# FORENSIC TOXICOLOGY: FROM CRIME SCENE TO VIRTUAL LAB

## MODULE 2

### CHAPTER 3: Confirmatory Testing

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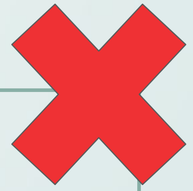
# INTRODUCTION

01.

# CONFIRMATORY TESTING

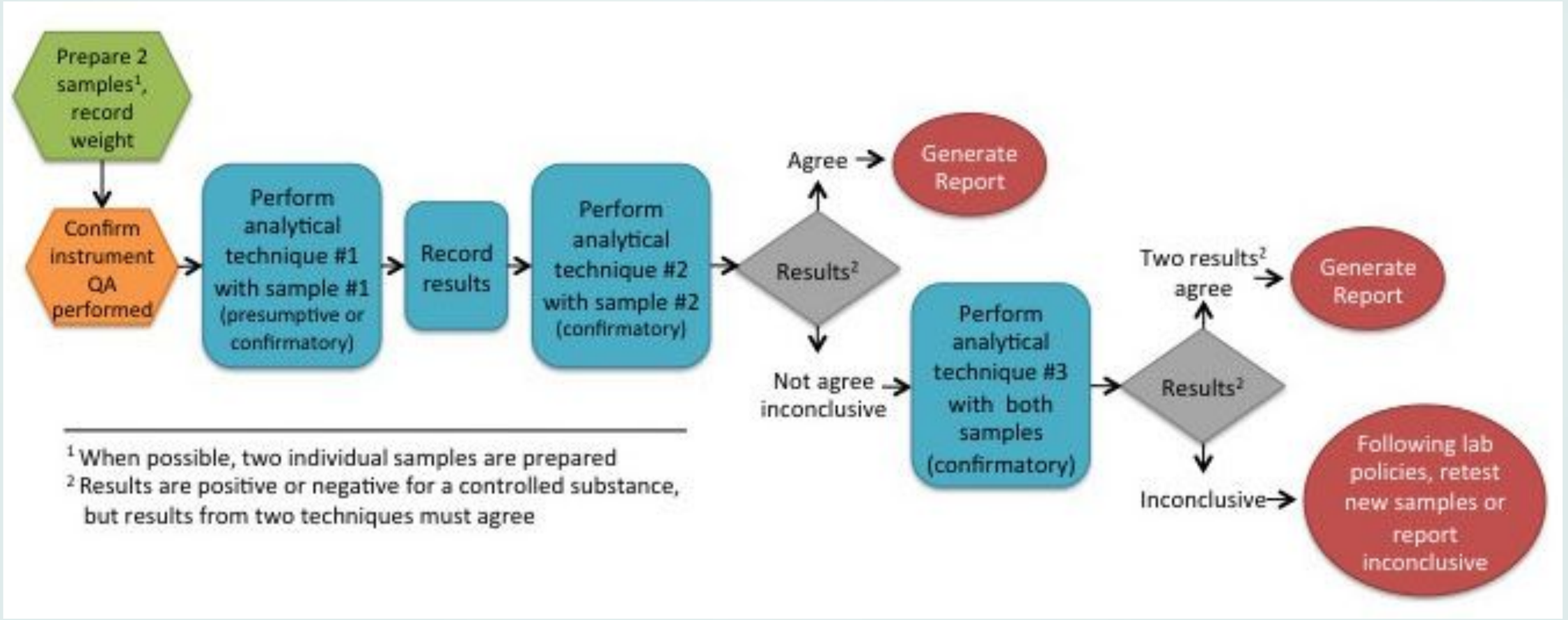
**Why?**
If drug test results need to be used in a **legal context**, positive findings from presumptive testing should be followed up by a confirmatory test of **greater or equal sensitivity and better specificity**

**Advantages**
- Provide more **definitive information** about the **quantitative concentrations** of specific drug and their metabolites
- **More accurate** due to **higher specificity** and **sensitivity**



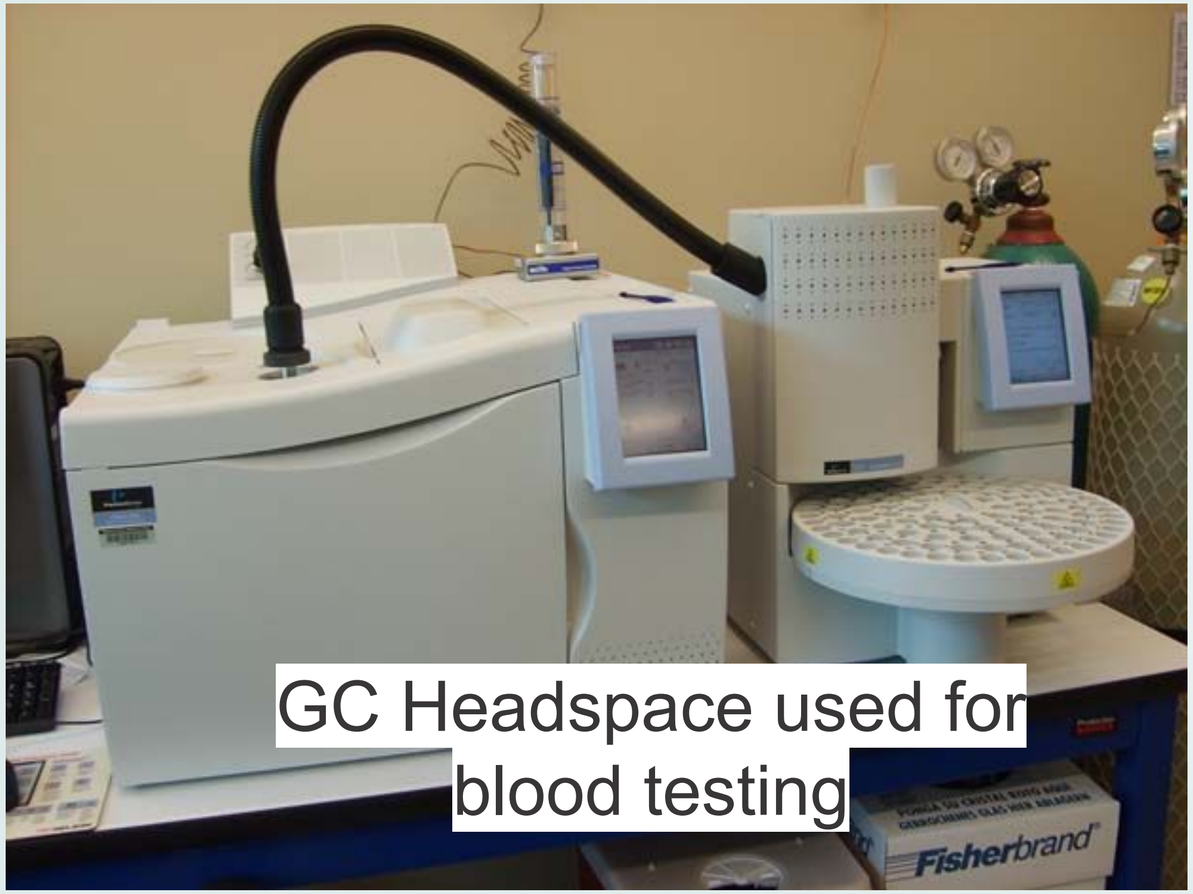
**Disadvantages**
- **Expensive**
- Technically **complex**
- **Labour intensive**
- **Time consuming** (can take days)

