Interpretation of analytical toxicology results for psychotropic substances

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- It is important in forensic pharmacology and toxicology to be aware of the extent to which samples can be interpreted on the basis of the known *pharmacology of a drug*.

Two-Step Testing Approach

- Screening test – designed to separate negative samples from samples that are "presumptively" positive

- Confirmation test – follow-up procedure designed to validate positive test results

  - distinctly different analytical technique
  - more *specific* and more *sensitive*

- When a biological drug screen is performed for clinical purposes, with the intention of detecting a specific drug, there are four possible outcomes:

- 1. True Positive (TP): The result of the test is positive, and the drug is present in the sample.
- 2. False Positive (FP): The result of the test is positive, but the drug is not present in the sample.
- 3. True Negative (TN): The result of the test is negative, and the drug is not present in the sample, or is present below the threshold concentration.
- 4. False Negative (FN): The result of the test is negative, but the drug is present in the sample above the threshold concentration.

Drug tests & cross reactivity

- Screening tests can and do react to "non-target" compounds
- Obtain list of interfering compounds from lab
- Initial screening ("instant" tests) are only 60-70% accurate
- Confirm positive results

Negative/none detected interpretation

- Client is not using a drug that can be detected by the test
- Client not using enough drug
- Client's drug use is too infrequent
- Collection too long after drug use
- Urine is tampered or adulterated
- Test being used not sensitive enough
- Client using drug not on testing list

Factors Influencing Detection Window

- Drug dose
- Route of entry into body
- Duration & frequency of use
- Rate of metabolism
- Testing sensitivity
- Specificity of testing method

Pharmacokinetics

- Pharmacokinetic processes of:

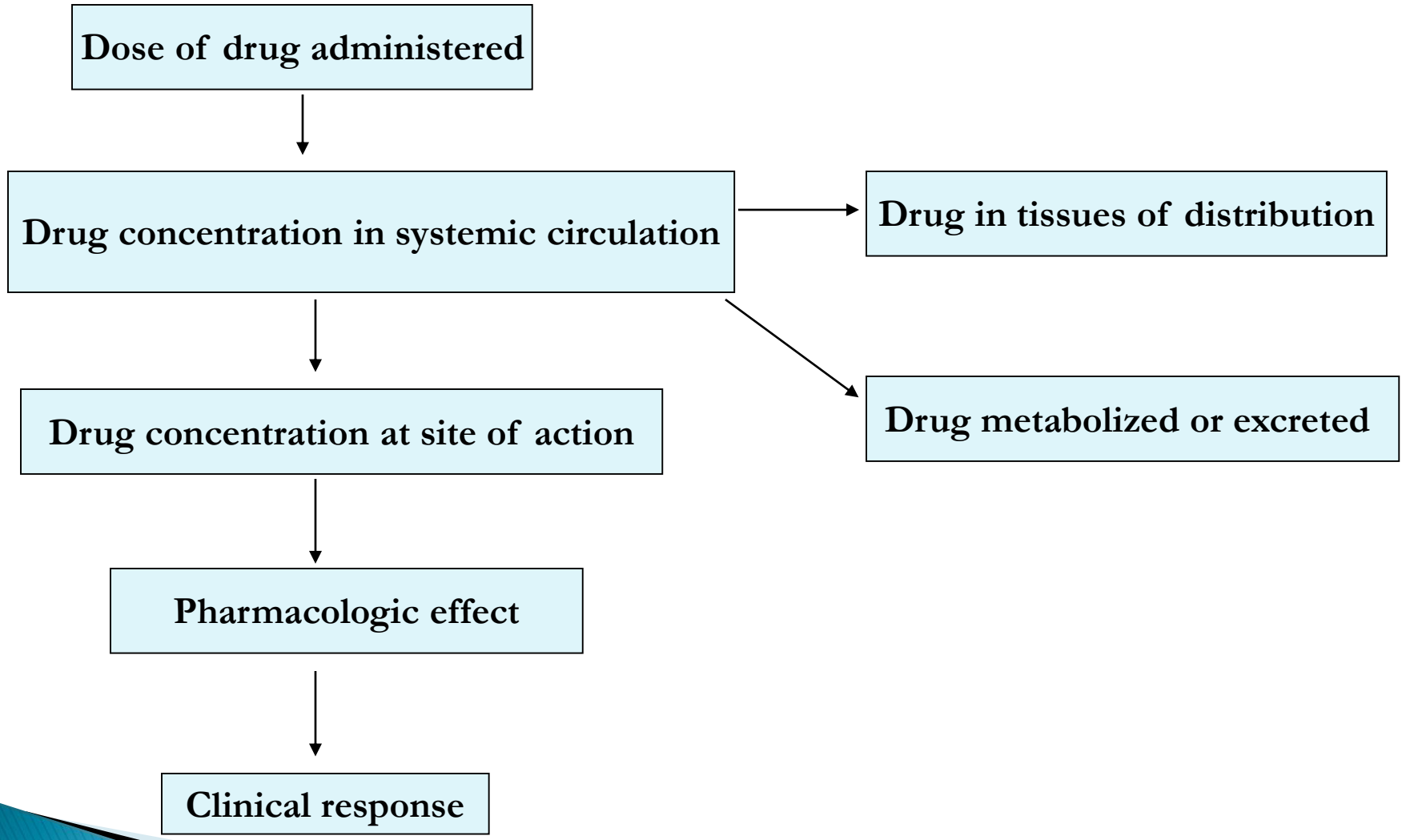
Absorption

Distribution

Metabolism

Elimination

determine *how rapidly* and for *how long* the drug will appear at the target organ.


Dose of drug administered
Drug concentration in systemic circulation
Drug in tissues of distribution
Drug concentration at site of action
Drug metabolized or excreted
Pharmacologic effect
Clinical response

Absorption

- Drug or poison absorption is an important process of pharmacokinetic.

- The route of administration is an important factor in determining the rate and extent of absorption.

Distribution of drugs into tissues

- The uptake of drugs into tissues depends on:

1) Blood flow to the tissue
2) The partition coefficient of the drug between blood and the tissue
3) The degree of ionization of the drug at the pH of plasma
4) Molecular size of the drug
5) Extent of tissue and plasma protein binding

Why is drug biotransformation necessary?

- Renal excretion plays important role in terminating the biological activity of drugs that have smaller volume and possess polar characteristics that are ionized at physiologic pH.
- Most drugs with lipophylic and unionized structure should be biotransformed to terminate their activity.
- Metabolites may be pharmacologically inactive or active.

Pathways of drug metabolism

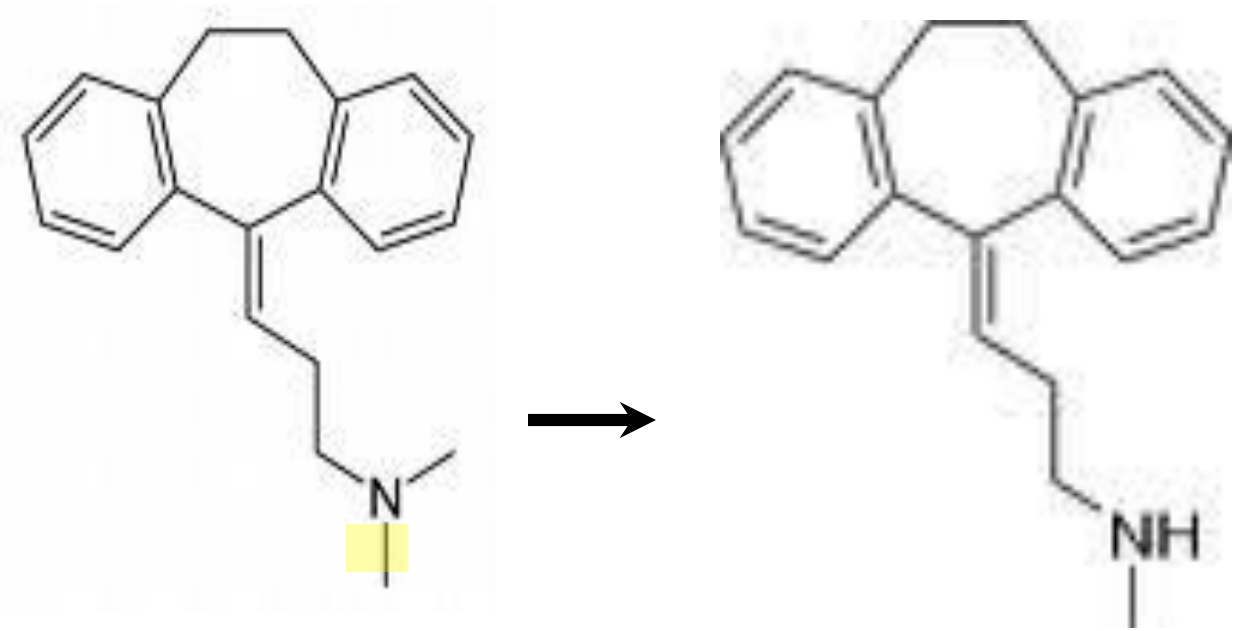
- Phase I reactions convert the parent drug to a more polar metabolite by introducing or unmasking a functional group (-OH, -NH, -SH).

