FORENSIC TOXICOLOGY:

FROM CRIME SCENE TO VIRTUAL LAB

MODULE 2

CHAPTER 1: Sample Collection and Storage

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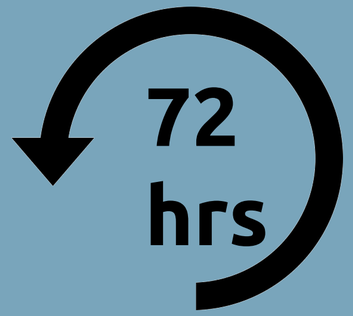
01. COLLECTION

01.

BLOOD

Blood samples from the deceased, suspect, or victim will always be collected from a **medical professional**.

If possible, a **Forensic Blood Collection Kit** should be used. If unavailable, collect blood using **two 10 mL grey-stoppered vacuum tubes**. Ensure all vials are sealed and labeled correctly.

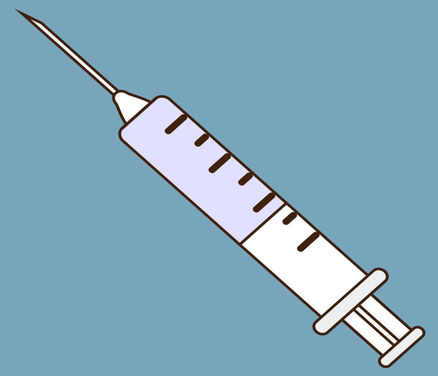


For **drug analysis**, blood samples must be collected no more than **72 hours** after the incident

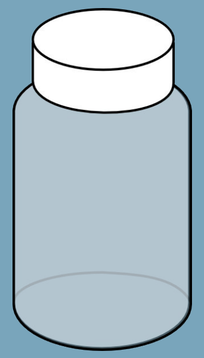
For **alcohol analysis**, blood samples must be collected no more than **24 hours** after the incident

01. SYRINGES, PIPES, POWDERS, DRUGS OR DRUG RESIDUE

Drug paraphernalia can include **syringes, pipes, powders, tablets, capsules,** or **other residues**. These can be collected and submitted for analysis



For **sharp objects** such a syringes and pipes, package separately in a **puncture-resistance container,** seal and label



For **powders, tablets or capsules,** place in a **plastic or glass vial,** seal and label

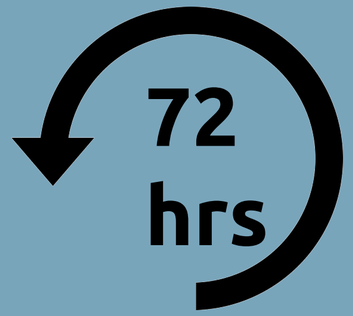
All drug paraphernalia should be treated as **biohazardous** and labeled as such

01.

URINE

Urine samples should be collected in a leak proof **urine collection cup.** Ensure it is sealed, closed tightly, and labelled.

Urine samples can also be collected in 10 mL **grey-stoppered vacuum tubes**, sealed and labeled

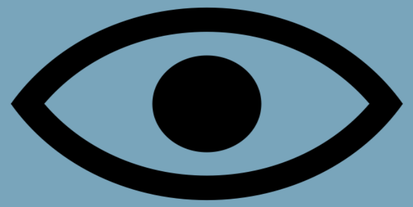


For **drug analysis**, urine samples must be collected no more than **72 hours** after the incident

For **alcohol analysis**, urine samples must be collected no more than **24 hours** after the incident

01.

VITREOUS HUMOUR



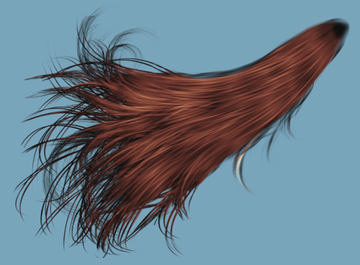
Vitreous humour is the **transparent, jelly-like tissue** behind the lens that fills the eyeball

The vitreous humour is collected **during the autopsy** by a medical professional in a 10 mL **grey-stoppered vacuum tube**. Ensure the tube is sealed and labeled.

The sample should be **refrigerated** and submitted for testing **as soon as possible**

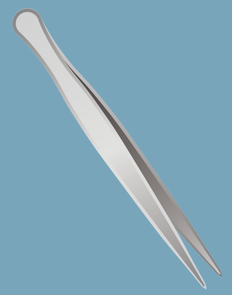
01.