# 01. OVERVIEW OF CHEMICAL ANALYSIS PROCESS FOR CONTROLLED SUBSTANCES



01.

# Gas chromatography




GC Headspace used for blood testing

- All samples must be subjected to additional tests after the screening process to confirm the presence of any drugs.
- All confirmatory testing performed by the Toxicology section utilizes a process called gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS).

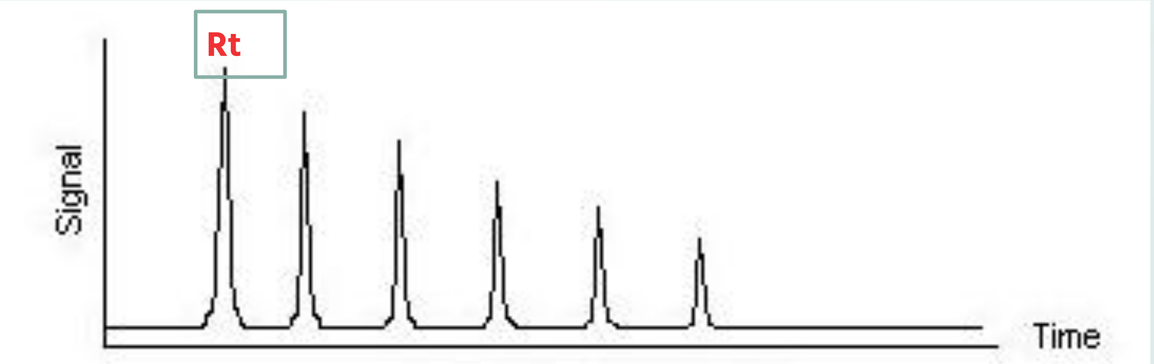
# 01. GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GC-MS)

**Positive presumptive tests are confirmed by methods such as GC-MS**
**The specificity of GC-MS in drug analysis is because there are two distinct analytical methods associated with GC-MS confirmation methods**

## GAS CHROMATOGRAPHY

- The analytical column **separates drugs and metabolites** from each other and from other impurities
- Retention times are a **reproducible identifier** for a certain drug or drug metabolite since the **individual retention time** varies for each drug/metabolites

- The time window at which the drugs or metabolites elute from the GC column into the mass spectrometer is called the **retention time**



# 01. GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GC-MS)

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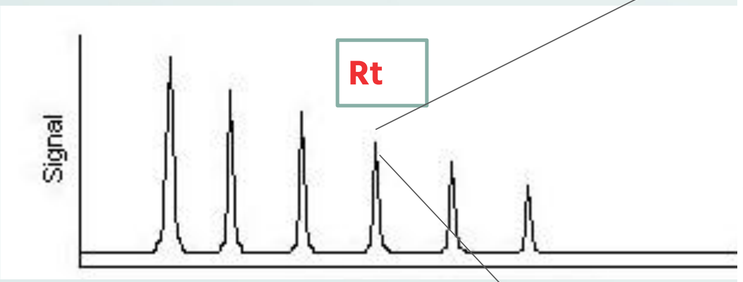
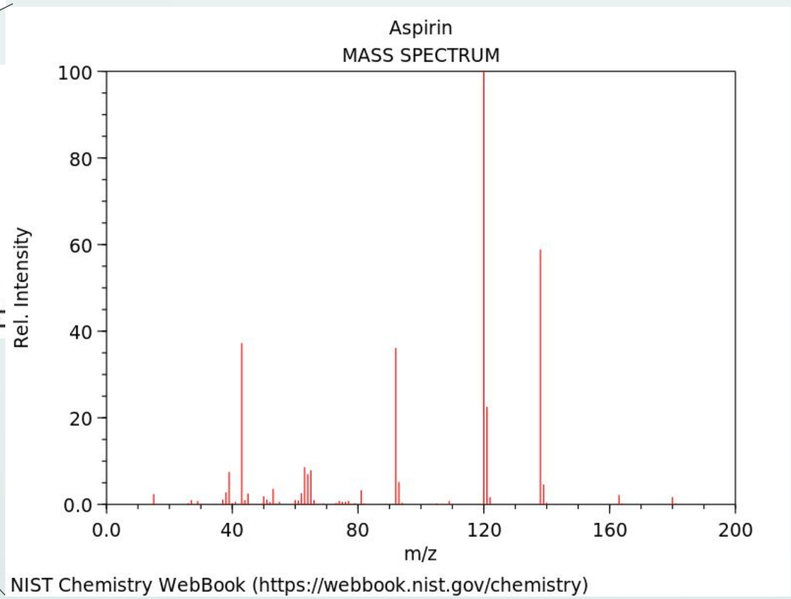
**The specificity of GC-MS in drug analysis is because there are two distinct analytical methods associated with GC-MS confirmation methods**

## GAS CHROMATOGRAPHY




## MASS SPECTROMETRY

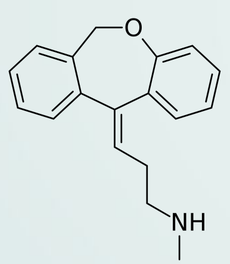
- The mass spectrometer separates the drug molecules into fragments, which are **unique for every drug/metabolite**
- The mass spectrum is **similar to a fingerprint** in that it is unique to an individual molecule
- The intensities of these mass fragments measured are **proportional to the amount of the drug present in a sample**

01.

