

How the laboratory solves unknown cases

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The Toxicology Detective

March 1, 2017

Toxicology testing times

Death: June 25, 2009.

Tox report released Feb 13, 2010
[propofol]



Death: April 21, 2016

Tox report released June 2, 2016
[fentanyl]



What took so long?

Clin tox testing strategy?

Forensic toxicology:

Definitive and slow (weeks, months)

Clinical toxicology:

Presumptive and fast (<4 h)



Clin tox testing strategy?

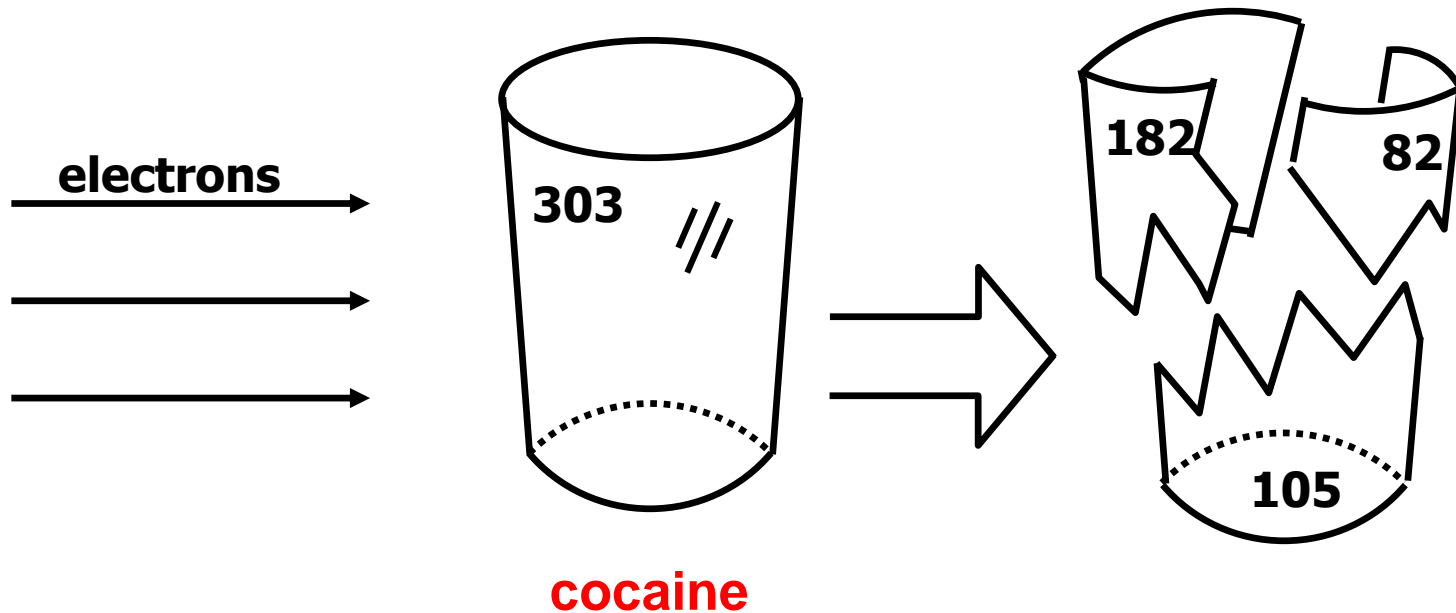
Targeted: suspected list



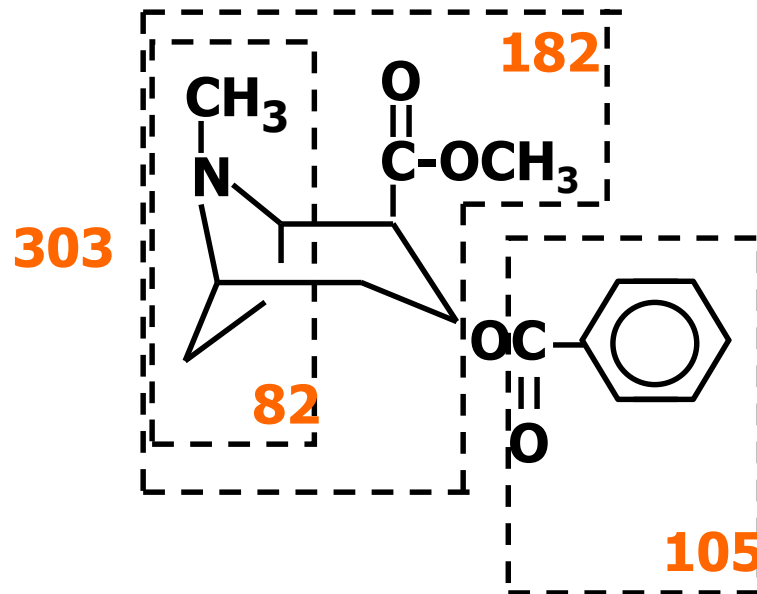
Untargeted:

“Targeted” compound testing by LC-tandem mass spectrometry

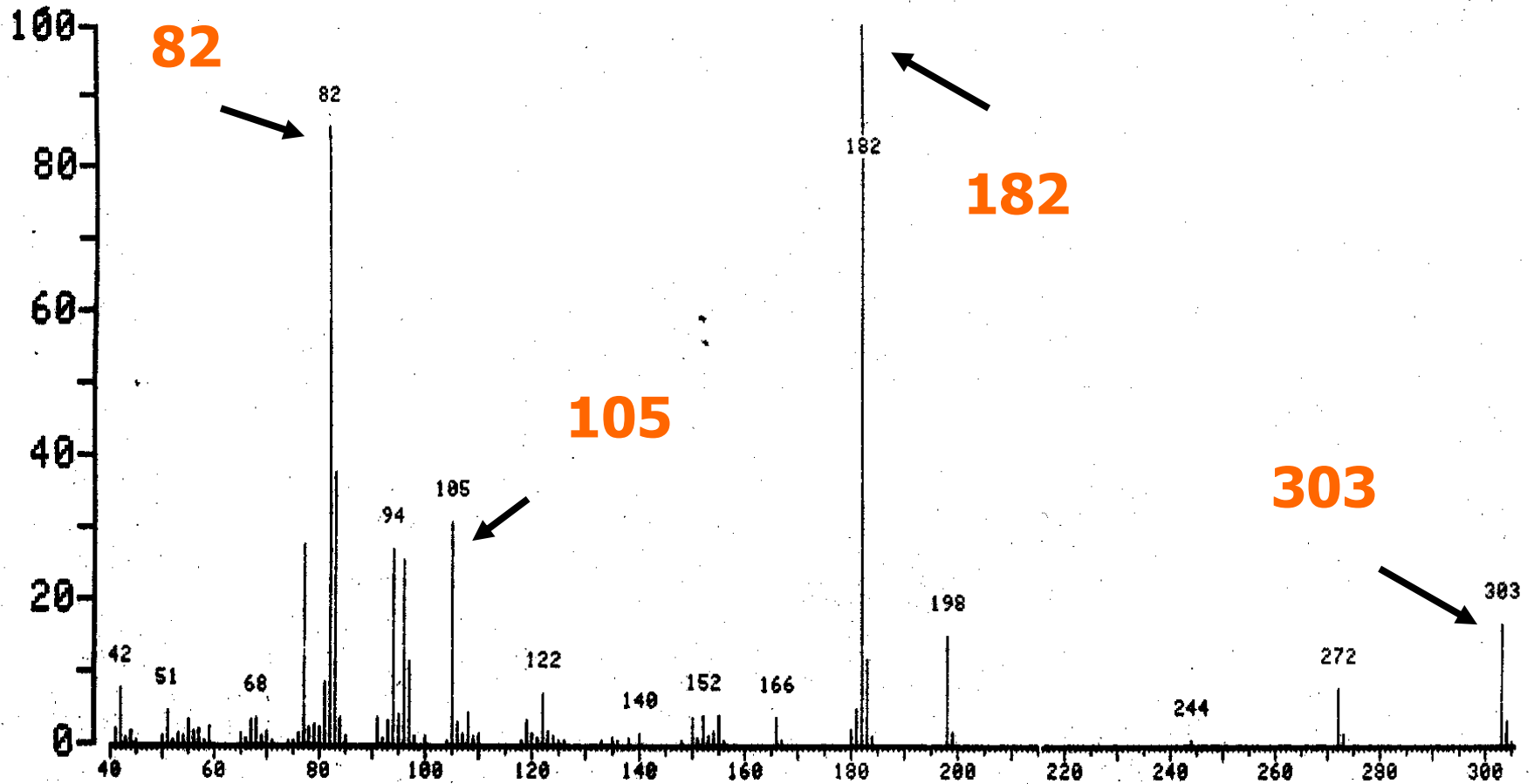
Molecular fragmentation by electrons



Fragmentation of cocaine

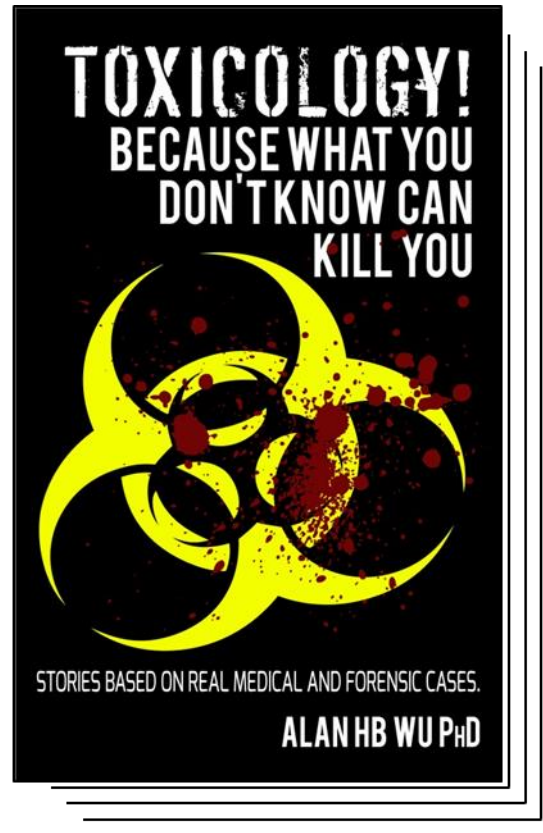


Library matches: cocaine mass spectrum



Paperbacks with real stories

Available through:
www.alanhbwu.com/



*Tox testing that
solved clinical and
forensic cases*



How the Legal and Medical Systems failed Patricia and Ryan Stallings.

Riglesbergen [Justice:Denied](#) 2(8), 1989.

Life seemed complete for the Stallings who had just moved to a house overlooking Lake Wauwanoka. Mom: "He had my hair and David's face. He had huge dimples and big blue eyes. That was the happiest time of my life. A new house, a healthy and happy baby, what could be wrong?"



Everything was about to change for Patricia. On July 7, 1989, she gave Ryan his evening bottle before she put him to bed. Ryan threw up immediately after he drank it.....

The clinical lab found ethylene glycol in the child's blood and in the baby's bottle nipple. Patricia v accused of poisoning her son. When Ryan died, she was convicted of murder.