HAIR



From a known source: the next preferred comparison sample after blood and buccal samples

When possible, known samples should be collected using the RCMP's hair collection kit, which has been specifically prepared for **DNA sample collection.**



Approximately **6-8 hairs** should be **pulled** from the scalp (NOT CUT)

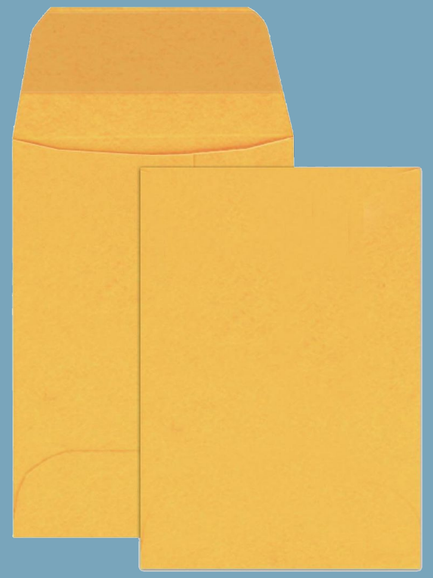
Place hair in a folded sheet of paper and place in an **envelope or plastic bag**. **Package**, **seal** and **label separately**

01.

HAIR

From an unknown source: If hair is recovered from a crime scene, they may be able to be submitted for DNA analysis

Place hair in a folded sheet of paper and place in an **envelope or plastic bag**



Package, seal and label **each sample separately**

02. STORAGE

02.

TEMPERATURE GUIDELINES

20°C

Fully dried exhibits can be stored at room temperature provided it is **not excessively hot or humid.**

-18°C

The **best storage** for most exhibits is a freezer. **Liquid samples** and **human tissue** should be stores in a frozen state where possible.

4°C

If a freezer is unavailable, a fridge can be used. Biological samples for **toxicological analysis** should be **refrigerated immediately** to prevent the potential **breakdown or loss of drugs.**

02.

TEMPERATURE GUIDELINES

Frozen: temperature maintained at or below -10°C

Refrigerated: temperature is maintained between 2°C and 8°C with less than 25% humidity

Temperature controlled: temperature maintained between 15.5°C and 24°C with less than 60% humidity

Room temperature: ambient temperature, may lack temperature and humidity control methods

02.

SHORT-TERM STORAGE CONDITIONS

Type of Evidence[2]	Frozen	Refrigerated	Temperature Controlled	Room Temperature
Liquid Blood[3]	Never	Best	Less than 24 hours	
Urine	Best	Less than 24 hours		
Dry Biological Stained Item[4]			Best	Acceptable
Wet Bloody Items (if cannot be dried)	Best	Acceptable	Less than 24 hours	
Bones	Acceptable		Acceptable	Acceptable
Hair			Best	Acceptable
Swabs with Biological Material		Best (wet)	Best (dried)	
Vaginal Smears			Best	
Feces	Best			
Buccal Swabs			Best	Less than 24 hours

02.

LONG-TERM STORAGE CONDITIONS

Type of Evidence[2]	Frozen	Refrigerated	Temperature Controlled	Room Temperature
Liquid Blood	Never	Best		
Urine	Best			
Dry Biological Stained Items			Best	
Bones			Best	
Hair			Best	Acceptable
Swabs with Biological Material			Best (dried)	
Vaginal Smears			Best	
Feces	Best			
Buccal Swabs			Best	
DNA Extracts	Best (liquid)	Acceptable (liquid)	Acceptable (dried)	

03. PRESERVATION

03.

PRESERVATION METHODS

Anticoagulants: prevent blood from clotting upon storage
E.g., potassium oxalate, EDTA, heparin, citrate

Antioxidants: sometimes used to prevent oxidative losses, but have the potential to act as reducing agents towards some drugs. For example, antioxidants can cause N-oxide metabolites to be transformed into the parent drug
E.g., ascorbic acid or sodium metabisulfite

Adjustment of specimen pH: generally not favoured routinely, because just as some drugs are alkali labile (cocaine, 6-acetylmorphine), others are acid labile

Sodium azide (a preservative): should not be used if samples are to be analyzed by ELISA because it can interfere with horseradish peroxidase-mediated colorimetric detection

Photolabile Specimens: should be stored in amber vials or foil-covered containers, or otherwise protected from direct sources of light

04. DEGRADATION

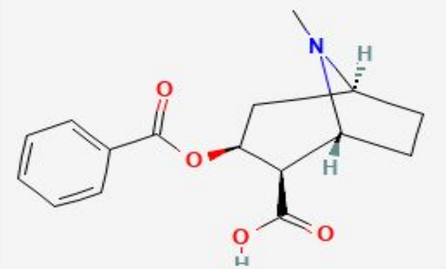
04.