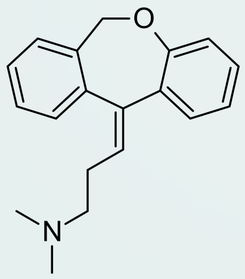
# Structurally similar drugs

1

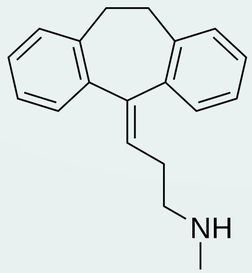
**The tricyclic antidepressant drugs and metabolites such as imipramine, desipramine, amitriptyline, nortriptyline, doxepin, and nordoxepin have close GC retention times. High concentrations may cause overlap and misidentification of peaks.**



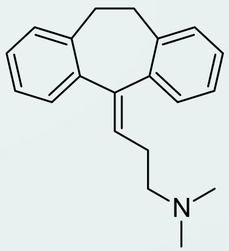

Nordoxepin



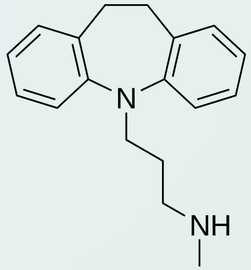

Doxepin



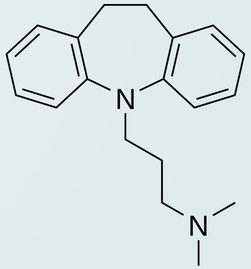

Nortriptyline




Amitriptyline




Desipramine




Imipramine

2

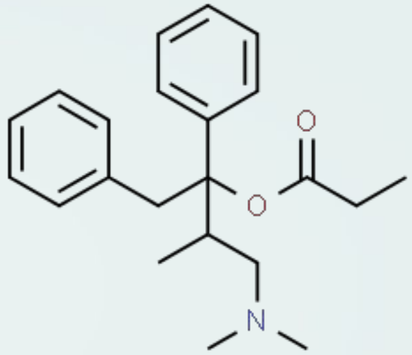
**Quinine and quinidine are isomers and would have the same retention times in TLC or GC procedures.**

01.

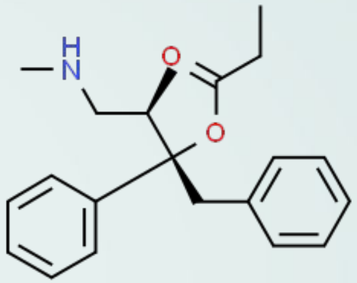
# Structurally similar drugs

3

When assaying propoxyphene, the thermolability of norpropoxyphene may produce additional confusing GC peaks.



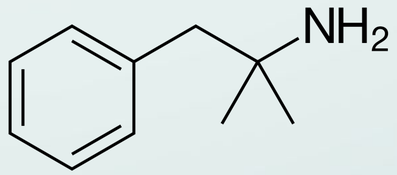

propoxyphene



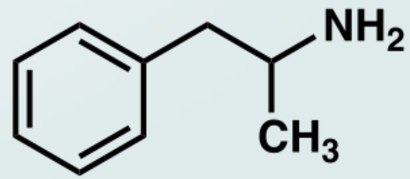

norpropoxyphene

4

Immunoassays for the amphetamines cross react with phentermine and sympathomimetic amines such as ephedrine and phenylpropanolamine.




phentermine




amphetamine

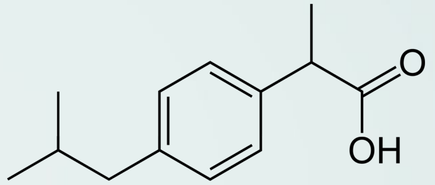
01.

# Structurally similar drugs

5

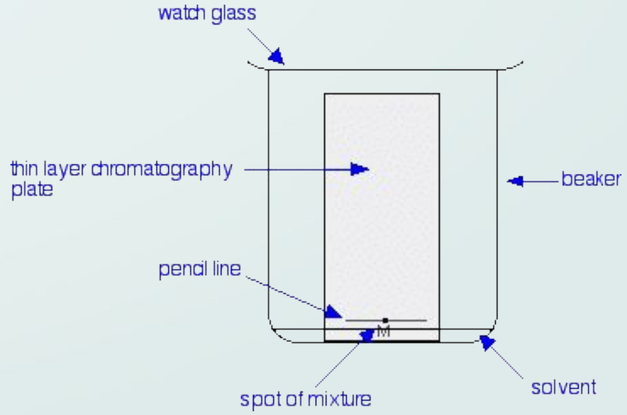
Immunoassays may exhibit cross reactivity to endogenous substances which may vary from batch to batch; reports of cross reactivity with ibuprofen and other non-steroidal anti-inflammatory drugs have been noted in the literature.

Ibuprofen




6

TLC commonly has co-migrating or closely migrating compounds, which have similar color characteristics.



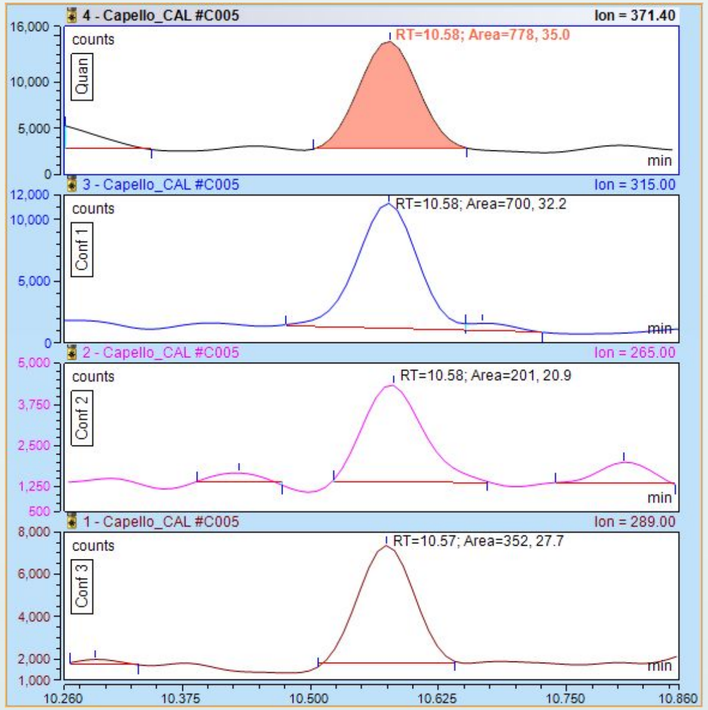
# 02.

