Testing for Drugs of Abuse

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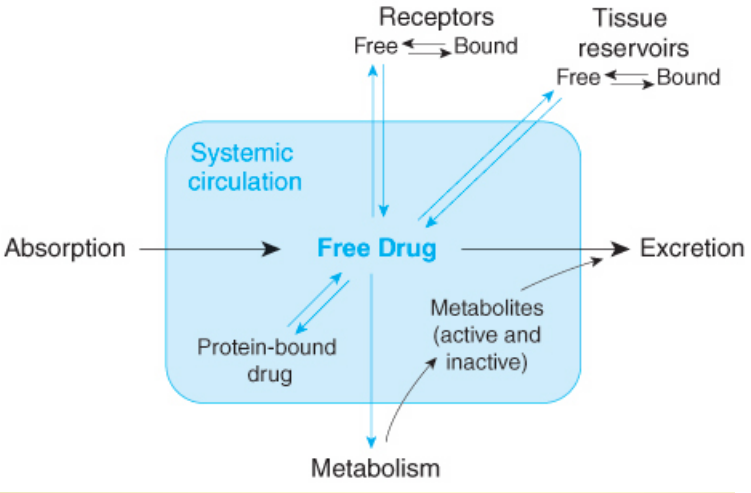
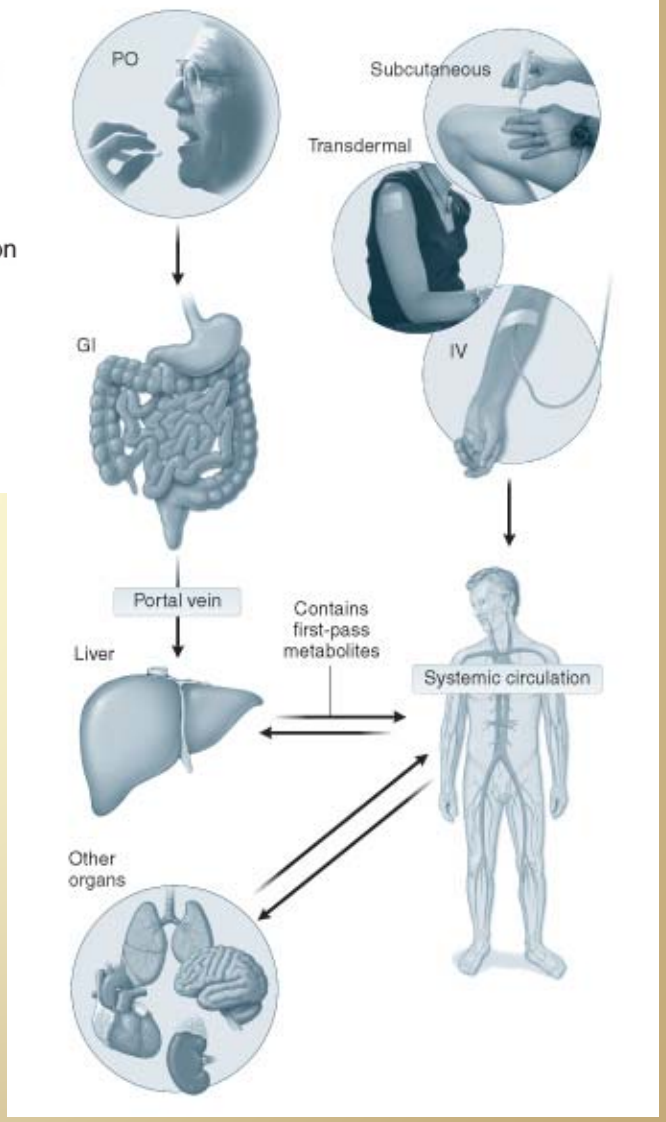
hodsdon.com/wiki

http://www.hodsdon.com/wiki/pmwiki.php?n=Main.Medicine

Drug 'Lifecycle' in Humans

- Absorption
  - Ingestion, injection, inhalation, etc.
- Distribution
- Metabolism
  - Metabolites usually inactive
  - Determines length of efficacy
- Excretion
  - What goes in must come out
  - Usually in the urine
  - Also in feces or breath
  - Most often modified
  - Can be quite delayed





Choice of Specimen

Blood

- Generally target active compounds
- Correlates well with current clinical effect
- Quantitation useful (just like therapeutic drug monitoring)
- Not that useful for detecting past use
- Obviously, more invasive
- Hard to adulterate

Urine

- Generally target inactive metabolites
- Not specific for current use
- Renal concentrating ability increases sensitivity
- Detectable for days after use (variable)
- Quantitation varies with hydration status; hence, generally not useful
- Less invasive
- Easier to adulterate

Testing Methods

Immunoassays

- Used for screening
- Testing is generally automated and results are available rapidly
- Can be instrument-based (lab) or simple 'dipstick' (bedside)
- Sensitivity and specificity vary greatly (dipsticks generally perform poorly)
- Less expensive
- Commercially available but dominated by workplace drug testing needs, not clinical

Confirmation

- Includes HPLC, TLC, GC/MS, LC/MS/MS (defined in handout)
- Generally used to confirm screening results, but can be ordered directly
- Often relies on more complicated and expensive equipment (but reagent costs can be less)
- Almost always labor-intensive with turn-around times taking days to weeks
- Sensitivity as good or better than immunoassays
- Specificity is much better; can often provide list of exact compounds

Enzyme-Multiplied Immunoassay Technique (EMIT)