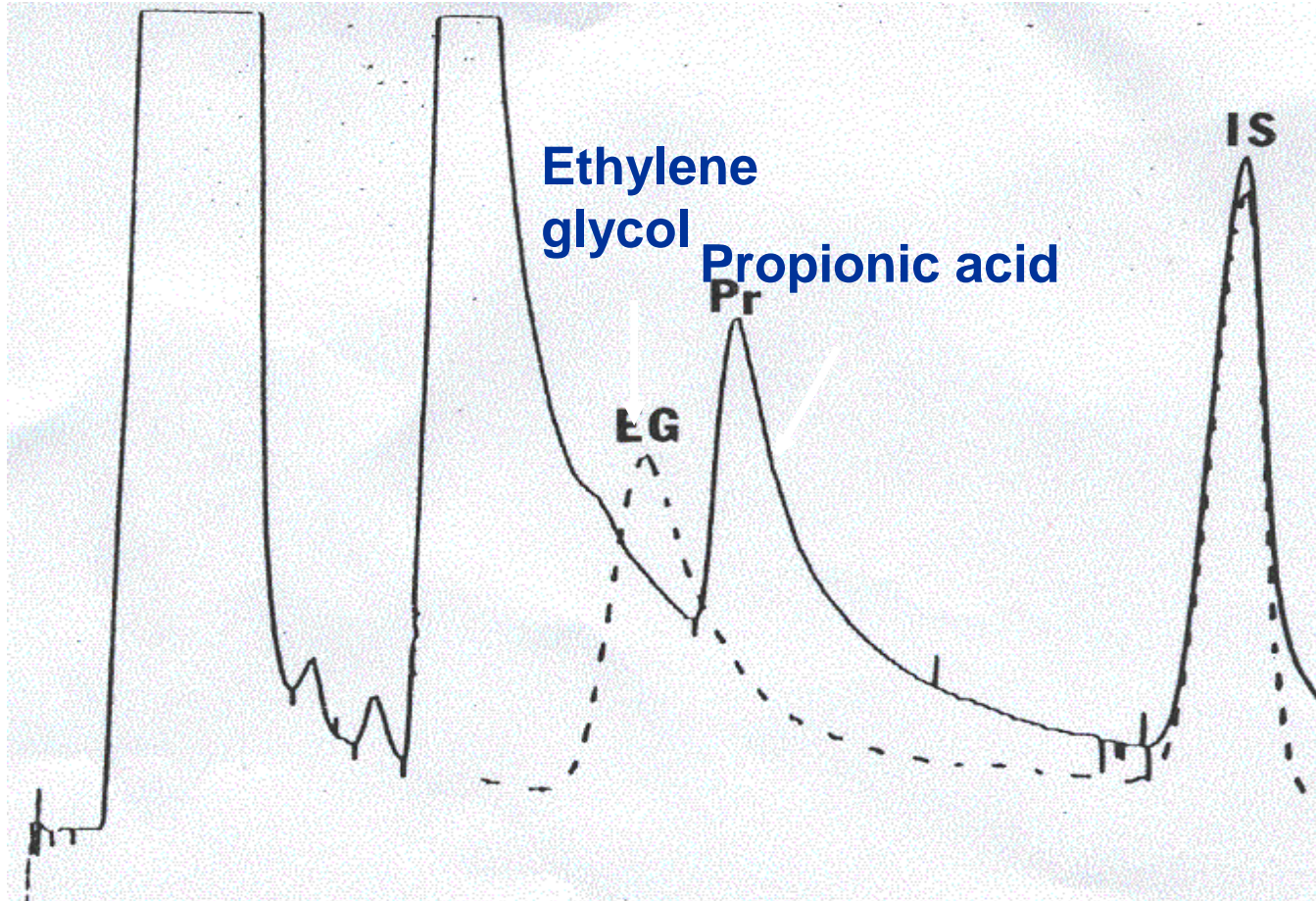
While in prison, she has a 2nd child who develops the same symptoms as Ryan. After an investigation, a mistake was made in the lab.

Misidentification of propionic acid as ethylene glycol in a patient with methylmalonic acidemia

James D. Shoemaker, MD, PhD, Robert E. Lynch, MD, PhD,
Joseph W. Hoffmann, PhD, and William S. Sly, MD

From the Metabolic Screening Laboratory, E. A. Doisy Department of Biochemistry and Molecular Biology, and the Departments of Pathology and Pediatrics, St. Louis University Medical Center, and the Department of Pediatrics, St. Louis University and Cardinal Glennon Children's Hospitals, St. Louis, Missouri

Why mass spectrometry?



Internet pharmacy

- 22 y-o Russian female
- Chief complaint: lethargy
- S/P ingestion of “Adderall.” Sample from India
- PE: lethargy, hypertension (143/83), tachycardia (HR 124)
- Negative urine drug screen for amphetamine and all other targets

Adde Cure 30 ingredients?

ADDE CURE - 30	
Each Tablet Contains	
Dextroamphetamine Saccharate	: 7.5 mg
Amphetamine Aspartate Monohydrate	: 7.5 mg
Dextroamphetamine Sulfate USP	: 7.5 mg
Amphetamine Sulfate USP	: 7.5 mg
Total amphetamine base equivalence	: 18.8 mg



ADDERALL XR® CAPSULES

EACH CAPSULE CONTAINS:	5 mg	10 mg	15 mg	20 mg	25 mg	30 mg
Dextroamphetamine Saccharate	1.25 mg	2.5 mg	3.75 mg	5.0 mg	6.25 mg	7.5 mg
Amphetamine Aspartate Monohydrate	1.25 mg	2.5 mg	3.75 mg	5.0 mg	6.25 mg	7.5 mg
Dextroamphetamine Sulfate USP	1.25 mg	2.5 mg	3.75 mg	5.0 mg	6.25 mg	7.5 mg
Amphetamine Sulfate USP	1.25 mg	2.5 mg	3.75 mg	5.0 mg	6.25 mg	7.5 mg
Total amphetamine base equivalence	3.1 mg	6.3 mg	9.4 mg	12.5 mg	15.6 mg	18.8 mg

Amphetamine toxicity?

Hypertension ✓

Tachycardia ✓

~~Agitation~~

~~Seizures~~

~~Hyperthermia~~

~~(+) Utox~~



ADDE CURE - 30

Each Tablet Contains
Dextroamphetamine : 7.5 mg
saccharate : 7.5 mg
Amphetamine
Asparate
Monohydrate : 7.5 mg
Dextroamphetamine : 7.5 mg
Sulfate USP : 7.5 mg
Amphetamine
Sulfate 'JSP : 18.8 mg
Total amphetamine
base equivalence

B.No : AD-709

MFD : 04.05.2009

Exp : 05.06.2011

Marketed by
Euro Biotech Health Care
No.1, Near Petrol Pump,
Mira Bhayander Road,
Mumbai - 400 105.

Manufacturer's address in India





POSTED:NOV 10 2015 07:17PM PST
UPDATED:NOV 10 2015 07:48PM PST

On Tuesday, the Santa Cruz County Sheriff's Office announced deputies had arrested a man who admitted to supplying counterfeit Xanax to one of the men who died recently.

The suspect is 22-year-old Daniel Meehan from Aptos. At his home, police say they found cocaine, guns, marijuana plants and a butane hash oil lab.

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drug looked nearly identical to Xanax, which treats anxiety and panic disorders.



Deaths from fake Xanax attributed to rising prescription drug abuse

The pills actually contained Fentanyl, a potent opioid.

The epidemic of prescription drug abuse has taken a deadly toll around the country. KTVU talked with a police officer who focuses much of his attention on the prescription drug trade.