# CASE STUDIES

# 02.

# CASE STUDY 1.

THC, THCOH, and THCCOOH are analyzed in plasma and THC is analyzed in hair. Deuterated THC, THCOH, and THCCOOH are used as internal standards




**The quantitation and confirming ions for THC in hair at the lower concentration, provided by the calibration point at 0.05 ng/mL — well below the cutoff level for THC in hair defined for gas chromatographic confirmation tests. Both peak shape and signal-to-noise ratio are appreciable for such a low concentration.**

# 02. Analysis of elemental contaminants in cannabis and hemp using inductively coupled plasma mass spectrometry (ICP-MS)

## CASE STUDY 2.

Table 1. Applicable limits for the big four for inhaled drug products

| Element | Limit [µg·g$^{-1}$] |
|---|---|
| Arsenic (As) | 0.2 |
| Cadmium (Cd) | 0.5 |
| Mercury (Hg) | 0.2 |
| Lead (Pb) | 0.1 |

Metals are known to accumulate in plant material through normal metabolic processes and some plants (including cannabis and hemp) are capable of hyperaccumulating metals.

Table 4. Results of the analysis of hemp and cannabis samples and spike recovery tests

| Sample | Concentration unspiked [ng·g$^{-1}$] | | | | Spike recovery [%] | | | |
|---|---|---|---|---|---|---|---|---|
| | $^{75}$As | $^{111}$Cd | $^{202}$Hg | $^{208}$Pb | $^{75}$As | $^{111}$Cd | $^{202}$Hg | $^{208}$Pb |
| Hemp 1 | 0.13 | 0.26 | 0.04 | 0.65 | 104.7 | 103.9 | 119.9 | 107.5 |
| Cannabis 1 | n.d. | n.d. | n.d. | 0.02 | 122.9 | 116.4 | 115.7 | 103.9 |

***One major set of contaminants that require testing are heavy metals due to their toxicity and/or carcinogenicity.***

# 02.

# CASE STUDY 3.

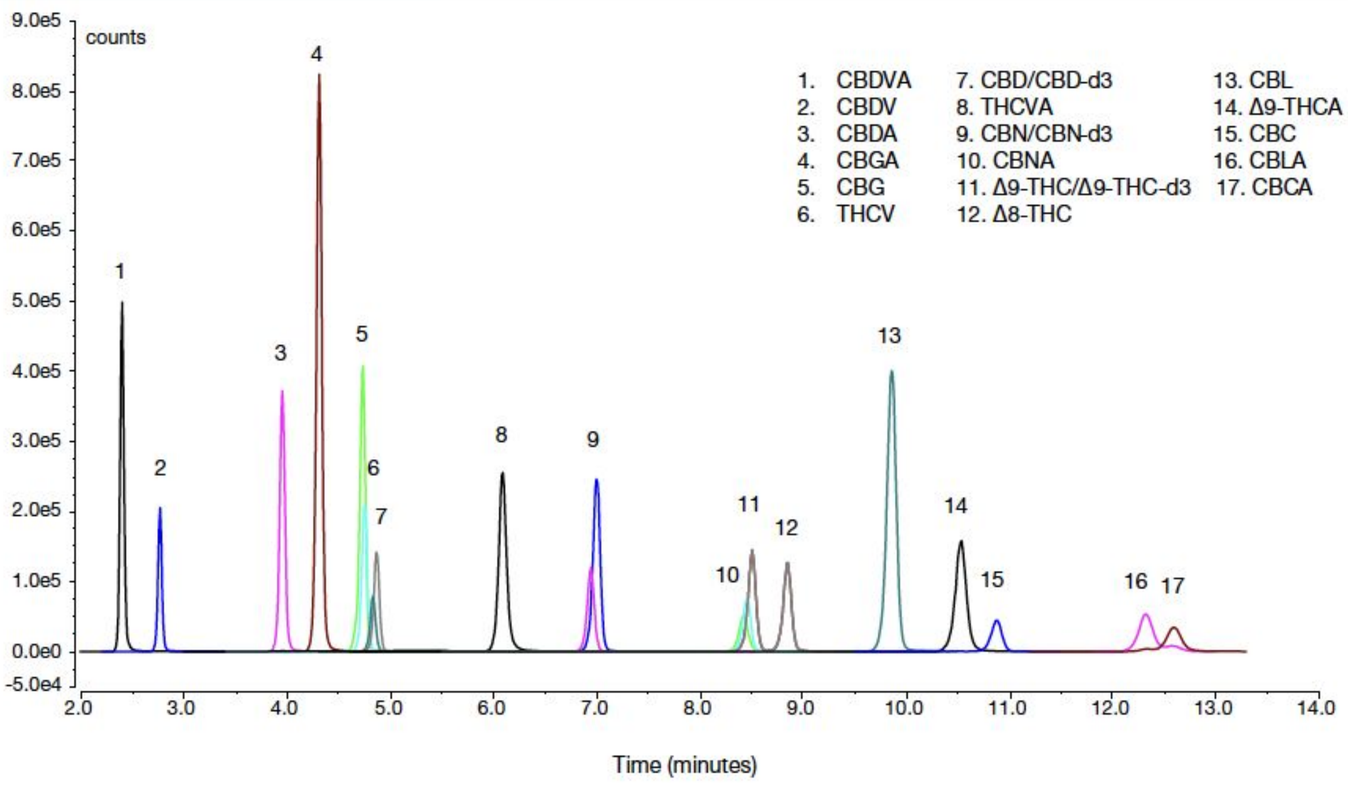



Figure 1. LC-MS/MS ion chromatogram of a calibration standard containing 17 cannabinoids

**Quantitation of seventeen cannabinoids in dried cannabis, hemp and vape oils by LC-MS/MS**

- *The method described has been shown to provide reproducible and accurate results for cannabinoids in dried cannabis, hemp, and vape oil samples.*
- *Separation of the key cannabinoids has been demonstrated in both calibration solutions and extracted matrix samples.*

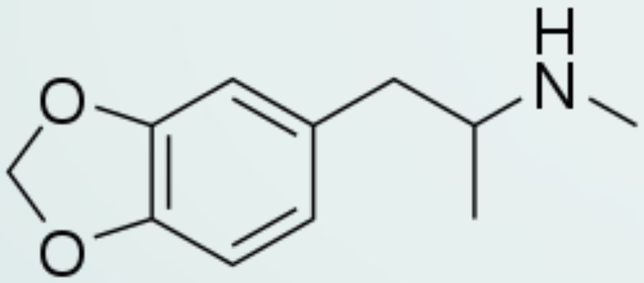

# LITERATURE EXAMPLES

# 03.

# The Detection of Novel Stimulants in Oral Fluid From Users Reporting Ecstasy, Molly and MDMA Ingestion

## Overview

In order to evaluate hypothesized non-specific and interchangeable use of the terms **Ecstasy, Molly** and **MDMA**, this study compared **self-reported drug** use with **toxicological findings** in **biological specimens.**