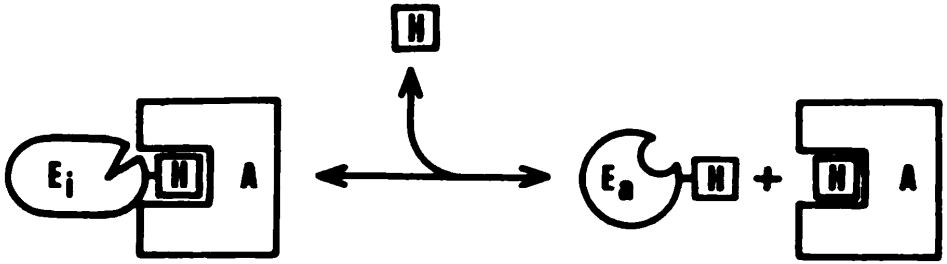


Fig. 1. Principle of Homogeneous EIA

A, antibody; E_a, active enzyme; E_i, inhibited enzyme; *H*, hapten

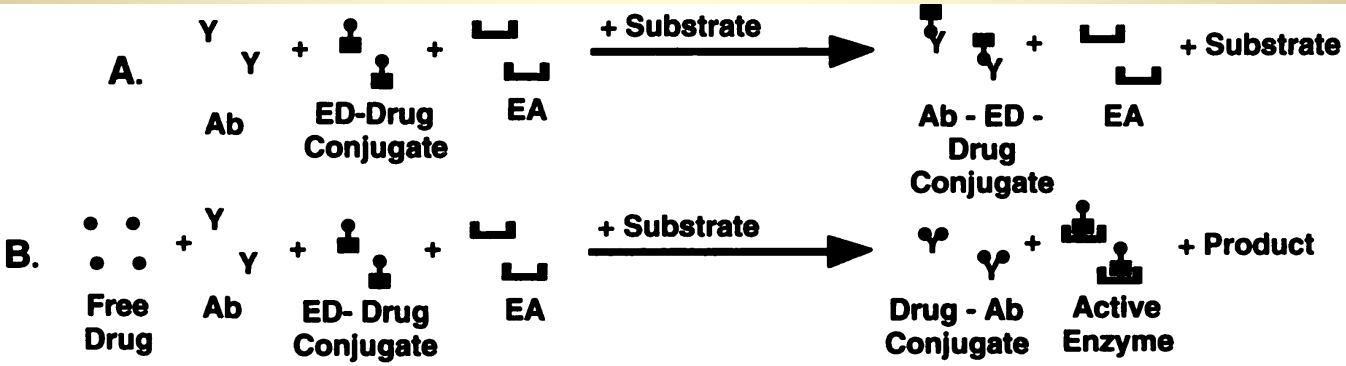


Fig. 1. Principle of the CEDIA (cloned enzyme donor immunoassay) methodology.

(*A*) In the absence of free drug or drug metabolite in a urine specimen, formation of a complete tetrameric β-galactosidase enzyme is inhibited, and no product is generated after addition of substrate to the reaction mixture. (*B*) Drug or drug metabolite in a urine specimen competes with the enzyme donor (ED)–drug conjugate for anti-drug antibody (Ab) binding sites; complete, active β-galactosidase molecules are formed in proportion to the amount of drug or drug metabolite present, and the conversion of substrate to product is also proportional to the drug concentration in the specimen. EA, enzyme acceptor.

Importance of cross-reactivity and matrix effects!

Detection Thresholds

- In most analyzers, antibody reactivity results in a proportional spectrophotometric signal.
  - UV/Vis absorption
  - Fluorescence
  - Chemiluminescence
- Each assay is calibrated using a single drug solution at a fixed concentration (e.g. 300 ng/ml morphine).
  - Reactivity greater (less) than this threshold is considered positive.
  - Reactivity less (greater) than this threshold is considered negative.
  - Note that signal quickly becomes non-linear at concentrations above the detection threshold.
- Many factors alter the reactivity of clinical samples.
  - Variable reactivity of similar drugs (e.g. codeine, hydrocodone, oxycodone, etc.).
  - Conjugated metabolites generally have poorer reactivity (e.g. glucuronidated opiates).
  - Unrelated cross-reacting drugs can add (or subtract) to the final signal.
  - Matrix effects: although clinical samples are diluted (1:20 – 1:40) into standard solutions, variations in urine components can affect final signal.
    - pH, salt concentration, protein, enzyme inhibitors, chromatographic molecules, etc.