HYDROLYSIS OF COCAINE

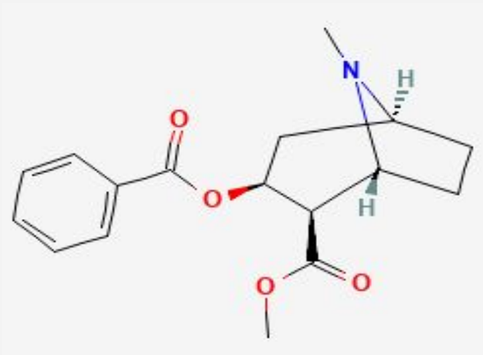
In **whole blood and plasma**, cocaine is **rapidly and extensively degraded** in vivo and in vitro

Cocaine generally undergoes hydrolysis via two mechanisms

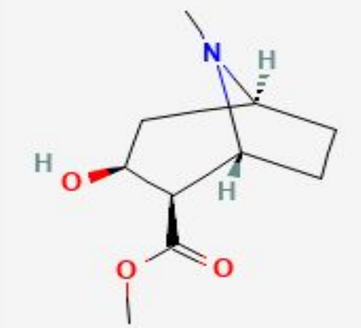
1. **Benzoylecgonine (BE)** is formed by the **chemical hydrolysis** of the methyl ester groups
2. small amounts of **ecgonine methyl ester (EME)** are formed **enzymatically by hydrolysis** of the benzoyl ester