Fake Norco: acetaminophen and hydrocodone

Centers for Disease Control and Prevention

MMWR

Morbidity and Mortality Weekly Report

Early Release / Vol. 65

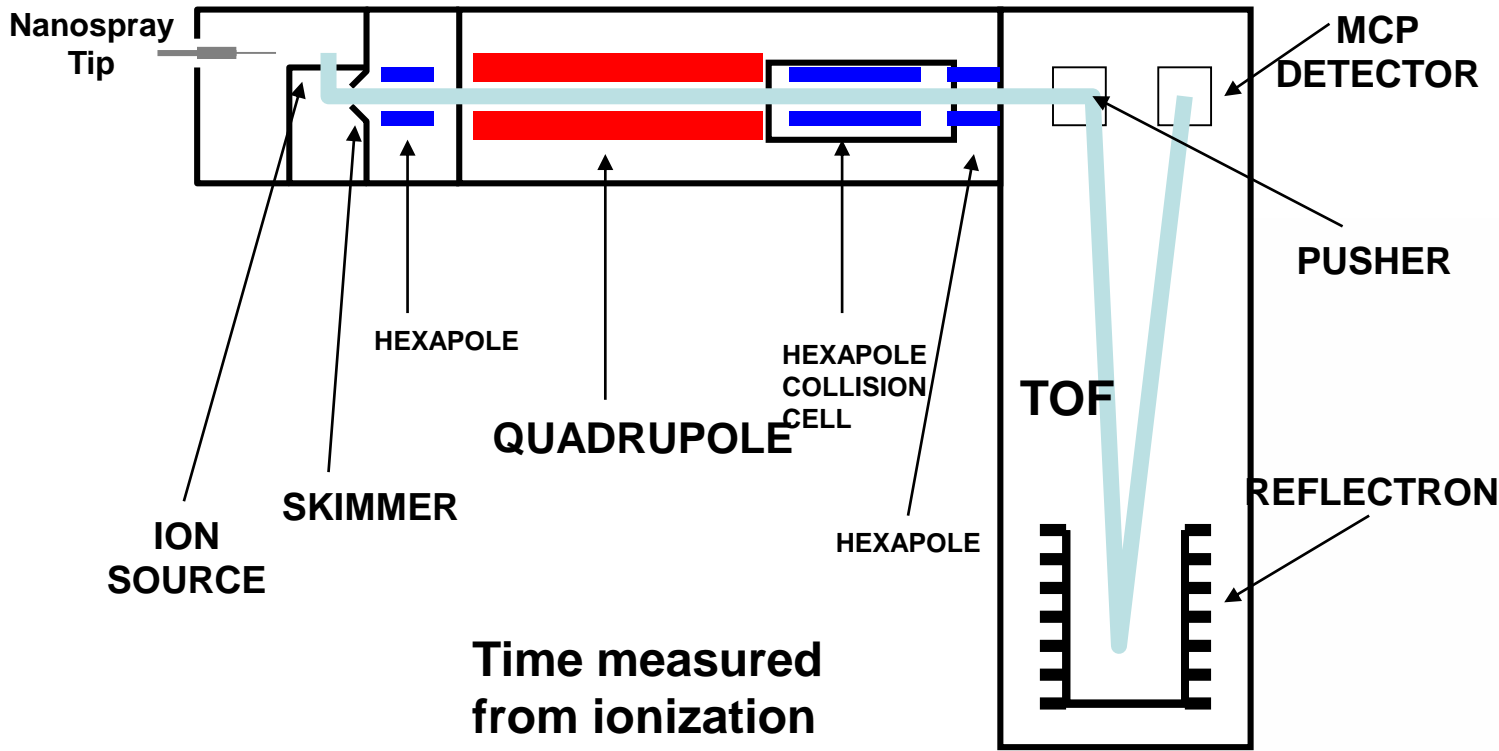
April 26, 2016

Counterfeit Norco Poisoning Outbreak — San Francisco and Bay Area, California, 2016

Kathy T. Vo, MD^{1,2}; Xander M.R. van Wijk, PhD³; Kara L. Lynch, PhD³; Alan H.B. Wu, PhD³; Craig G. Smollin, MD^{1,2}

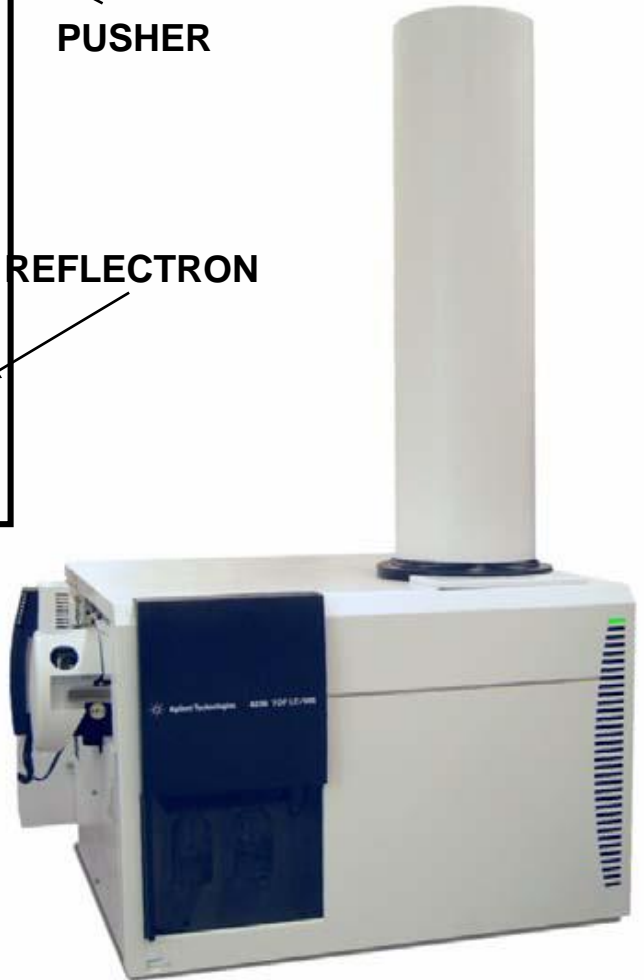


“Untargeted analysis”: High resolution MS-TOF



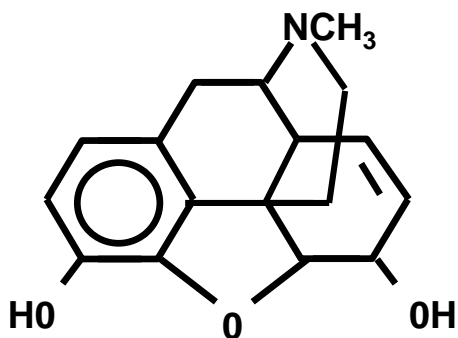
Time measured from ionization to detector

Measurement of mass to 4 decimal places



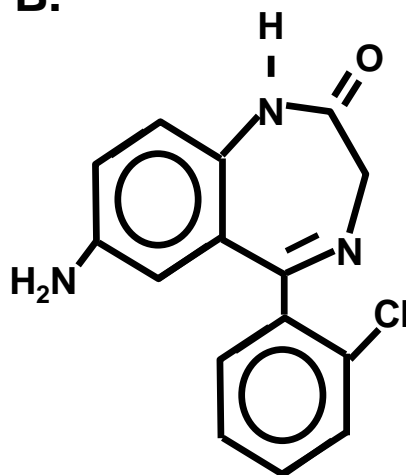
Example for TOF-MS

A.