Detection Thresholds

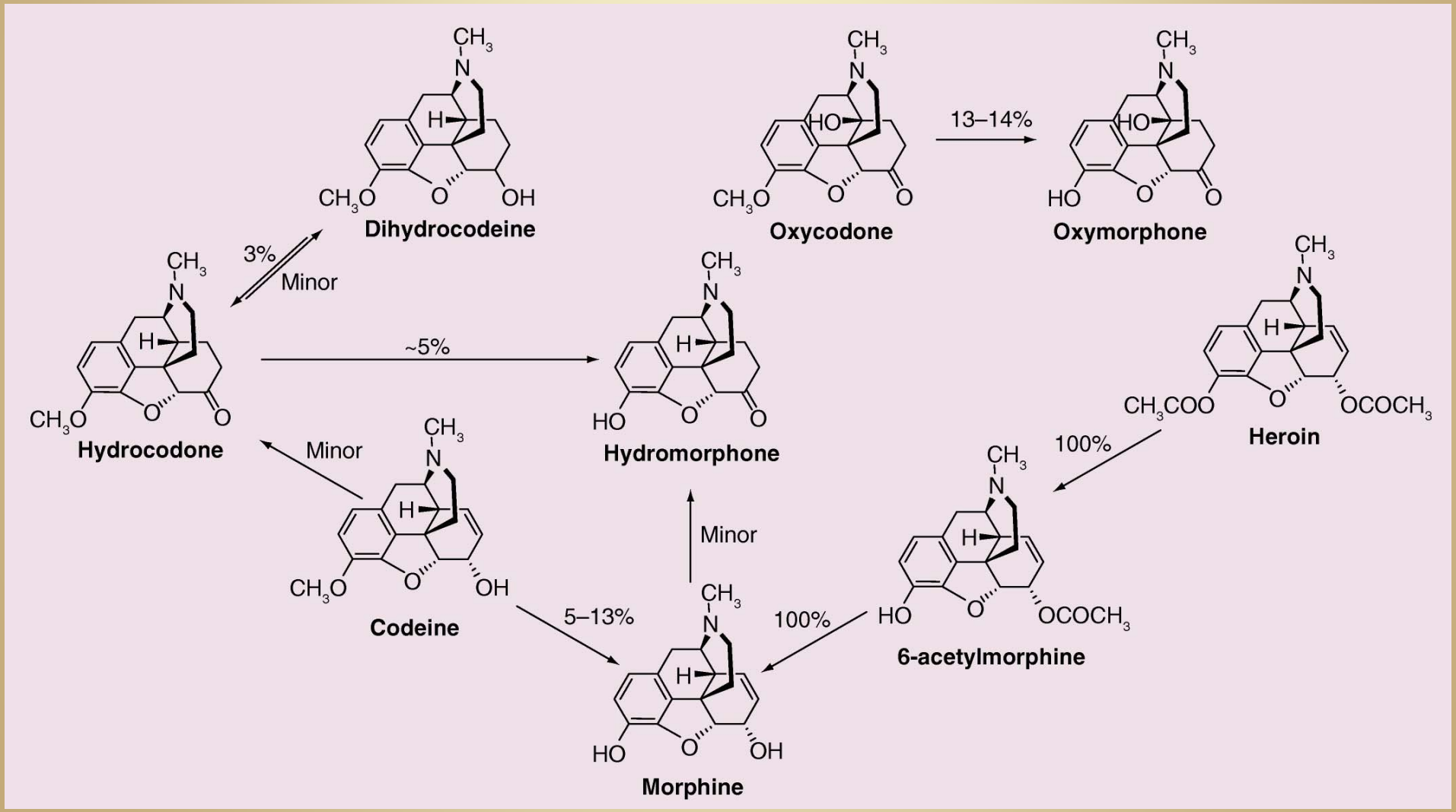
Assay	Calibrator
Cocaine	300 ng/ml benzoylecognine (BZE)
Opiates	300 ng/ml morphine
Oxycodone	100 ng/ml oxycodone
Methadone	300 ng/ml methadone
Barbiturates	200 ng/ml secobarbital
Benzodiazepines	200 ng/ml oxazepam
Amphetamines	1000 ng/ml methamphetamine
Phencyclidine	25 ng/ml phencyclidine (PCP)
Cannabinoids	50 ng/ml 11-nor-Δ9-THC-9-carboxylic acid

Opiate Detection Limits

Opiate	Minimum Detection Limit (in water)
Morphine	300 ng/ml (calibrator)
Codiene	12.5 ng/ml
Hydrocodone	400 ng/ml
Hydromorphone	700 ng/ml
Meperidine	160,000 ng/ml
Oxycodone	26,000 ng/ml
Oxymorphone (estimated)	(>100,000 ng/ml)
Morphine-3-glucuronide	1050 ng/ml

- Also need to consider
  - Dosage / Dosing Interval
  - Metabolism
  - Urine Concentration

Opiate Metabolism



Opiate Metabolism Profiles

1. ***Pharmaceutical Morphine:*** Morphine and *sometimes* oxymorphone when the morphine is very high.
2. ***Codeine:*** Codeine, morphine and norcodeine/norhydrocodone (can't distinguish these two in our GC/MS method due to derivatization).
3. ***Hydrocodone:*** Hydrocodone, hydromorphone and norhydrocodone/norcodeine.
4. ***Hydromorphone:*** Hydromorphone.
5. ***Oxycodone:*** Oxycodone and oxymorphone.
6. ***Heroin Use:*** 6-monoacetylmorphine (6-MAM), morphine, codeine, and *sometimes* oxymorphone (same as with morphine).
7. ***Poppy seeds:*** Morphine and codeine. Difficult to distinguish past heroin use from poppy seed ingestion. NIDA recommends the following criteria.
   - If total morphine > 5000 ng/ml (maybe 10,000 ng/ml better), or codeine > 300 ng/ml,
   - or the morphine:codeine ratio < 2,
   - or total morphine > 1000 without any codeine present,
   - or the presence of any 6-MAM (specific for heroin),
   - then poppy seed ingestion is ***NOT likely*** the sole source of the urine opiates.

DAU Performance Summary

1) Opiates
   - Detects multiple opiates with varying sensitivity.
   - Poor sensitivity for oxycodone and meperidine
   - Does not detect methadone, propoxyphene or fentanyl at all
   - Fluoroquinolones reported to cross-react.

2) Methadone
   - Good sensitivity and specificity
   - Does not detect any opiate

3) Oxycodone
   - Very sensitive and specific

4) Benzodiazepines
   - Poor sensitivity for Ativan (lorazepam) and Xanax (alprazolam)
   - Good sensitivity for older benzodiazepines that are primarily metabolized to oxazepam
   - See hodsdon.com/wiki for more details, including metabolism pathways.
   - Oxaprozin (Daypro) reported to cross-react (rare)

1) Barbiturates
   - Good sensitivity and specificity

6) Cocaine (BZE)
   - Good sensitivity and specificity

7) Amphetamines
   - Depending on the assay, many drugs cross-react.
   - Anti-histamines most common at YNHH

8) Phencyclidine
   - Common cross-reactivity with dextromethorphan (metabolite)

9) Cannabinoids
   - Sensitivity to inactive metabolites vary; sometimes positive for weeks to months
   - Good specificity

Should Urine Opiates be Positive?