**MDMA**

**CAMH:**

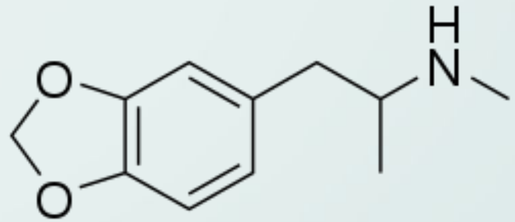
- **As with all illegal street drugs, the purity and strength of ecstasy can never be accurately gauged. When you take ecstasy, you don't know what you're taking, or how it will affect you.**

- **Combining ecstasy with other drugs, whether illegal or prescription, may cause a toxic interaction. Several prescription medications are known to interact with ecstasy, including a type of antidepressant called monoamine oxidase inhibitors (MAOIs) and ritonavir, a protease inhibitor used to treat HIV.**

# 03.

## The Detection of Novel Stimulants in Oral Fluid From Users Reporting Ecstasy, Molly and MDMA Ingestion

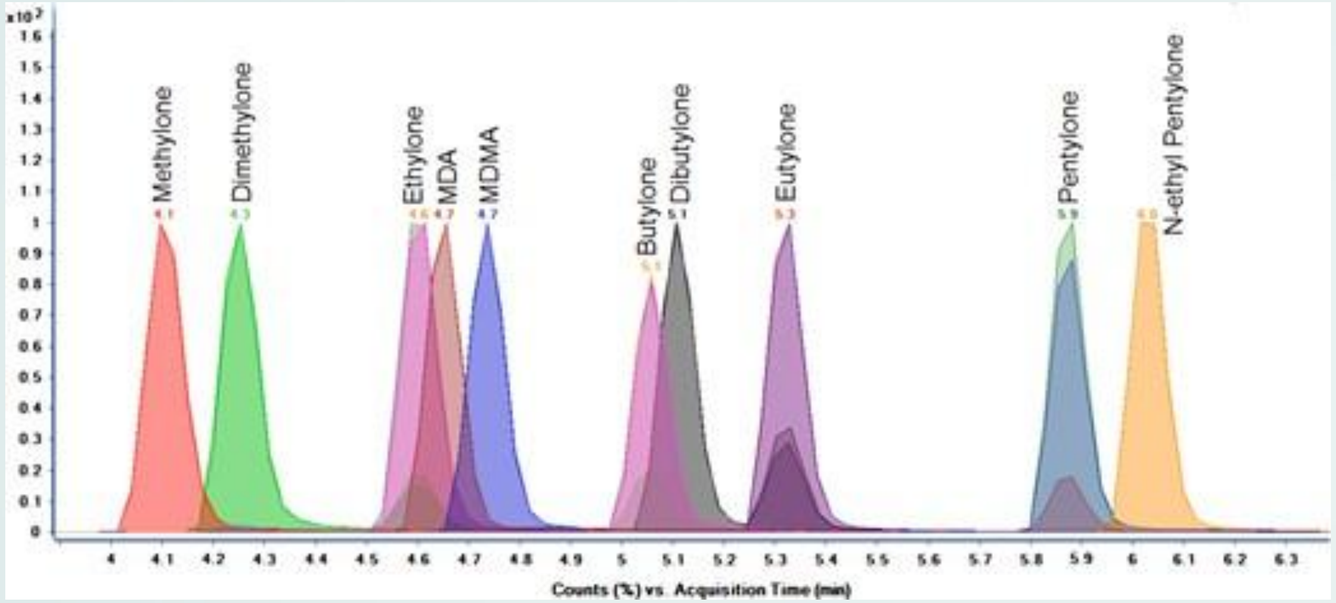
- Users of Ecstasy and Molly, preparations believed to contain MDMA, have increasingly tested positive for novel psychoactive substances (NPS).
- The current study collected oral fluid specimens from participants attending large music festivals
- Participants also completed a survey about recent recreational drug use.
- Collected specimens were screened for therapeutic drugs, common drugs of abuse, and NPS, using **liquid chromatography quadrupole time-of-flight mass spectrometry (LC QTOF).**
- Positive screen results were confirmed by validated **liquid chromatography–tandem mass spectrometry (LC–MS-MS)** methods for MDMA, MDA, methylone, dimethylone, ethylone, butylone, dibutylone, eutylone, pentylone, N-ethyl pentylone (ephylone), alpha-PVP and 4-fluoroamphetamine (4-FA).




**MDMA**

# 03.

# The Detection of Novel Stimulants in Oral Fluid From Users Reporting Ecstasy, Molly and MDMA Ingestion




A quantitative LC-MS-MS method for the analysis of MDMA, MDA, methylone, dimethylone, ethylone, butylone, dibutylone, eutylone, pentylone and N-ethyl pentylone was developed and validated for analysis of the oral fluid samples

Separation achieved between all species, specifically noting the separation of isobaric analytes (e.g., dimethylone, ethylone, butylone and dibutylone, eutylone, pentylone).

# 03. The Detection of Novel Stimulants in Oral Fluid From Users Reporting Ecstasy, Molly and MDMA Ingestion

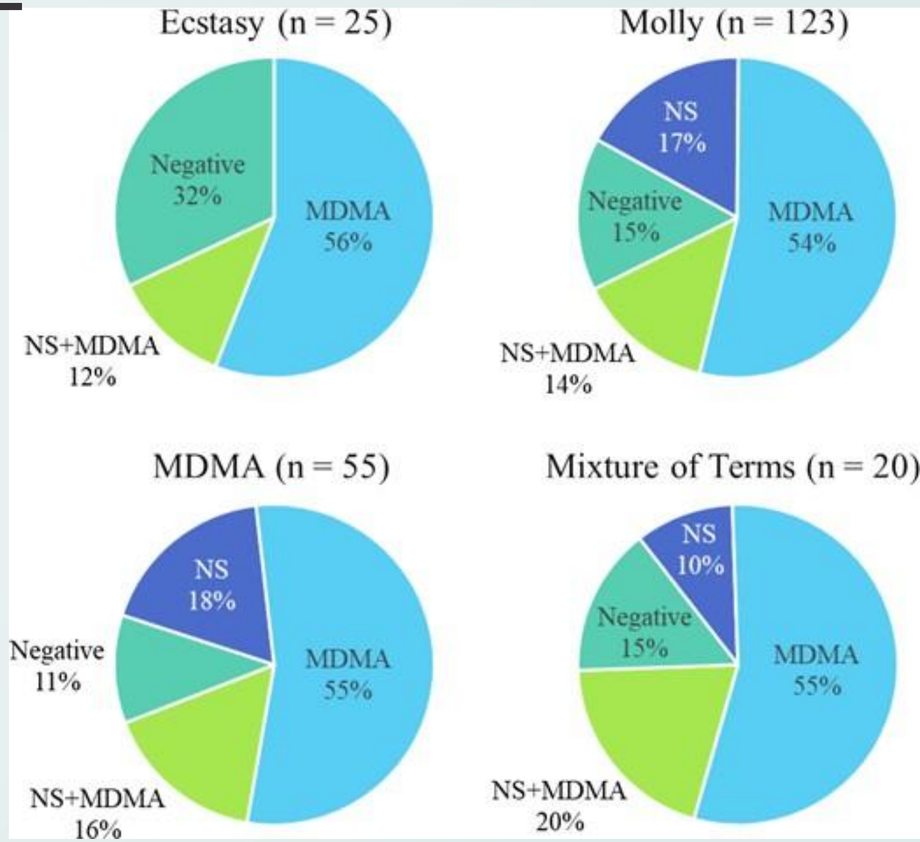
**Following analytical analysis, survey responses were compared to the confirmatory findings**