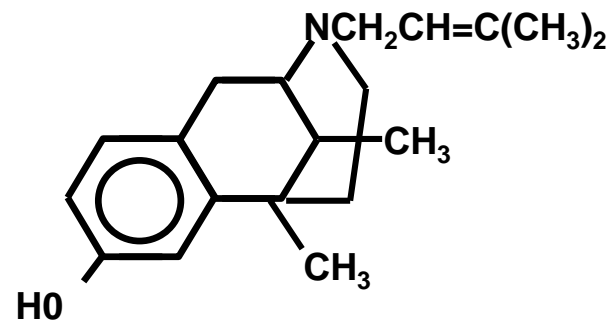
Morphine, $C_{17}H_{19}NO_3$
MW = 285.1365

B.



7-aminoclonazepam
 $C_{15}H_{12}ClNO_3$
MW = 285.0669

C.

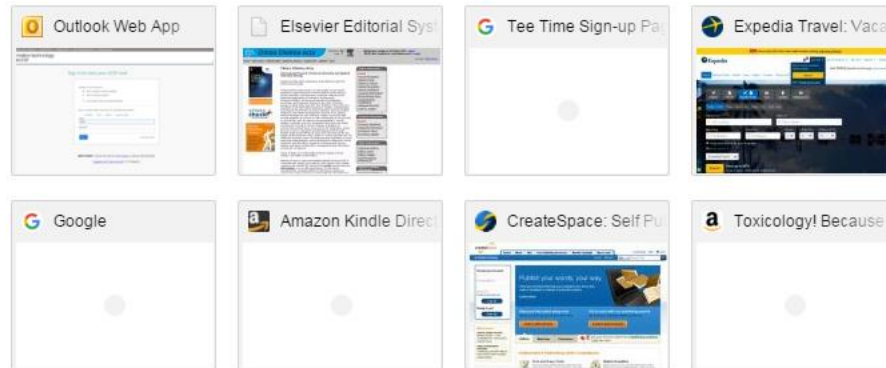


pentazocine
 $C_{19}H_{27}NO$
MW = 285.2093

Unknown searches: from mass spectrum libraries to Google



C19 H27 NO



Unknown searches: from mass spectrum libraries to Google to Wikipedia



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The Free Encyclopedia

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$C_{19}H_{27}NO$

From Wikipedia, the free encyclopedia

The molecular formula **$C_{19}H_{27}NO$** may refer to:

- [Ciprefadol](#)
- [Pentazocine](#)



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If an [internal link](#) led you here, you may wish to change the link to point directly to the intended article.

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Unknown acidosis

CASE 1

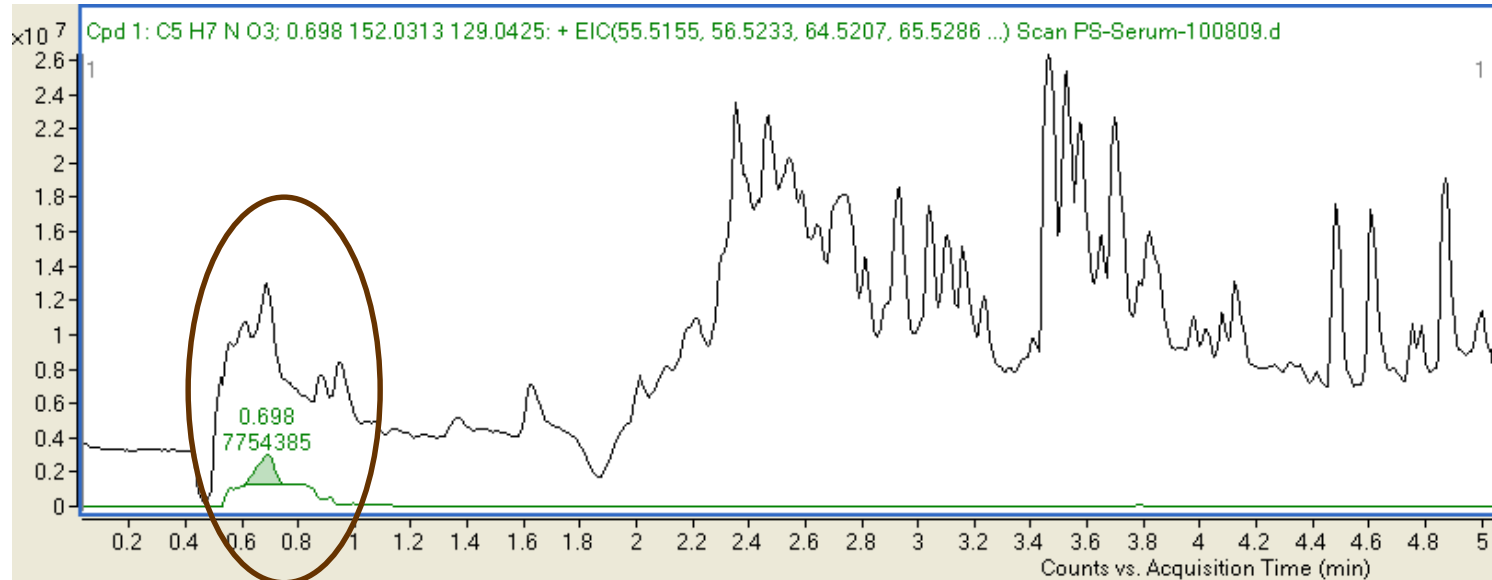
27yo F, Hx of chronic abdominal pain and vomiting, chronic Tylenol use. Acidotic, acetaminophen =3, but lactate, ethanol, methanol, ethylene glycol= neg

CASE 2

38yo F, Hx of depression and chronic pain, possible O/D of Vicodin. Acidotic pH= 6.9, acetaminophen = 371, ALT= 8045, AST=8026, but lactate, ethanol, methanol, ethylene glycol = neg

Cause of acidosis?