- 45 year old woman has been taking 10 mg oxycodone tabs every four hours (6 doses per day) for past few months for treatment of chronic pain.
- Oxycodone ~ 75% bioavailable and $t_{1/2}$ ~ 2 – 3 hours.
- At steady state, $Rate_{in} = Rate_{out,}$ divided into an average daily urine output of ~ 1.5 liters.
- (10 mg x 6/day x 0.75) / 1.5 liters = 30 mg/L
  - 30 mg/L = 60 x 10^6 ng / 10^3 mL = 30,000 ng/mL
  - If it is excreted entirely as unchanged oxycodone

Should Urine Opiates be Positive?

- However, studies have shown that oxycodone is excreted as
  - ~20 - 25% free oxycodone
  - ~ 50% conjugated oxycodone
  - ~ 20 - 25% free and conjugated (almost all) oxymorphone
- Relative cross-reactivity estimated as
  - 1/2 for conjugated oxycodone and
  - 1/8 for total oxymorphone.
- Effective, average signal can be calculated:
  - (0.25 x 30,000)/1 + (0.5 x 30,000)/2 + (0.25 x 30,000)/8 =
  - 7,500 / 1 + 15,000 / 2 + 7,500 / 8 =
  - 15,938 ng/mL

Should Urine Opiates be Positive?

- 16,000 ng/mL is below the threshold for detection for our Opiate EMIT at YNHH (~26,000 ng/mL).
- With an average urine output, this patient should generally be negative throughout her dosing cycle.
- However, what if she is dehydrated and her urine is maximally concentrated?
  - Minimal daily urine volume is 0.4 – 0.5 L, or ~ 1/3 of our '1.5 L'.
  - 16,000 ng/mL x 3 = 48,000 ng/mL
  - This is well above our detection threshold. Hence, a positive result is possible and does NOT necessarily imply presence of another opiate, such as morphine.
- Note that at higher doses such as 20 mg q4 hours, resulting in 32,000 ng/mL average reactivity, a positive result is much more likely.
- A sensitive and specific urine oxycodone assay was introduced a few years ago with a 100 ng/mL detection threshold.

Can Urine Oxycodone Ever be Negative?

- With its much lower detection threshold, can the urine oxycodone assay ever be ***negative*** in this patient?
  - Implication is that the patient is non-compliant (at best) or diverting (at worst) the prescribed opiates (e.g. selling them on the street).

- Safe to use the same 'relative' reactivity of ~16,000 ng/mL in an average, daily urine volume of 1.5 L.

- In this case, what if she is over-hydrated and her urine is maximally diluted?
  - Maximal daily urine volume is 12 – 15 L, or 8 – 10x of our '1.5 L'.
  - 16,000 ng/mL / 10 = 1600 ng/mL
  - This remains well above our detection threshold for our oxycodone assay (but notice is much lower than detection threshold in the opiate assay).
  - ***Therefore, a negative result in the oxycodone-specific assay implies a degree of non-compliance to some extent.***

How long should it be positive?

- Very common question, but not easy to answer.
- Two categories of research studies:
  - Drug administered to healthy, non-abusing volunteers and urine samples monitored (often less than 24 hours).
  - Daily urine samples collected from an inpatient drug rehabilitation facility (days to weeks).
  - Summarized for each drug in handout.
- Major difference is the accumulation of inactive metabolites in tissue (especially if use is much more frequent than elimination half-life, $t_{1/2}$).
- Also affected by all the previous considerations of variable urine concentration, reactivity of metabolites, and detection thresholds.

Difficulty with the Interpretation of Urine Drug Concentrations

- Though they generally trend towards lower values, the absolute concentrations of excreted compounds can vary and identification of "new use" is problematic.

- As the figure demonstrates, improved performance can be provided by normalizing drug levels to urine Cr.

- However, this is not common practice and firm guidelines have not been established.

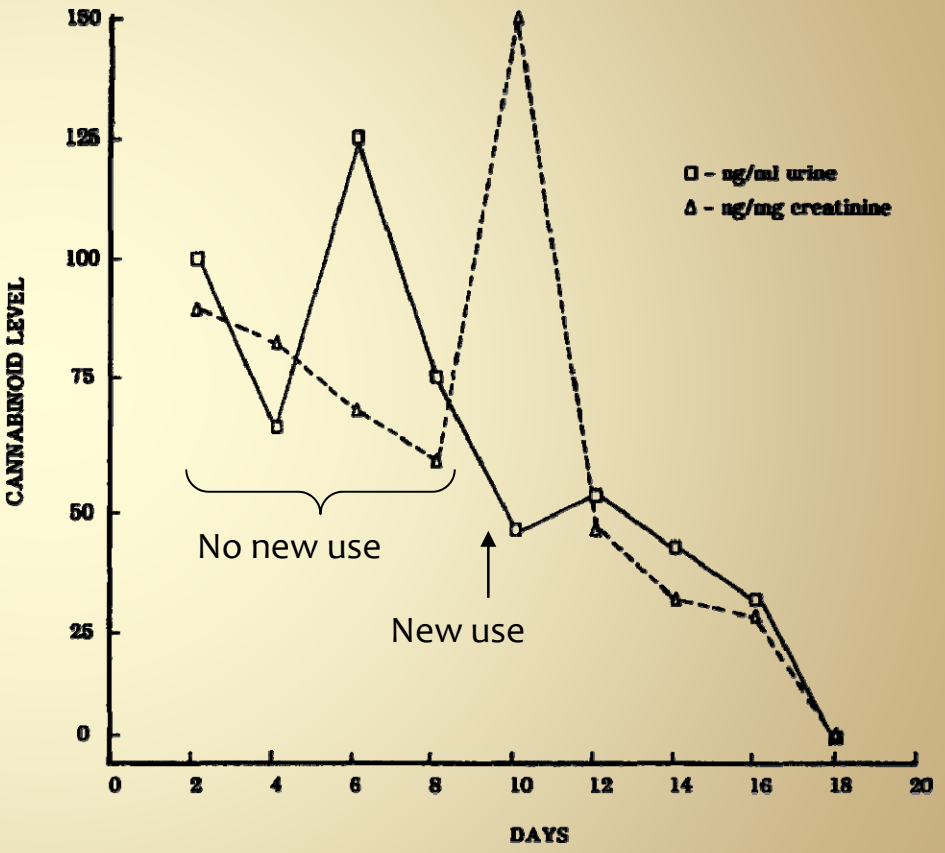


Figure 1. Urinary cannabinoid levels of specimens taken on alternate days after last marijuana use. □ – concentration of THC metabolite in ng/ml urine. Δ – THC metabolite concentration divided by the creatinine concentration expressed in ng metabolite/mg creatinine.