**The goal of this comparison was to pair self-reported drug use with analytical findings and to evaluate the agreement or lack of agreement.**

**The confirmatory data was broken into four categories:**

1. **Novel stimulant only (NS)**
2. **MDMA and/or MDA only (MDMA)**
3. **Novel stimulant and MDMA/MDA (NS + MDMA)**
4. **Negative** (meaning the collected specimen was negative for novel stimulants, MDMA and MDA).

Samples positive for MDA only were tallied with MDMA due to the relationship to MDMA as a metabolite




**The relationship between Ecstasy, Molly and MDMA responses vs. analytical findings of novel stimulants and/or MDMA and MDA.**

# 03.

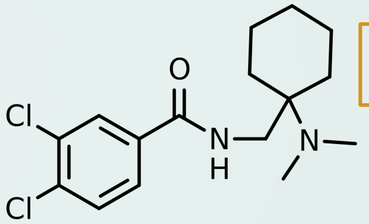

# QUANTITATIVE ANALYSIS OF NOVEL SYNTHETIC OPIOIDS, MORPHINE AND BUPRENORPHINE IN ORAL FLUID BY LC-MS-MS

## Overview

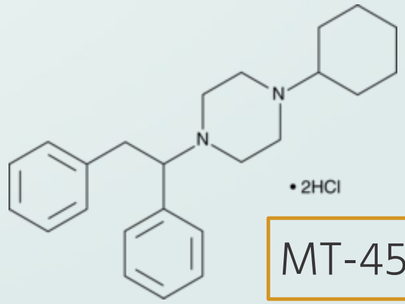
Goal of developing and validating a comprehensive analytical method for the detection and quantification of morphine, buprenorphine, and novel synthetic opioids in oral fluid collected via Quantisal.

## Background

- Synthetic opioids including AH-7921, MT-45, U-series and W-series have emerged in the drug market.
- These substances are not well studied in humans, and there are limited methods available for the detection and quantification of these drugs.
- **Oral fluid is useful for determining recent drug use, does not require a medical professional, and can be collected under direct observation, thereby determining adulteration.**




AH-7921




MT-45

03.


# QUANTITATIVE ANALYSIS OF NOVEL SYNTHETIC OPIOIDS, MORPHINE AND BUPRENORPHINE IN ORAL FLUID BY LC-MS-MS

Goal of developing and validating a comprehensive analytical method for the detection and quantification of morphine, buprenorphine, and novel synthetic opioids in oral fluid collected via Quantisal.

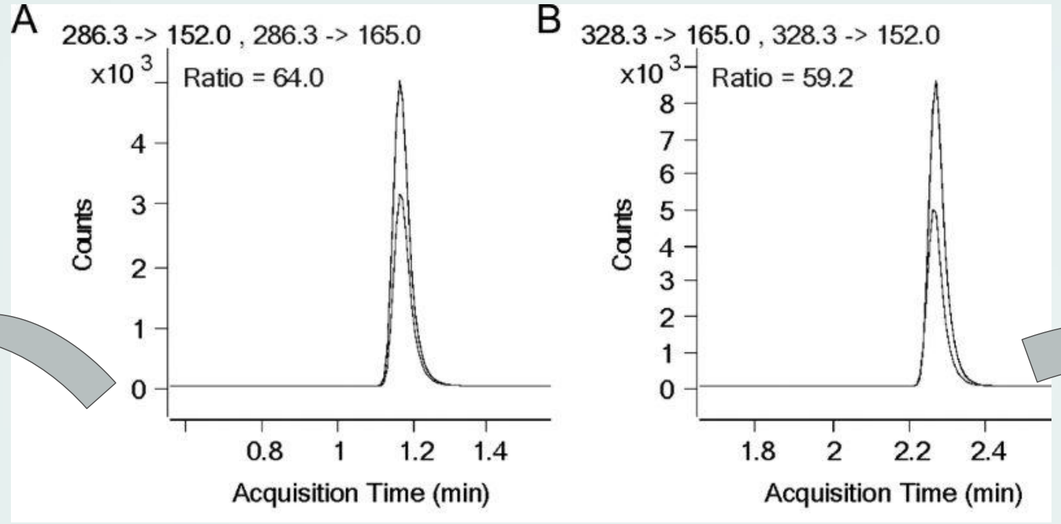
- All analytes were stable under all tested conditions (24 h at room temp, 72 h at 4°C, and in the autosampler for 60 h at 4°C).
- Used solid-phase extraction paired with liquid chromatography-tandem mass spectrometry

- **The LOD and LOQ were 5 ng/mL and 10 ng/mL, respectively.**
- **Linearity of 10 and 500 ng/mL ($R^2$ = 0.9959).**

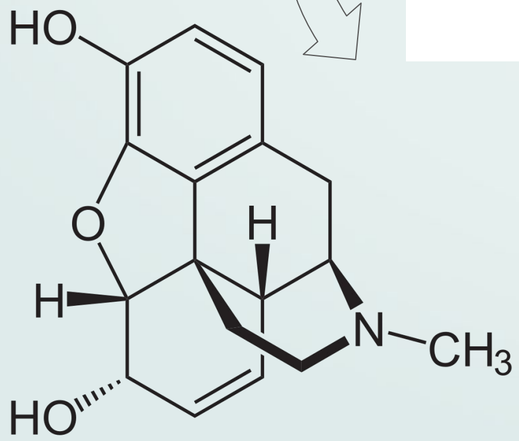
# 03.

