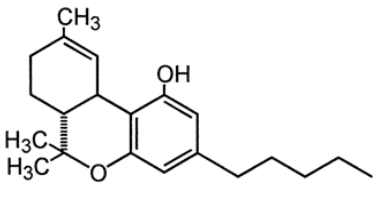
So much variability

Highly lipophilic, gets stored in body

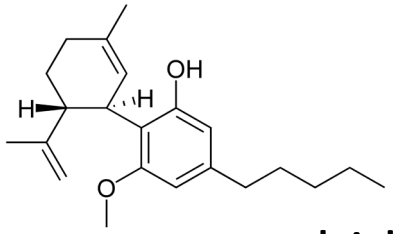
What about legal Delta-8 THC or Cannabidiol (CBD)?

Lower concentrations in hair than other drugs of abuse

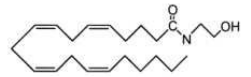
Controversy regarding if passive exposure will lead to positive urine screening test



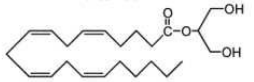
Δ-9-tetrahydrocannabinol (THC)



cannabidiol



Anandamide



2-Arachidonylglycerol (2-AG)

Endogenous cannabinoids

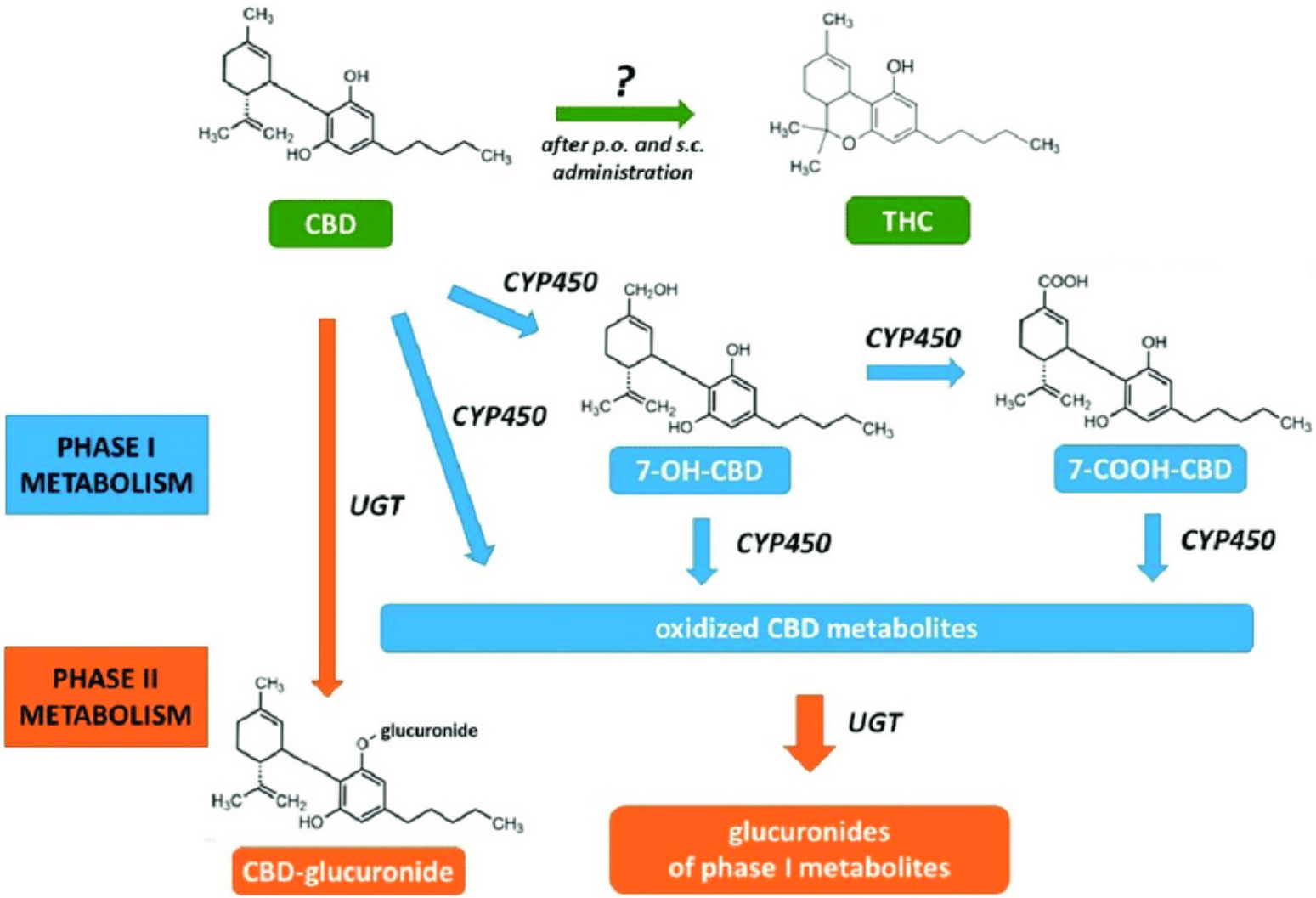
Inhaled:

60% of THC is absorbed (stored in adipose tissue)
Effects peak in about 20 min, can last 2-3 hours
THC becomes inactive when metabolized
Drug and its metabolites detected for weeks (can be months in chronic users)

Ingested:

Only 6-20% reaches systemic circulation (due to first pass effect)
3-10X greater dose than inhalation to get effect

Cannabis: Pharmacokinetics



Opioids

Opiates are naturally occurring alkaloid analgesics from the opium poppy
Opioids include the naturally occurring drugs as well as synthetics
Cross tolerance develops between opioids

Talk more about Opioid Use Disorder (OUD)

Different PK/PD (also called TK/TD) and highly lipid soluble

Withdrawal is not life threatening

Urine detection window: Approximately 48 hours, but can be detected as long as 3-4 days

Hair much longer due to lipophilic nature of opioids

Heroin has a specific marker of 6-AM (this short urinary metabolite half life limits detection to 2-8 hours after exposure)

False positive with poppy seeds; detection cutoffs vary and no federal cutoff

Amphetamines (Bath salts—cathinone, MDMA, MDA, etc.)

Numerous clinical uses for obesity, ADHD, narcolepsy, and cold medicine

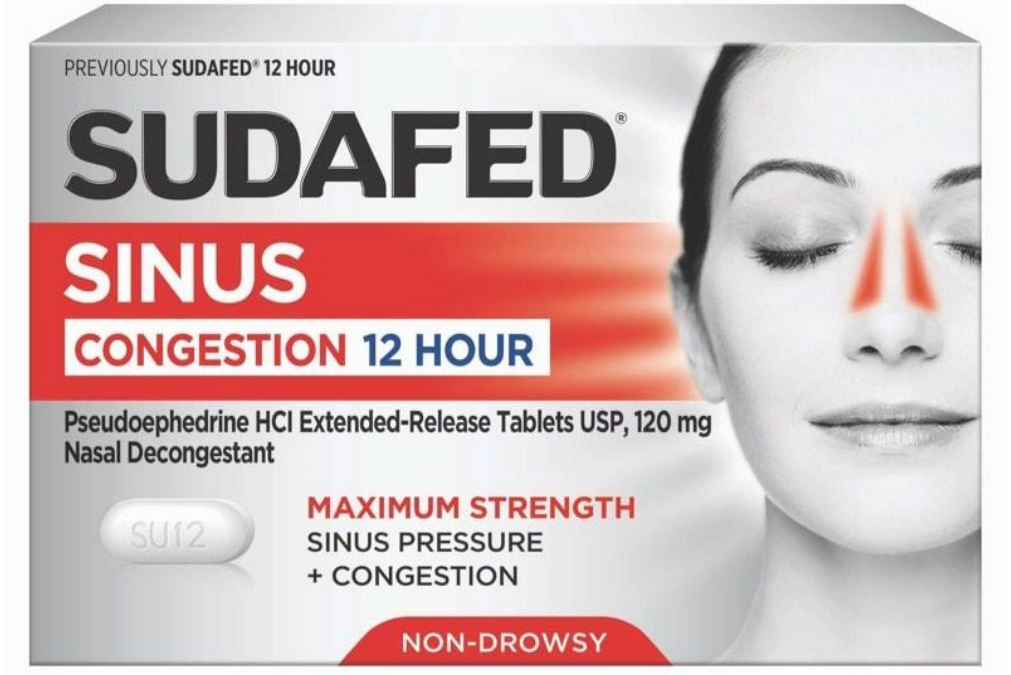
Numerous false positives on presumptive tests with drugs like phenylephrine, ephedrine, phentermine and pseudoephedrine

ADHD medications will test positive for amphetamines but not methamphetamines

Length of time depends upon route of administration and pH of urine

Highly lipid soluble

Difficult to analyze and test for, wide range of side chains



Cocaine

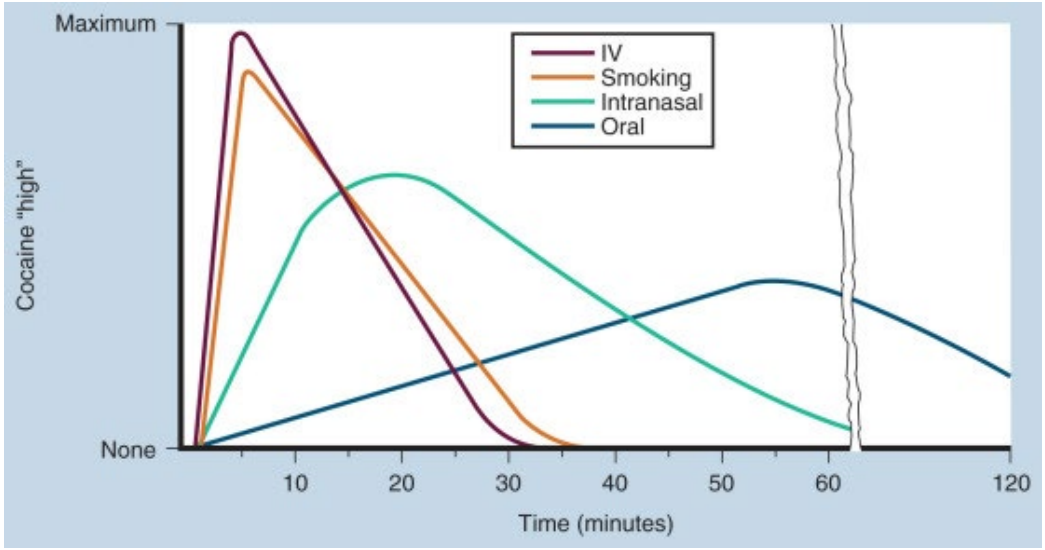
Most abused stimulant in US: “rush” followed by “crash”