From NIDA Monograph: Urine Testing for Drugs of Abuse

Current versus Past Use

- a case history

- A patient with a known history of IV drug abuse was admitted three days ago for treatment of deep vein thrombosis (DVT) with anticoagulation therapy.
- After returning from a smoking break, the patient had slurred speech, difficulty walking and 'pinpoint' pupils. The clinicians suspected heroin use and collected urine for drug screening.
- Immunoassay results:
  - opiates positive, all other drugs negative.
- Is this consistent with acute heroin intoxication?

Current versus Past Use

- a case history

- In a chronic heroin abuser, conjugated opiate metabolites can be found in urine for many days.

- If we have a previous urine sample for comparison (e.g. from before going outside), we could compare its relative reactivity with the current sample, but this is ***very risky***.

- Instead, we can search for evidence of 'active' opiates in either blood or urine.
  - Heroin is 'diacetylmorphine', which is rapidly deacetylated to 6-monoacetylmorphine (6-MAM).
  - 6-MAM is more slowly metabolized to morphine (both active),
  - And morphine is slowly glucuronidated to inactive forms (mostly).

- Blood levels should directly correlate with clinical effect, but testing is not generally available. Instead, can use GC/MS confirmation method on urine to identify presence of 6-MAM or unconjugated morphine.
  - 6-MAM should only be detectable in urine for ~ 6 hours after use, but can disappear much faster.
  - Unconjugated morphine should be detectable for up to ~ 12 hours after use.
  - GC/MS method normally includes a 'hydrolysis' step to remove the glucuronide, which is required for detection (glucuronidated compounds are not sufficiently volatile).
  - In this case, we can compare the GC/MS results with and without the hydrolysis step to measure total and unconjugated morphine.

Current versus Past Use
- a case history

- In this case we detected ~500 ng/ml morphine in the ***hydrolyzed*** urine.

- We did not detect any 6-MAM in either sample, nor any opiate in the non-hydrolyzed sample.

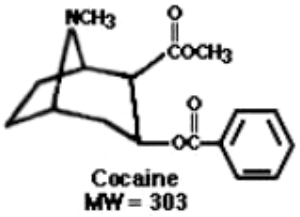
- Conclusion: ***no*** opiate use in past 12 hours.

- Later that evening when we reported these results to the clinicians, we found out that the patient's mental status had rapidly deteriorated; after being transferred to the ICU, a CT scan revealed a large subdural hematoma.

Current versus Past Use

- 6-monoacetylmorphine is highly specific for acute heroin use.
- Unconjugated opiates can generally be detected for up to 12 hours after use.
- In contrast, their conjugated counterparts can be detectable for days.
- Similarly, ‘cocaine’ (the actual parent compound) can be detected for 6 – 12 hours after use.
- The inactive cocaine metabolite, benzoylecognine, is detectable for days to weeks.

Cocaine GC/MS



Cocaine
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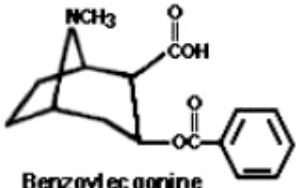

Benzoylecgonine
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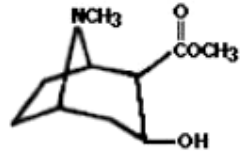

Ecgonine methyl ester
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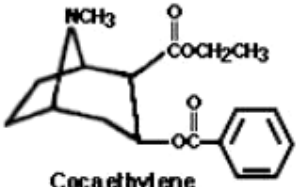

Cocaethylene
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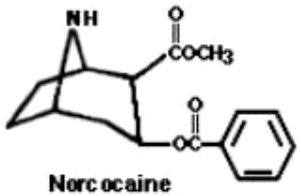