Benzoylecgonine

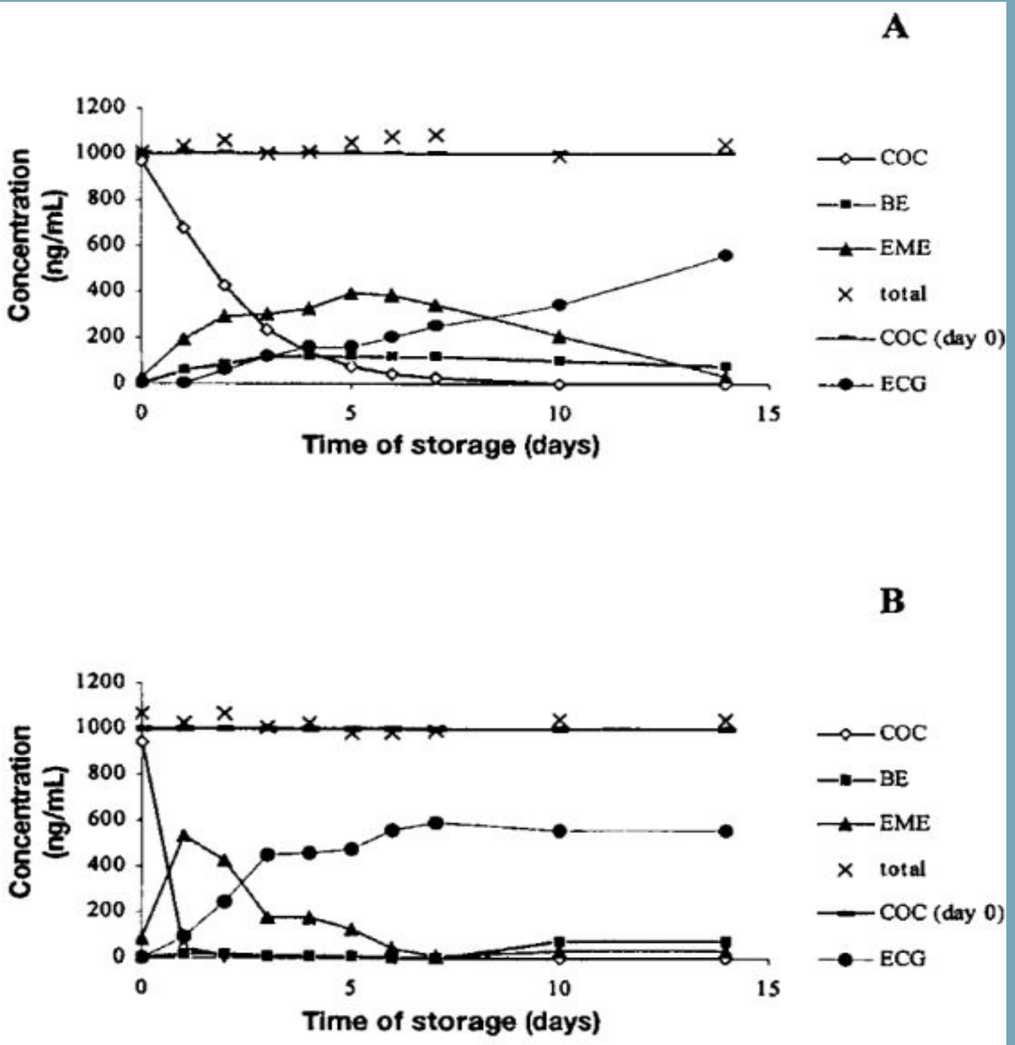


Cocaine



Ecgonine methyl ester

04. COCAINE HYDROLYSIS



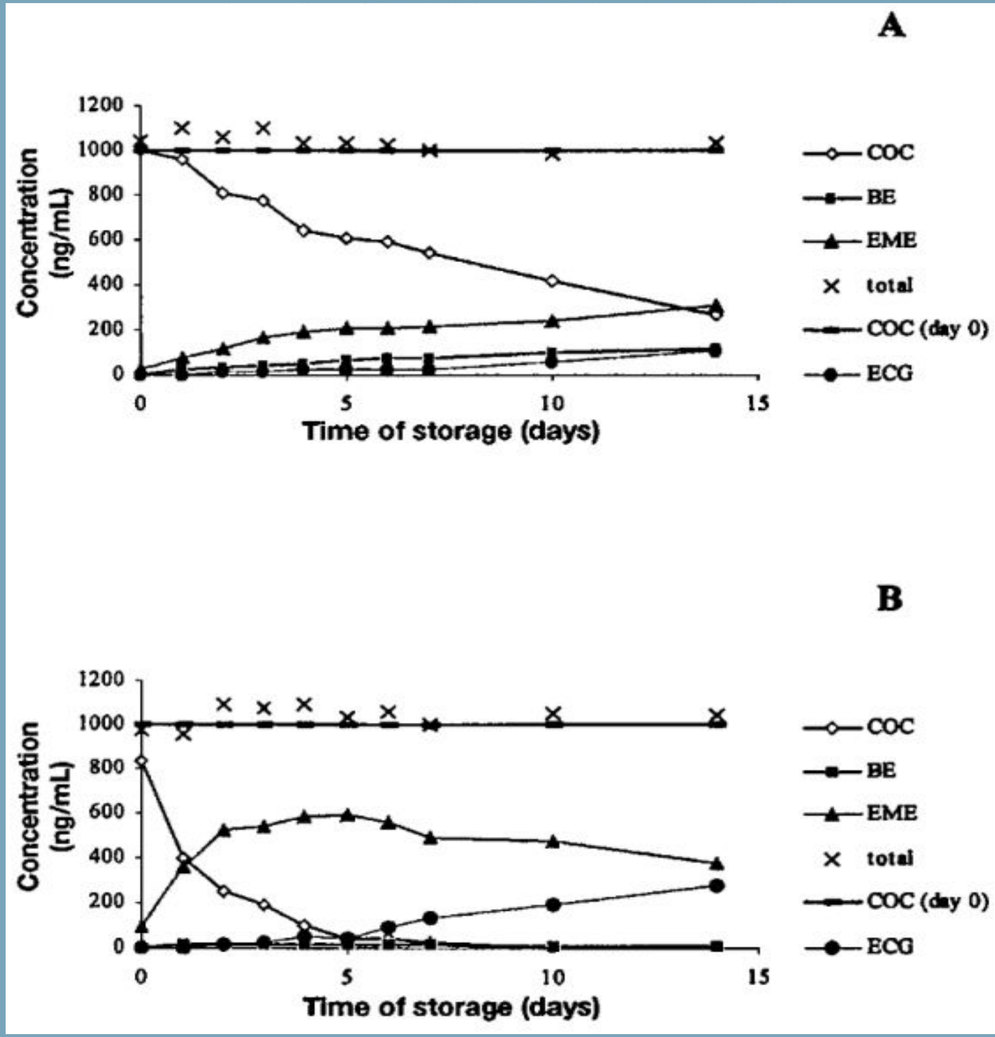
Changes in COC, BE, EME, and ECG concentrations. Storage temperature of 20°C: preserved plasma (0.25% fluoride) (A) and unpreserved plasma(B)