Prevents reuptake of neurotransmitters

Mostly excreted in urine (64-69%) within three days,
With (about 80% of that on the first day after use)

Chronic use increases the metabolites and parent drug concentrations in the urine

Cocaine Pharmacokinetics/Toxicokinetics



Urine is suitable only for determining exposure, positive results can be seen 5-16 days after last use in chronic users

Tests look for benzoylecgonine and metabolite has stability issues unless frozen

Passive exposure (from dermal exposure) can result in positive results without PPE

Takes 4-5 days before detected in hair, not useful in an acute sense

Benzodiazepines

Most widely prescribed CNS depressant in the US

Used as anxiolytics, anticonvulsants and muscle relaxants

*Often mixed with alcohol, opiates and illicit drugs

High lipid soluble and protein-bound

Peak concentration because rate of absorption depends upon the specific benzodiazepine

Ethanol (Alcohol)

Comparatively, ethanol concentrations in the blood are remarkably stable during short and long-term storage of specimens.

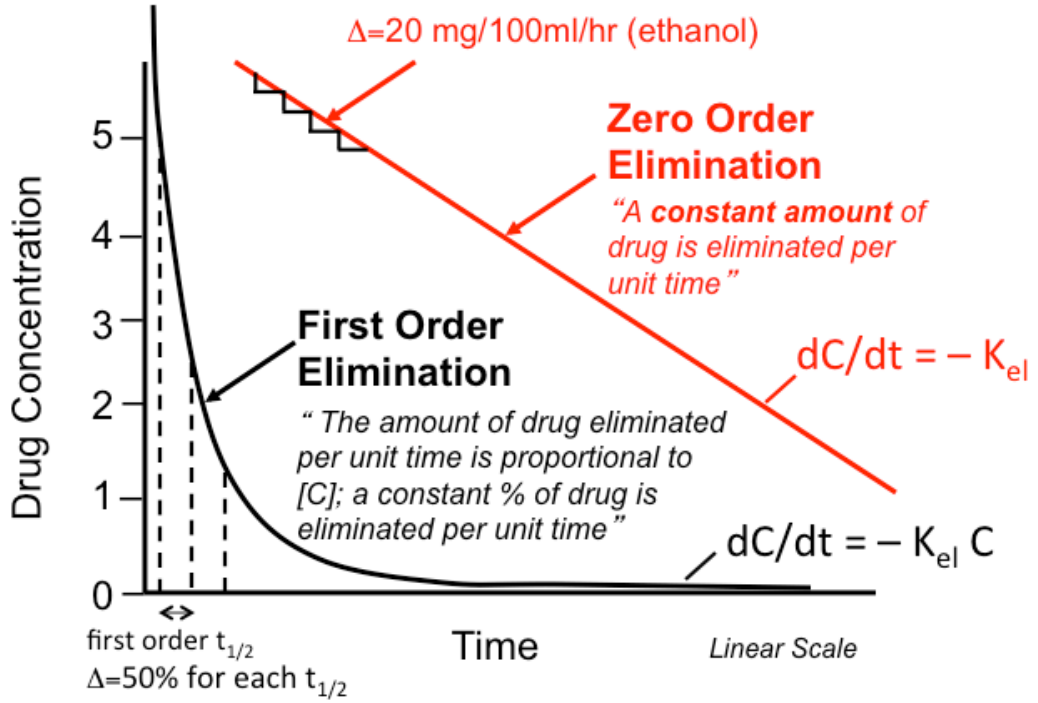
Primarily absorbed via passive diffusion in the small intestine

Withdrawal can be life threating in AUD

Concentrations in blood and breath are very highly correlated

Has a specific formula for elimination rate
0.015g/100mL per hour (men)
0.018g/100mL per hour (women)

Still a lot of variability based on race, age, chronic use, liver enzymes, etc.



Drug Analysis Basics: Resources

https://forensicresources.org

https://www.dea.gov/factsheets

National Highway Traffic Safety Administration,
Drug and Human Performance Fact Sheets, 2004

American Society of Addiction Medicine Consensus Statement,
Appropriate Use of Drug Testing in Clinical Addiction Medicine, 2017
(for informational purposes)

Questions? Comments?

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