Norcocaine
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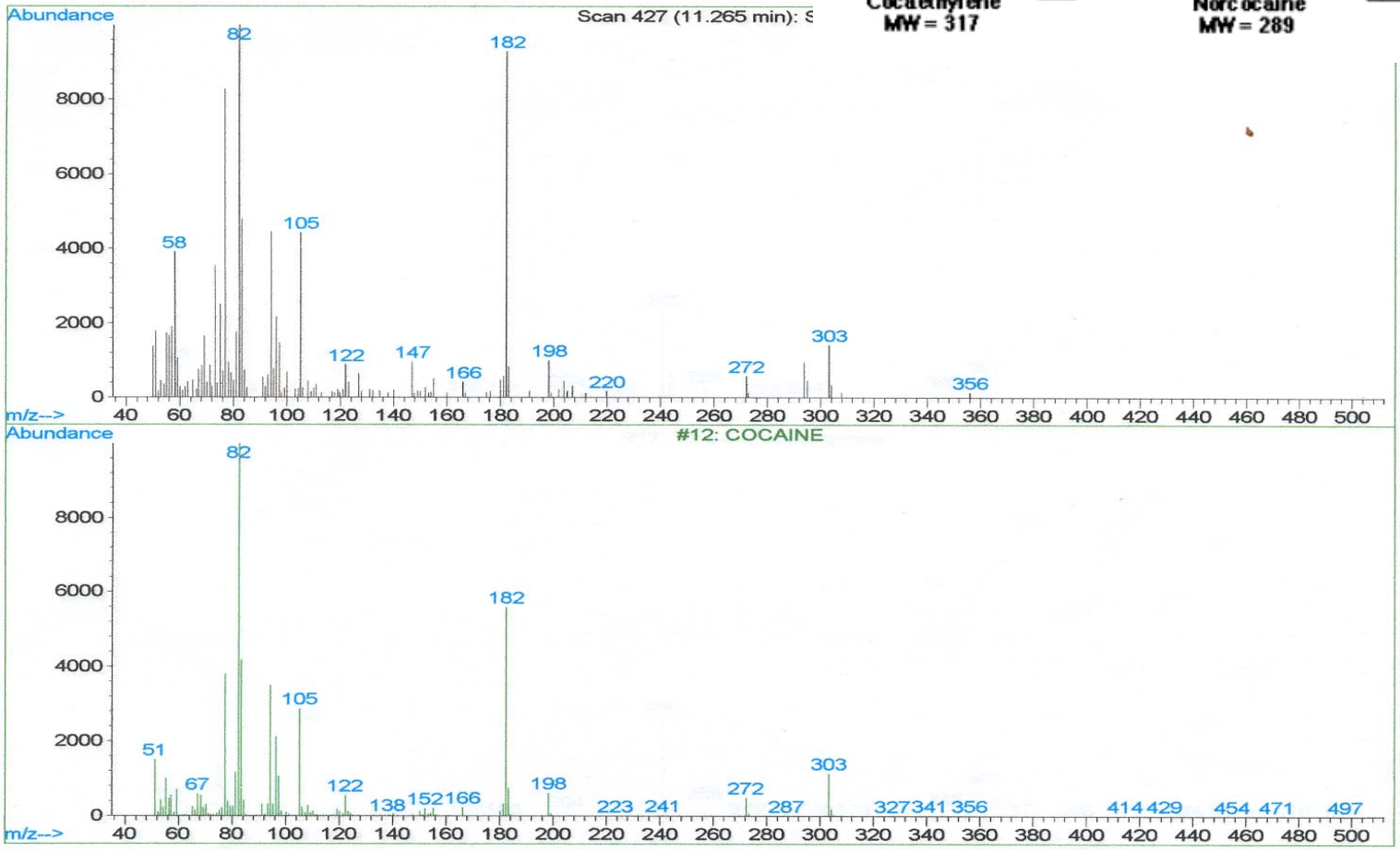
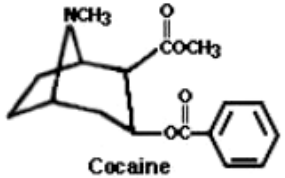


Benzoylecognine



Cocaine
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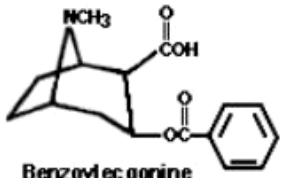

Benzoylecgonine
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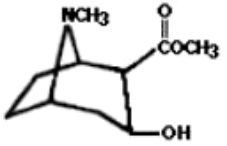

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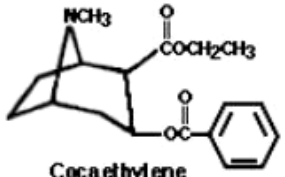

Cocaethylene
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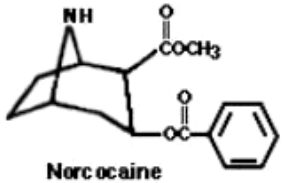