Findings:

- Cocaine (COC) had totally disappeared from unpreserved plasma on day 2
- COC was still detectable in preserved plasma on day 7
- The decrease in COC concentration was accompanied by an increase in the hydrolyzed species
- The predominant process was the enzyme-mediated conversion of COC to EME
- In unpreserve plasma, EME was formed in higher amounts and more rapidly compared to preserved plasma
- The concentration of ecgonine (ECG) was dependent on the concentration of EME, with its concentration continuously increased with the decrease of EME

04.

COCAINE HYDROLYSIS



Changes in COC, BE, EME, and ECG concentrations. Storage temperature of 4°C: preserved plasma (0.25% fluoride) (A) and unpreserved plasma(B)

Findings:

- COC was found to degrade more slowly at a storage temperature of 4°C
- In preserved plasma 27% of the initial concentration of COC was still present on day 14
- In unpreserved plasma, COC totally disappeared within 6 days of storage
- In unpreserved plasma, the rate of hydrolysis was similar to that obtained at a storage temperature of 20°C in preserved plasma

05. STABILITY

05.

CANNABINOID STABILITY IN BLOOD

WHY

In toxicological testing, drug stability is important when providing quantitative results and interpretation of findings

WHAT

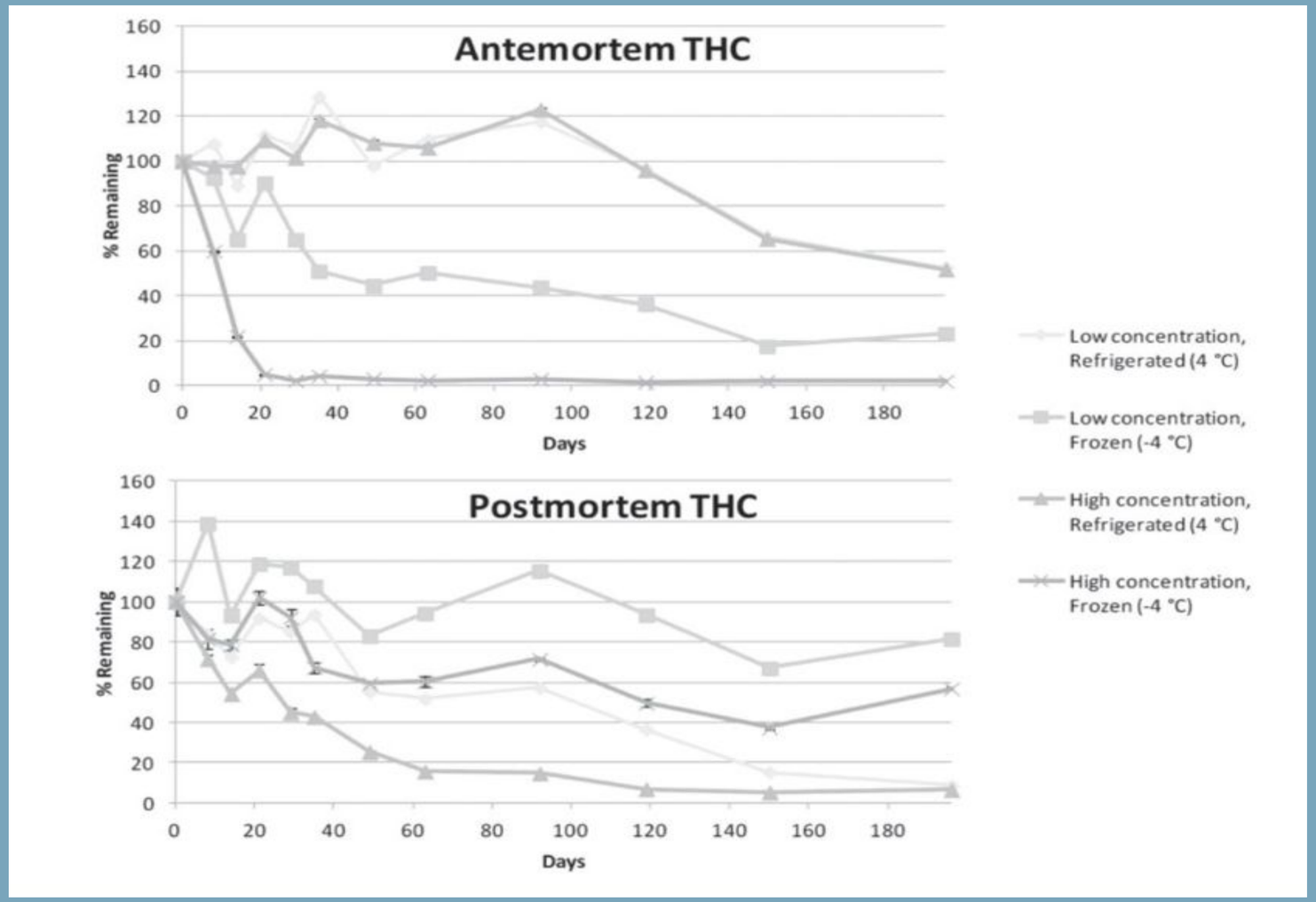
Stability of THC, 11-hydroxy-THC, 11-nor-9-carboxy-THC, Cannabinol and Cannabidiol in **antemortem** and **postmortem blood** was evaluated in **refrigerated (4°C)** and **frozen (-4°C)** storage conditions