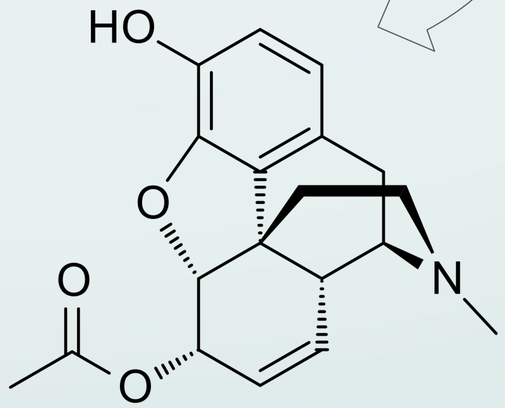
# QUANTITATIVE ANALYSIS OF NOVEL SYNTHETIC OPIOIDS, MORPHINE AND BUPRENORPHINE IN ORAL FLUID BY LC-MS-MS

Chromatograms of each analyte




Extracted Ion Chromatograms of morphine (A), 6-acetylmorphine (B), at 10 ng/mL.



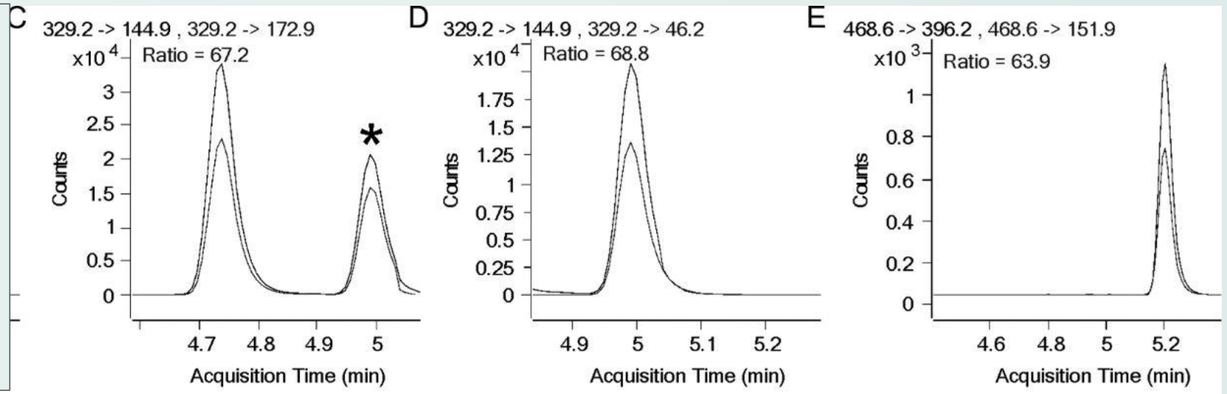



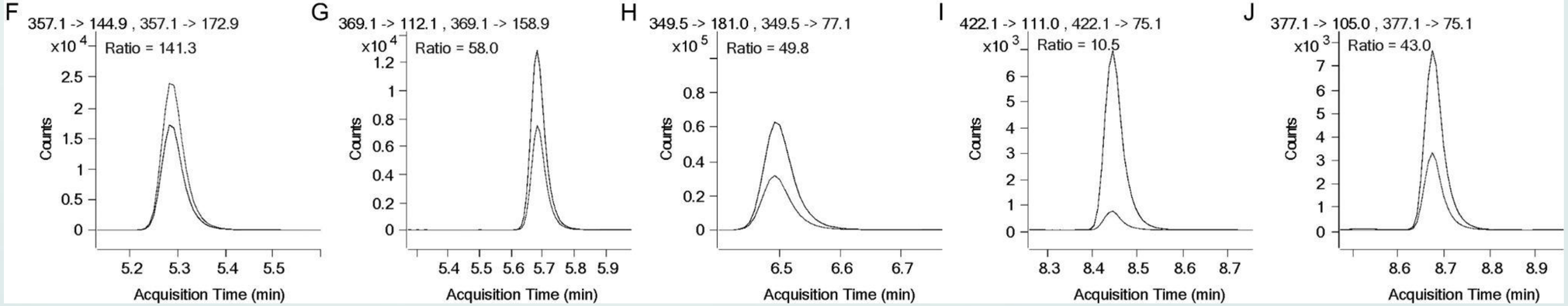
# 03.

# QUANTITATIVE ANALYSIS OF NOVEL SYNTHETIC OPIOIDS, MORPHINE AND BUPRENORPHINE IN ORAL FLUID BY LC-MS-MS

Chromatograms of each analyte

Extracted Ion Chromatograms of U-47700 (C), AH-7921 (D), buprenorphine (E), U-49900 (F), U-50488 (G), MT-45 (H), W-18 (I) and W-15 (J) at 10 ng/mL.



# 04.

# BIOLOGICAL MATRICES

# 04.

# URINE TOXICOLOGY DETECTION PERIODS FOR DIFFERENT SUBSTANCES

| Substance | Typical Urine Detection Period |
|---|---|
| Amphetamine or methamphetamine | 2–4 days |
| Barbiturates | |
| Short-acting—Secobarbital | 1–2 days |
| Long-acting—Pentobarbital | 2–4 days |
| Phenobarbital | 10–20 days |
| Benzodiazepines | |
| Therapeutic dose | 3–7 days |
| Chronic dosing | Up to 30 days |
| Cocaine | 1–3 days |
| Cannabinoids | |
| Casual use | 1–3 days |
| Daily use | 5–10 days |
| Chronic use | Up to 30 days |
| Ethanol (alcohol) | 12–24 hours |
| Opioids (e.g., codeine, morphine) | 1–3 days |
| Methadone | 2–4 days |
| Propoxyphene | 6–48 hours |
| Ecstasy/euphorics | 1–5 days |
| PCP | |
| Acute use | 2–7 days |
| Chronic use | Up to 30 days |

## Confirmation urine testing methods include:

1. Gas Chromatography (GC)
2. High Performance Liquid Chromatography (HPLC)
3. Gas Chromatography - Mass Spectrometry (GC-MS)
   The gold standard for drug detection, but expensive

04.