- Phase II processes involve conjugation with glucoronic acid, acetylation, conjugation with amino acids and sulfate.

- Parent drugs or phase I metabolites undergo coupling or conjugation reactions with an endogenous substance to yield drug conjugates.

Metabolism of amitriptyline

- Phase I



Amitriptyline **Nortriptyline**

Amphetamines - Results Interpretation

- Screening tests - drug class assays
- Interpret positive results with caution
- Some screening assays often have cross-reactivity with structurally similar compounds:
- Fluoxetine
- Selegiline
- Ranitidine
- Trazodone
- Bupropion
- Chlorpromazine
- Promethazine
- Ephedrine
- phenylpropanolamine - PPA

Confirmation of positive results with sensitive and specific methods is necessary

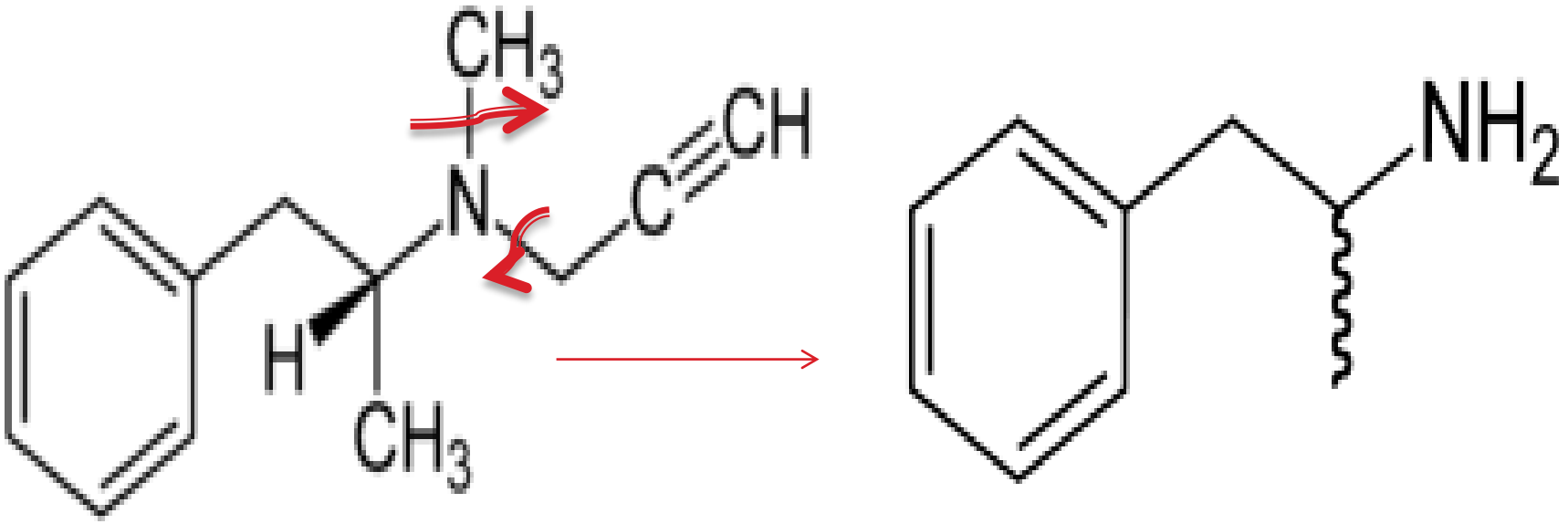
- Detection time: up to 4 days

Important note

- If anyone is prescribed and takes **selegiline**, they can and will test positive for amphetamine/methamphetamine on most drug tests.
- The prescription for **selegiline** would explain why they test positive for amphetamine/methamphetamine.

Selegiline metabolism to amphetamine

- The major metabolite of selegiline is (*R*)-methamphetamine.