LC-TOF



**So what is this compound? Can we match a mass spectrum?
What database is available?**



C5H7NO3

Google Search

I'm Feeling Lucky

https://www.google.com/#pq=c25h34n6o3&hl=en&cp=4&gs_id=k&xhr=t&q=c5h7no3&tok=Ej5kt1vq

File Edit View Favorites Tools Help

c5h7no3

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wualan@labmed2.ucsf.edu

Google

c5h7no3



Search

About 18,100 results (0.09 seconds)

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Find information on molecules with molecular formula **C5H7NO3**. 80 chemical structures have molecular formula **C5H7NO3**.

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[ChemSpider](#) | [PCA](#) | **C5H7NO3**

www.chemspider.com/Chemical-Structure.7127.html

Std. InChI: InChI=1S/**C5H7NO3**/c7-4-2-1-3(6-4)5(8)9/h3H,1-2H2,(H,6,7)(H,8,9)/t3-/m0/s1. Copy Copied. Std. InChIKey: ODHCTXKNWHHXJC-VKHYHEASA-N ...

[ChemSpider](#) | [GLP](#) | **C5H7NO3**

www.chemspider.com/Chemical-Structure.485.html

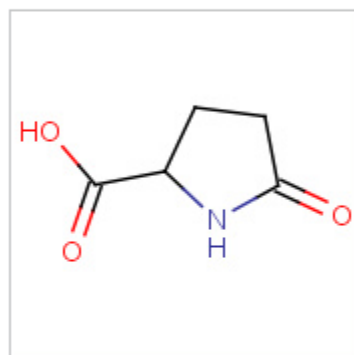
ChemSpider is a free chemical structure database providing fast access to ...

[Show more results from chemspider.com](#)

San Francisco, CA

Change location

3. 🔍

**Compound Number**

MolPort-001-791-750

[Buy From Stock](#)**Availability**

in stock

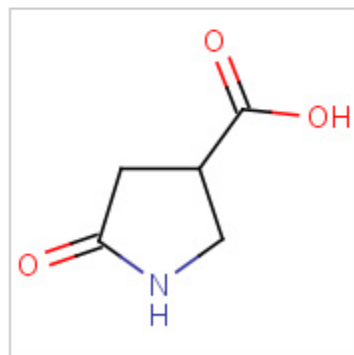
IUPAC

5-oxopyrrolidine-2-carboxylic acid

Names

(5-oxo-DL-prolinato-N1,O2)silver; (±)-2-Pyrrolidinone-5-carboxylic acid; 2-PYRROLIDONE CARBOXYLIC ACID; 2-Pyrrolidinone-(R)-5-carboxylic Acid; Pyroglutamic Acid; bis(5-oxo-prolinato-N1,O2)mercury; bis(5-oxo-DL-prolinato-N1,O2)tin; bis(5-oxo-L-prolinato-N1,O2)mercury; bis(5-oxo-L-prolinato-N1,O2)zinc; tris(5-oxo-DL-prolinato-N1,O2)yttrium;