Norcocaine
MW = 289

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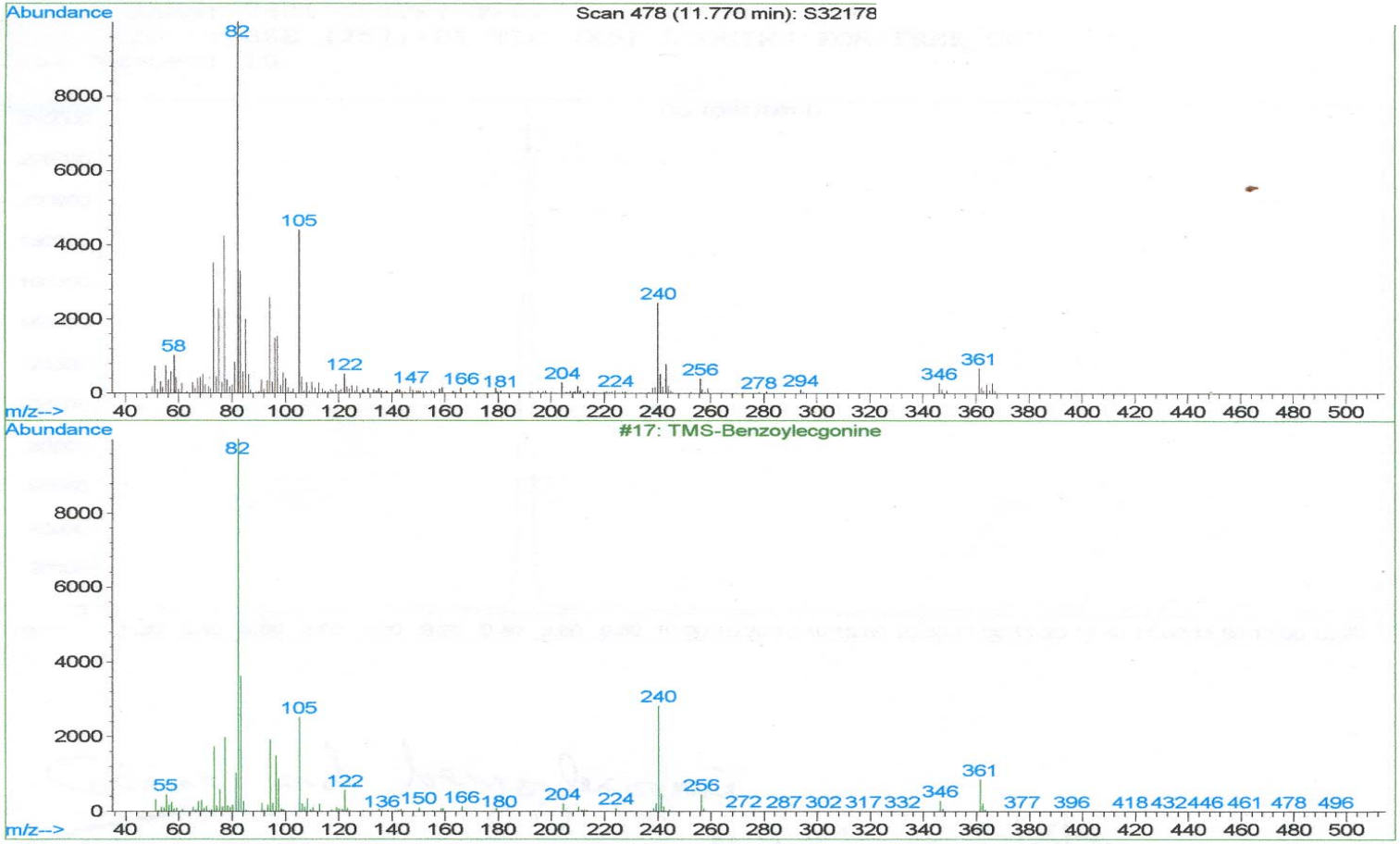
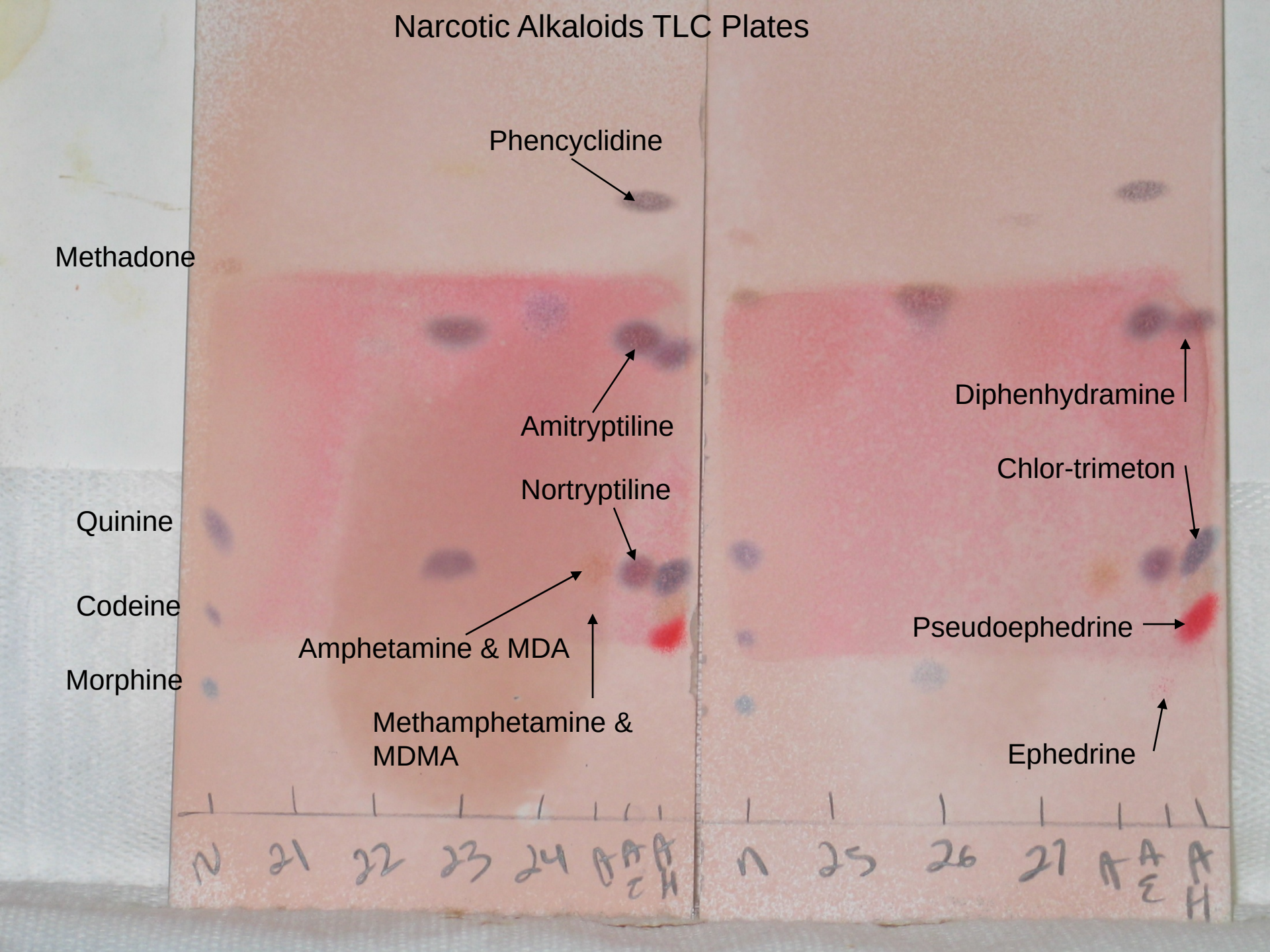