Selegiline **Amphetamine**

Ritalin and amphetamine

- The concentration of methylphenidate or ritalinic acid, its major metabolite, may be quantified in plasma, serum or whole blood in order to monitor compliance, to confirm the diagnosis in potential poisoning victims or to assist in the forensic investigation in a case of fatal overdosage.
- Methyl phenidate produces a false-positive urine *amphetamine* screen tests.

Is methylphenidate mistaken for amphetamines?

- When laboratory screening procedures are followed by confirmation of potential positives by gas or liquid chromatography with mass spectrometric detection, methylphenidate will not be mistaken for amphetamine class compounds or vice versa, nor will the presence of methylphenidate in a urine sample mask abuse of amphetamines when **proper analytical methods** are used.

Metabolism of Methylphenidate vs. Amphetamine

Methylphenidate

Hydrolysis & Deesterrifcation

- Parahydroxy-methylphenidate
- * Ritalinic Acid

Amphetamine

Oxidative Deamination

Ring Hydroxylation

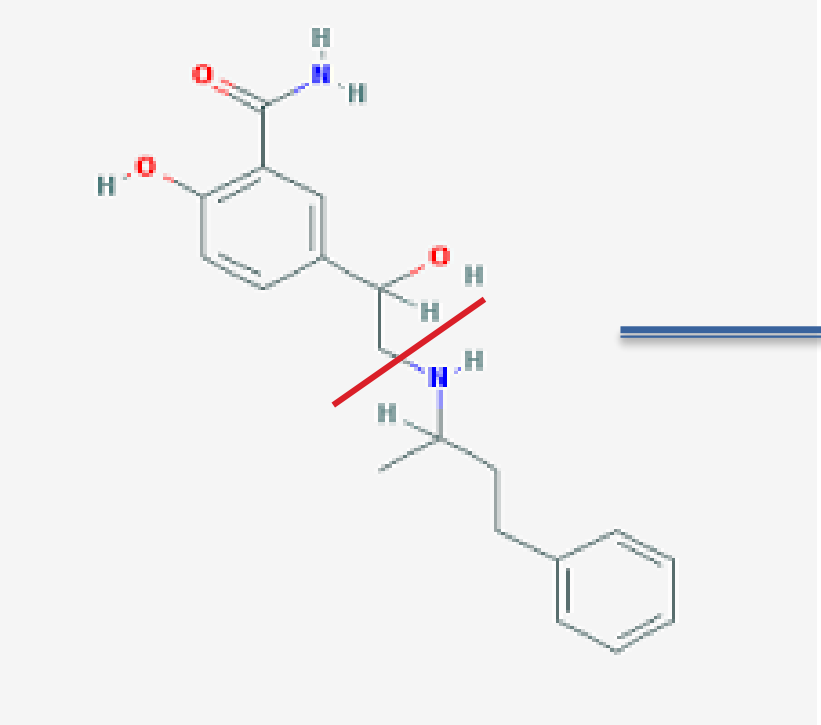
- 80% unchanged in urine
- Hipuric Acid
- Benzoic Acid
- Hydroxyamphetamine metabolite

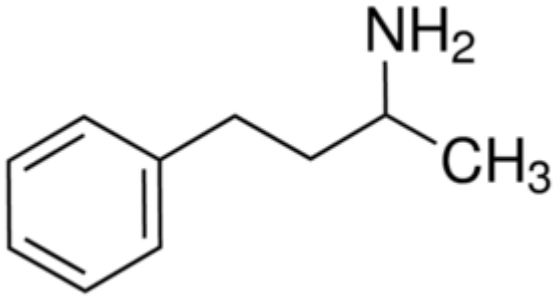
MPH does not usually show on routine urine drug screening

Labetalol metabolism

- Patients on labetalol can have a false positive amphetamines screen due to a metabolite of labetalol (1-Methyl-3-phenylpropylamine) structurally resembling amphetamine.
- In these cases, confirmatory testing will be negative.