4. 🔍

**Compound Number**

MolPort-000-005-185

[Buy From Stock](#)**Availability**

in stock

IUPAC

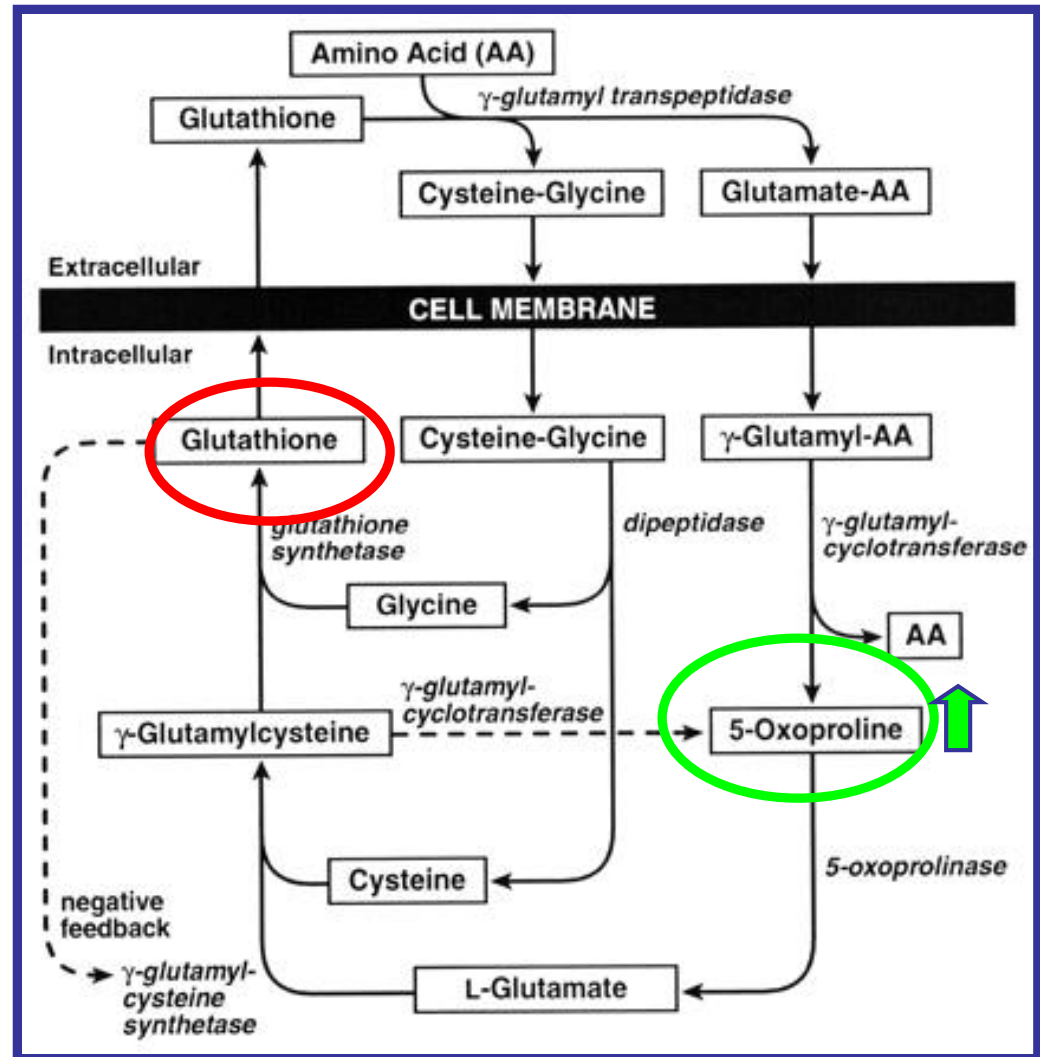
5-oxopyrrolidine-3-carboxylic acid

Names

-; 3-pyrrolidinecarboxylic acid, 5-oxo-; 5-Oxo-3-pyrrolidinecarboxylic acid; 5-oxopyrrolidinecarboxylic acid; 5-Oxo-pyrrolidine-3-carboxylic acid; 5-Oxo-pyrrolidine-3-carboxylic acid; 5-oxopyrrolidine-3-carboxylic acid; 5-oxopyrrolidine-3-carboxylic acid;

Oxoproline acid accumulation

- Chronic ingestion and acute intoxication of APAP depletes the body of glutathione
- Accumulation of γ -glutamylcysteine and overproduction of 5-oxoproline



“Slide Toxin”: what’s wrong with this picture?

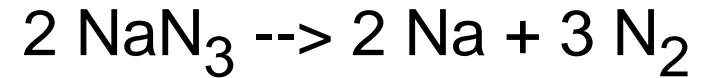


FAA: Evacuation of all in 90 seconds?



Two evacuation chutes deployed inside trapping individuals to the floor. Bags were sliced open. Several passengers admitted to SFGH-ICU with significant delayed acidosis.

How do airplane chutes deploy?



130 grams sodium azide ignited within msec producing 67 l of N_2

50 kg in airplane chute.

Death following sodium azide ingestion. J Foren Sci 1990;35:193-6.

Herbals: natural, but safe? “Jacked!”

- 24-y old US soldier in Iran buy a muscle supplement in the Marine Corp. base shop.
- During a workout, develops chest pain and dies of a sudden-death MI.
- Autopsy conducted shows totally clean arteries.
- What was in that supplement?

dimethylamylamine



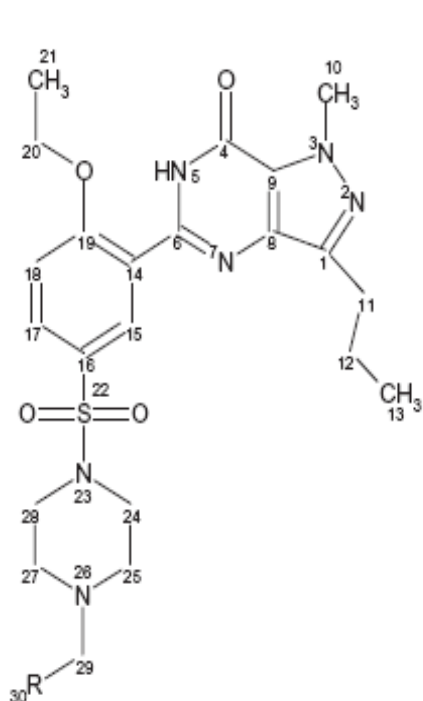
“Duffer dysfunction!”

- An senior professional golfer orders an erectile dysfunction herbal over the internet
- After an event, he hooks up with a female fan. He uses an extra dose of the ED drug.



Analysis of herbal supplements

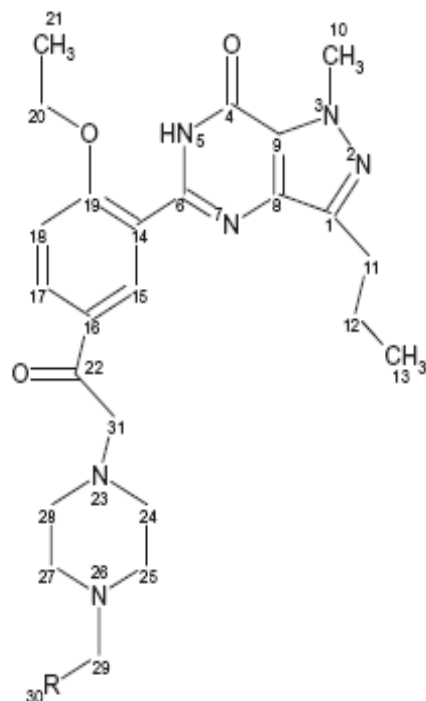
Name	Score (MFG)	RT	Mass	Diff (MFG, ppm)	Formula (MFG)	Ions	Height
Acetildenafil	84.05	4.147	466.2700	-1.7	C ₂₅ H ₃₄ N ₆ O ₃	4	1548390
Hydroxyhomosildenafil	91.08	4.602	488.2208	-0.53	C ₂₃ H ₃₂ N ₆ O ₄ S	7	1118384



Sildenafil: R=H

Homosildenafil: R=CH₃

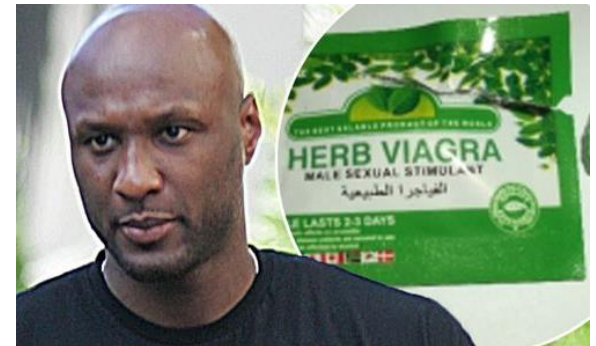
Hydroxyhomosildenafil: R= CH₂OH



Acetildenafil

Life imitates art

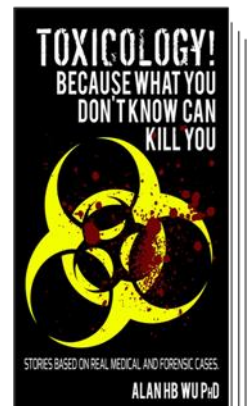
Can this happen?
Story written in 2013



“The Spice of Dyslife”

A 36-y old female actress is preparing for a part. Her agent tries to relax her by baking “cookies.” The actress eats them and becomes hallucinogenic. Calls husband says, “I want to jump out of a window.” Husband rushes home, puts wife to bed. While he is in bathroom, wife jumps out of 3rd floor window. Dies of traumatic injuries.