# EFFECTIVENESS OF DRUG DETECTION METHODS THAT USE DIFFERENT BIOLOGICAL PRODUCTS

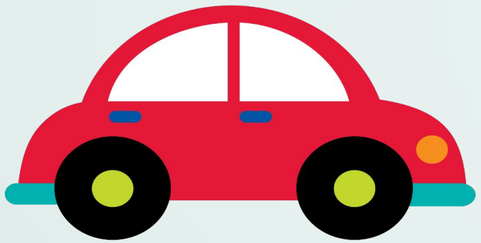
| Body Product | Drug Detection Time | Major Advantages | Major Limitations | Primary Use |
|---|---|---|---|---|
| Urine | 2–4 days | Mature technique; established cutoffs for detecting many drugs of abuse | Detects only recent use; needs costly confirmation to be accurate | Monitors recent drug use in many populations |
| Breath (alcohol) | 12–24 hours | Easy to use; readily available and well-established method | Short detection time | Confirms observed intoxication or impairment |
| Saliva | 12–24 hours | Easy to obtain samples; good correlation with blood levels for some substances | Very short detection time; new method; oral cavity is contaminated easily | Links positive drug test to behavioral impairment and intoxication |
| Sweat | 1–4 weeks | Cumulative measure; relatively tamper-proof collection method | High potential for contamination; new technique | Detects recent and less recent drug use |
| Blood | 12–24 hours | Accurate results; established method | Invasive method; expensive; detects only current use or intoxication | Detects drug effects on crashes, medical emergencies |
| Hair | 4–6 months | Measures long-term drug use; readily available samples; accurate results | New technique; costly and time-consuming; no dose-response relation established | Confirms drug use in past 4 to 6 months; prevalence studies |


# CANLII

05.

# R.v. SINGH

**Issue: has the Crown established beyond a reasonable doubt whether Mr. Singh had care and control of his vehicle?**



## BACKGROUND

1. At 2:55 pm on February 4th 2007, a witness noticed Mr. Singh's van pointed on a diagnoal in the middle of the road

2. Mr. Singh was observed to be unconsciousness in his running vehicle with saliva or foam coming from his mouth

3. The witness called 911, and Mr. Singh was transported to the hospital, where his blood was drawn

05.

# R.v. SINGH

**Presumptive Testing:**

- Blood sample analyzed at the hospital by Ms. Braun
- Used the **Beckman-Coulter** method
- Reading was **128.8 mM/L**
- This value is "**critically high**"

**Confirmatory Testing:**

- Sample analyzed by Dr. Marie Elliott, an expert forensic witness
- Used **Headspace Gas Chromatography**
- Serum result = 662 mg alcohol in 100 mL of blood
- Converted to whole blood = **571 mg alcohol in 100 mL of blood**
- The hospital result was translated to **589 mg alcohol in 100 mL of blood**

**The Beckman-Coulter test is accurate enough for hospital purposes, where the required speed of a result trumps the need for scientific perfection**

**Legal BAC Limit = 80 mg alcohol in 100 mL blood**

**Headspace Gas Chromatography is the gold standard of analysis for four reasons:**

1. It can analyze minute amounts of blood
2. It is specific enough to identify other components
3. Accuracy of results
4. One can also analyze post mortem samples

05.

# R.v. SINGH

## INTERPRETATION

Dr. Elliot related the blood result taken from the hospital at 4:05 pm back to the time of discovery of Mr. Singh in his vehicle at 2:55 pm

**Assumed:**

- No large quantities of alcohol were consumed shortly prior to the questioned time
- That no alcohol was consumed between the questioned time and taking the blood sample
- An **elimination rate** of **10-20 mg** of alcohol in **100 mL** of blood **per hour**
- At 2:55 pm, Mr. Singh's BAC would be **571-596 mg alcohol in 100 mL blood**
- This reflects **~28 oz liquor** in his system

## FINDING

The Court found Mr. Singh to be in **care or control** of a motor vehicle while **impaired by alcohol.** Mr. Singh was also found to have a blood alcohol concentration **exceeding the legal limit.** His BAC was in fact **over 7x the legal limit.** Mr. Singh was found **guilty on both counts.**

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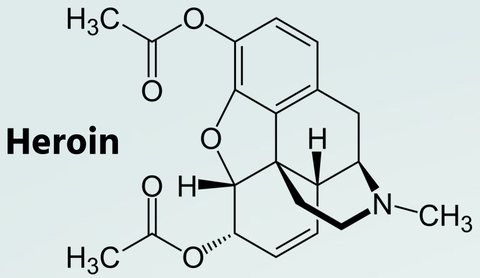


# R.v. JORDAN

## The Case

An appeal convicting the appellant of importing heroin into Canada

## Grounds of Appeal

1. Did the trial judge err in giving any weight to Mr. Clark's opinion that the unknown substance was heroin?
2. Did the trial judge err in holding that the search was reasonable?

## Background

- On Sept 18, 1982, appellant arrived by air **from Tokyo at Vancouver International Airport**
- Earlier that day, and RCMP sergeant had spoken with customs at the airport and indicated he was **interested in Jordan**, and that Jordan was connected with travel to Japan and with **purchasing heroin**
- **"Watch for" slips** were distributed, indicating that Jordan was to be sent for **secondary inspection**
- Upon the search, Superintendent Flagel found a **brown manila envelope** containing a substance that **appeared to be a narcotic** in Jordan's briefcase
- **Several other envelopes** with a **similar substance** were also found
- The envelopes were turned over to a **designated analyst, Mr. Clark.**
- Mr. Clark analyzed the substances found in the envelopes and **found them to contain heroin**

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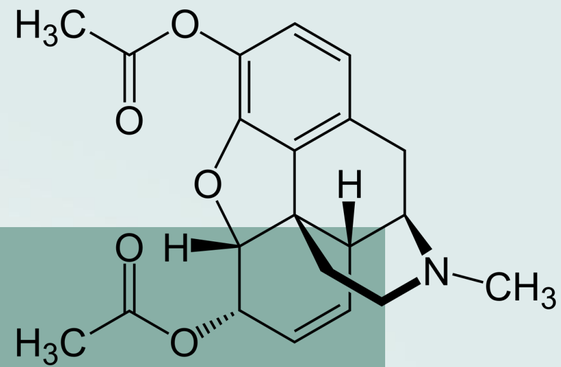
# R.v. JORDAN




## Confirmatory Testing

- **Quantitative** analysis using **gas liquid chromatography** test on Sept 20th
- Mr. Clark used what he believed to be a **known standard of heroin** and then **compared the results of the known to the unknown**
- **Another analys**t analyzed the **known standard** and **certified it to be heroin**
- **No presumptive tests** were performed
- **Identification** analysis using **gas chromatography - mass spectrometry** on October 4, 1982
- Mr. Clark formed his opinion that the **sample contained heroin**

## Ruling

1. The **tests** employed by the analysts were ruled to be **correct in every respect**
2. With respect to border searches, it is not necessary to prove the source of the information or the basis of the belief of the police officers. Therefore, the search was reasonable

**Appeal dismissed**

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# REFERENCES

06.

# REFERENCES


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