RESULTS

Cannabinoids in **antemortem blood** were **more stable in refrigerated conditions than frozen conditions**, with 11-hydroxy-THC, 11-nor-9-carboxy-THC and Cannabinol having **more than 80%** of the original concentration remaining by the end of the study. Cannabinoids in **postmortem blood** showed **improved stability in frozen conditions** with THC, 11-hydroxy-THC, 11-nor-9-carboxy-THC and Cannabinol having **more than 80%** of the original concentration remaining

05.

CANNABINOID STABILITY IN BLOOD



Percent THC remaining after 196 days in storage

05.

CANNABINOID STABILITY IN BLOOD

Post mortem samples

- All showed a decrease in concentration at the end of the study periods
- In contrast to the AM samples, all refrigerated PM samples had less than 50% remaining of the original drug concentration while all frozen PM samples had more than 50% remaining after 196 days

Antemortem Samples

- Most refrigerated samples decreased over time, but all of the drugs had greater than 50% of the original concentration remaining
- After 196 days, only refrigerated Carboxy-THC and CBN met stability criteria (80% or more of initial concentration remaining)
- Refrigerated THC samples were stable for both concentration pools up to 119 days
- Refrigerated Hydroxy-THC bloods showed stability up to 119 days
- CBD samples stable until Day 120

Overall showed a decrease in concentration after 6 months, regardless of storage condition or type of blood

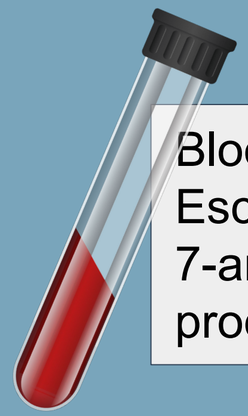
06. CANLII

06.

R.v. Nguyen

Ms. Elizabeth Hird is a Forensic Toxicologist Specialist
Ms. Hird is qualified to give expert testimony in the toxicology of drugs, specifically regarding the analysis of exhibits for drugs and/or alcohol, and pharmacology of drugs and/or alcohol, including the effects of drugs and/or alcohol in humans

She analyzed two biological samples taken from the complainant: a blood and a urine sample



Blood sample showed: Citalopram or Escitalopram, Tramadol and 7-aminoclonazepam (breakdown product of Clonazepam)