Narcotic Alkaloids TLC Plates



Finding Unexpected Drugs

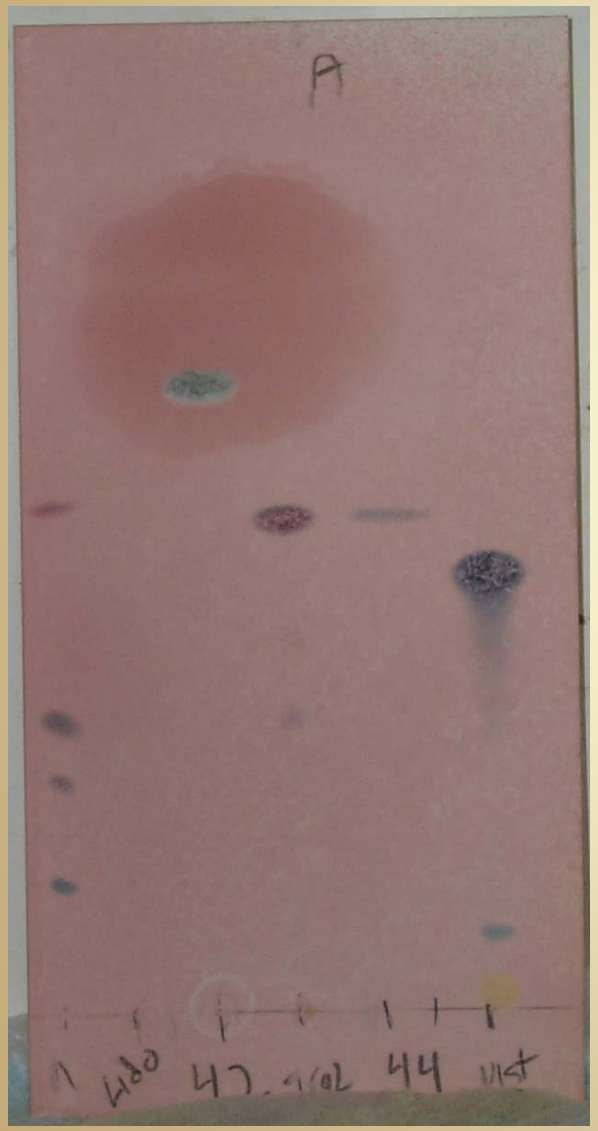
- **Urine Thin Layer Chromatography (TLC):** used for confirmation of DAU and also to "screen" for about 30 other drugs (when present in high or overdose concentrations).

- **Urine Gas Chromatography/Mass Spectrometry (GC/MS):** mainly for confirmation of DAU, also good for opiates, methadone, meperidine, "free" cocaine, PCP, and dextromethorphan.

- **HPLC of serum or urine by "TCA method":** good for a variety of drugs, especially TCAs, SSRIs, beta blockers, Ca-channel blockers, and benzodiazepines.

- Other specific drug assays intended for routine therapeutic drug monitoring.

Finding Unexpected Drugs



- A 45 y.o. male brought to ED with unexplained "acute respiratory distress".
- Patient intubated and unable to provide clear history. No family available.
- TLC of urine showed a "spot" recognized as consistent with lidocaine.
- Normally would request a sample of lidocaine from the pharmacy and run again as a control side-by-side with patient.
- However, since we have a plasma assay for lidocaine TDM, we were able to confirm an unusually high level.
- Respiratory distress/arrest is an unusual (<1%) adverse effect of lidocaine

Adulteration

- I don't know of any substance a person can ingest safely (i.e. non-toxic) that can 'mask' or interfere with drug screening immunoassays.
  - Except for a diuretic taken with lots and lots of water.
  - http://www.erowid.org/psychoactives/testing/testing.shtml
- However, there are LOTS of things one can add to a urine sample (*ex vivo*) that do interfere.
  - Strong acid, base, detergents (bathroom soap is #1), or any potent protein denaturant (after a 1:40 dilution).
  - Can also buy "clean urine" online.
- Basis for detection of an adulterated specimen is either to
  - test directly for the adulterant or
  - test if chemical characteristics of urine exceed physiologic limitations

Specimen Validity Testing

1. Temperature measured immediately after void
   - Should be between 32 – 38 °C (90 – 100 ° F)
   - Detects substitution with another urine sample
2. 3 < pH < 11
   - Detects acids, bases, and detergents (often change pH)
3. Concentration
   - A urine Cr < 2 mg/dL and a specific gravity < 1.001 is considered ***inconsistent*** with human urine.
   - A urine Cr < 20 mg/dL and a specific gravity < 1.003 is considered overly ***dilute***. May want to request a second, hopefully more concentrated, sample.
4. Specific additives monitored in forensic settings
   - Nitrites, Chromium, Peroxidase, Halogens, Glutarldehyde

Summary of Teaching Points

- Major difference between blood and urine testing.
- Characteristics of screening immunoassays and confirmatory methods.
- Applying detection thresholds to interpretation of patient results.
- Familiarity with general sensitivity and specificity characteristics of immunoassays.
- Challenges distinguishing current from past use.
- Adulteration and sample acceptability criteria.

References

- Goldfrank L. R. et al. (2006) *Toxicologic Emergencies*, 8th Ed. (Edited by Neal E. Flomenbaum, Lewis R. Goldfrank, Robert S. Hoffman, et al) Norwalk, CT: McGraw-Hill, New York.
  - In particular, Chapter 7 provides a very nice overview of the role of the clinical laboratory in the toxicologic evaluation of a patient. Chapters 64 – 73 cover specific classes of illicit substances.
- Karch, S. B. (2007) *Drug Abuse Handbook*, 2nd Ed. CRC Press, Taylor & Francis Group, Boca Raton, FL.
  - A weighty but readable tome on nearly all aspects of drug abuse clinical pathology.
- Golan, D. E. et al. (2005) *Principles of Pharmacology*, 1st Ed. Lippincott, Williams & Wilkins, Baltimore, MD.
  - Chapter 47 covers the broad area of toxicology, but does not spend much time on drugs of abuse. I include it as I know it is a textbook you have used in other courses.