Labetalol conversion to 1-Methyl-3-phenylpropylamine

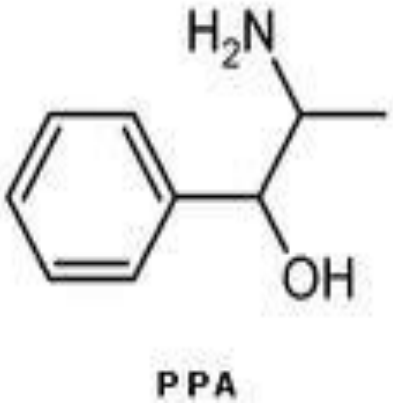




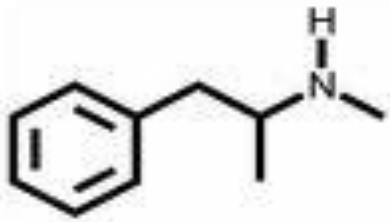
Labetalol

1-Methyl-3-phenylpropylamine

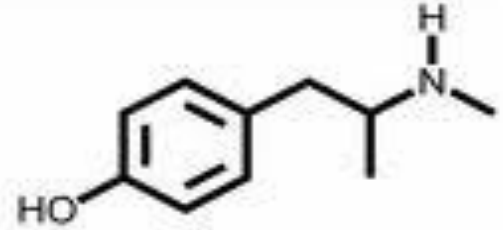
Similarities in chemical structure



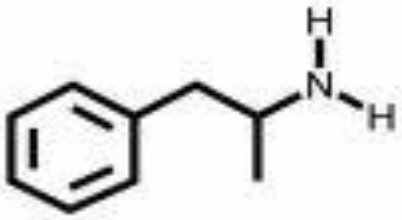
PPA



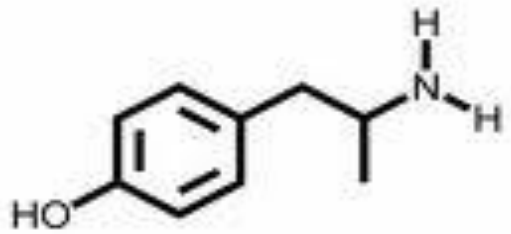
Methamphetamine



4-Hydroxymethamphetamine



Amphetamine



4-Hydroxyamphetamine

Expected duration for a positive urine drug screen

AMPHETAMINE	2 - 4 DAYS
METHAMPHETAMINE	2 - 4 DAYS
BARBITURATES (SHORT ACTING)	2 - 4 DAYS
BARBITURATES (LONG ACTING)	UP TO 30 DAYS
BENZODIAZEPINES	UP TO 30 DAYS
COCAINE	1 - 3 DAYS
HEROIN/MORPHINE	1 - 3 DAYS
MARIJUANA (CHRONIC USE)	UP TO 30 DAYS
MARIJUANA (OCCASIONAL USE)	1 - 3 DAYS
METHADONE	2 - 4 DAYS
PCP (CHRONIC USE)	UP TO 30 DAYS
PCP (OCCASIONAL USE)	2 - 7 DAYS

Results reporting

- Laboratory reports will provide a qualitative interpretation for the laboratory's specific panel of DOA, based on the testing laboratory's established cut-off concentrations.
- These cut-offs (usually given in ng/mL or µg/L) may or may not be listed on the laboratory report, but are readily available from the laboratory performing the testing.
- Qualitative results (positive/negative) will be reported for each requested drug.
- Some laboratories may provide semi-quantitative results for these drugs and may report the drugs' concentrations in ng/ml.

Cut-Off level definition

- The **defined** concentration of an analyte in an employee drug test specimen at or above which the drug test is called positive and below which it is called negative.

Items to be mentioned in results reporting

- Date of preparing test request.
- Date of admission to laboratory.
- Specimen type (urine, blood, etc.).
- Type of analytical technique used (immunochromatography, TLC, HPLC and GC/MS).
- Analysis result (analyte found: methamphetamine, amphetamine, …).

Interpretations of drugs of abuse test results

- Patient-specific factors such as weight, dose, level of hydration, time lag between drug ingestion and urine voiding, can influence the amount of drug excreted in the urine.

Pharmacokinetic properties, such as the **half-life** of the substance being detected and the individual rate at which the substance is metabolized by the patient, will also affect the ability of the tests to detect the drug(s) in question.

- When interpreting drugs of abuse test results, physicians need to consider:

☐ The level/concentration at which an individual drug is detected by the testing system (the cut-off concentration)

☐ The drugs that are detected by the testing system and more importantly those that are not detected and

☐ Substances that may give false-positive results (in immunoassays).

Concluding remarks

- All the available evidences must be taken into account when investigating any death or other incident where poisoning or drug use is suspected.

- Interpretation of analytical toxicology results must be made in the knowledge of the possible effects of:

  - The time elapsed since drug use
  - Site of sample collection
  - Analyte stability