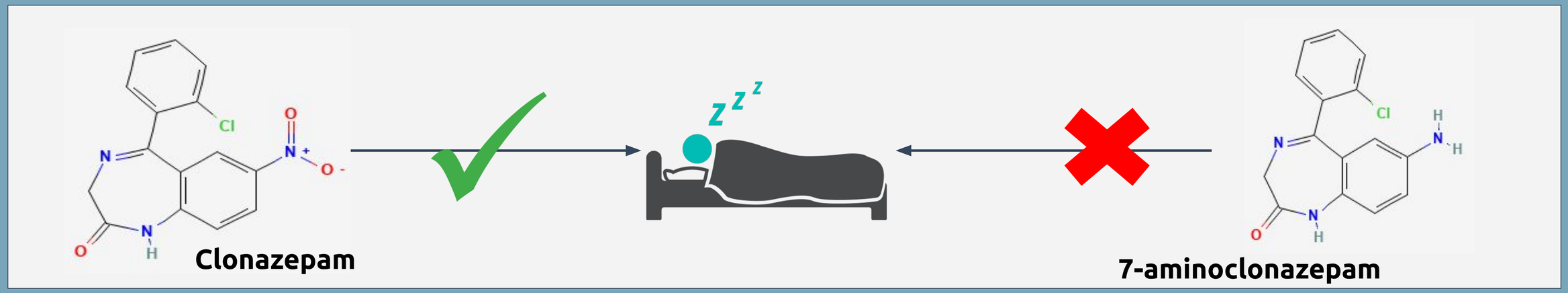
Urine sample showed: Citalopram or Escitalopram, Tramadol, 7-aminoclonazepam, Norquetiapine (a breakdown of the drug Quetiapine)

06.

R. v. Nguyen

Interpretation

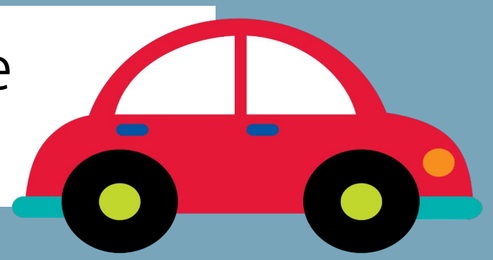
- 7-aminoclonazepam is an **inactive metabolite** of the drug Clonazepam
- Clonazepam is a drug used to treat seizures
- Because **only the metabolite is present**, it can mean that **Clonazepam was not used recently,** although Ms. Hird states she could not say with certainty that Clonazepam was not present when the sample was collected as it could have **broken down during storage**
- If Clonazepam **had been present** at collection of the sample, the side effects of the drug include **drowsiness and excessive sleepiness as well as anti-anxiety and relaxation.**



06.

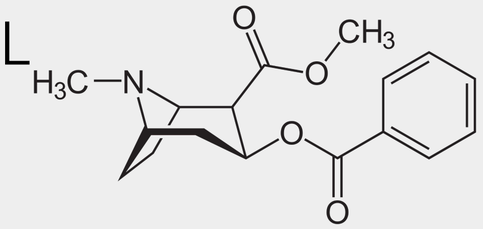
R. v Vanlerberghe

The accused, Mr. Vanlerberghe, was charged with criminal negligence causing death after he struck a pedestrian while driving



A blood sample was taken from Mr. Vanlerberghe approximately two hours after the incident. The sample was subsequently analyzed by Jeffrey D. Caughlin, a forensic toxicologist.

The blood was found to contain .03 ug/mL cocaine and 2.75 ug/mL benzoylecgonine. Methylecgonine was also present.



06.

R. v Vanlerberghe

Testimony of Jeffrey Caughlin

1. Peak blood levels after intranasal or intravenous injection range from **90 to 470 ng cocaine per mL blood**
2. Cocaine disappears from blood **within 3 to 10 hours** depending on the dose
3. Cocaine may **break down upon storage after sampling**
4. Therefore, blood cocaine levels **reported may be lower than those present at the time of sampling**
5. Benzoylecgonine arises in the body from **metabolism of cocaine** as well as upon **storage from the decomposition of cocaine**
6. Methylecgonine arises in the body from the **metabolism of cocaine**
7. Neither is pharmacologically active
8. Eithers' presence indicates **prior use of cocaine**, but it is not possible to accurately say at what time or to what extent based on their presence alone

07. REFERENCES

07.

REFERENCES

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2. NISTIR 7928 The Biological Evidence Preservation Handbook
3. Sampling, storage and stability
4. [PDF] Analysis of cocaine, benzoylecgonine, ecogonine methyl ester, and ecgonine by high-pressure liquid chromatography-API mass spectrometry and application to a short-term degradation study of cocaine in plasma. | Semantic Scholar
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7. R. v. Vanlerberghe, 1993 CanLII 231 (BC SC)