Urine drug screen is negative for THC.





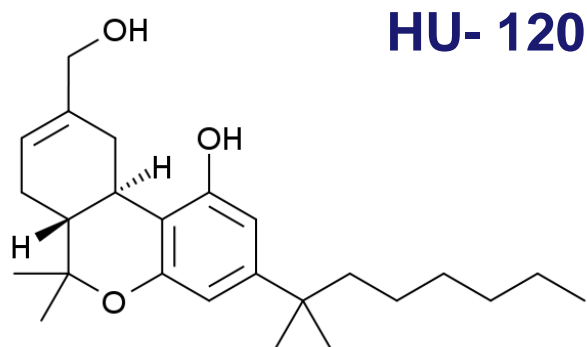
SPICING it up with Synthetic Cannabis



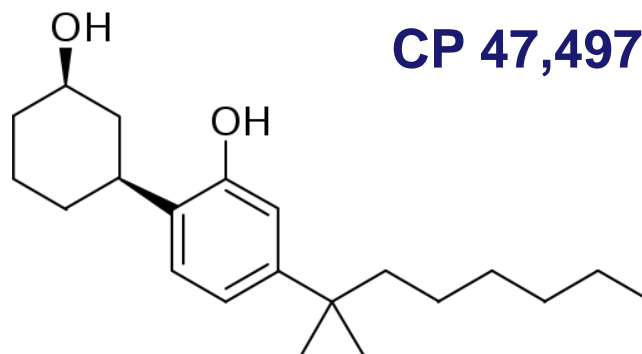


Synthetic cannabinoids

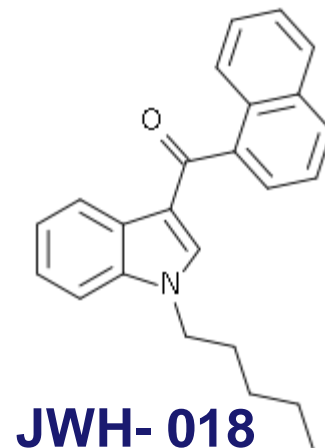
Classical cannabinoids



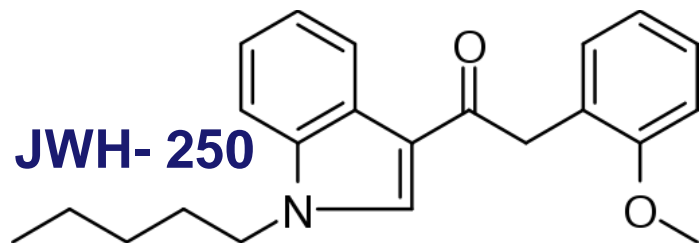
Cyclohexylphenols



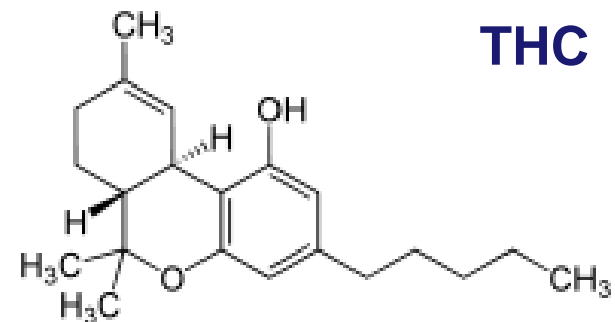
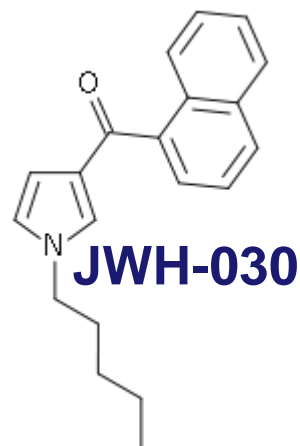
Naphthoylindoles



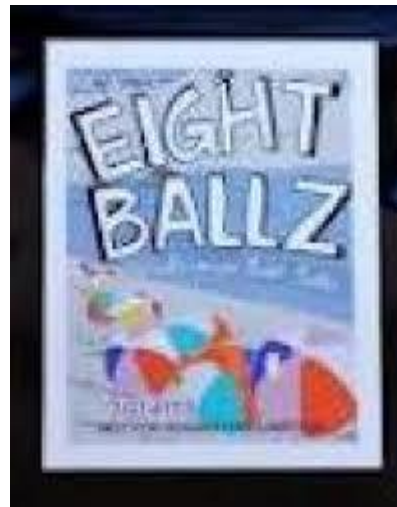
Phenylacetylindoles



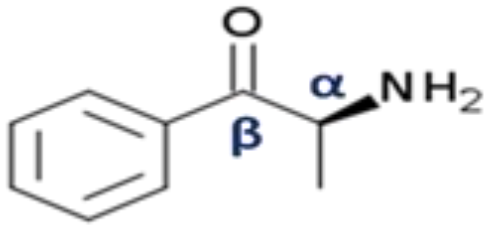
Naphthoylpyrroles



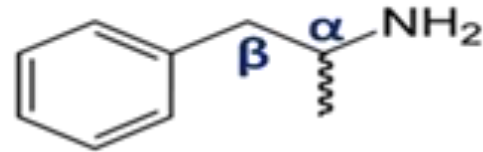
Bath salt products



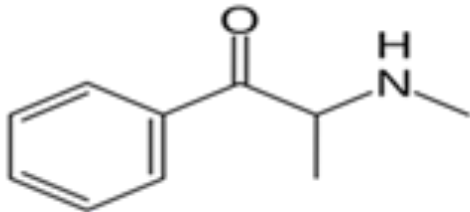
Bath salt chemistry



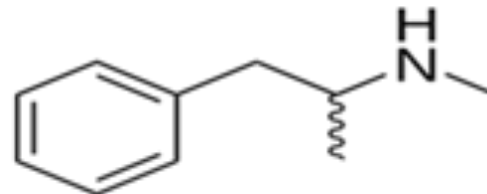
Cathinone



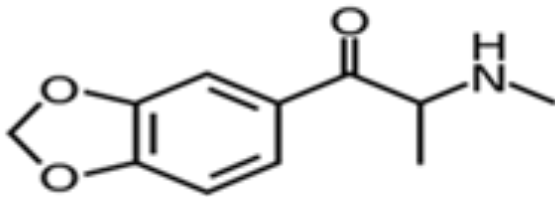
Amphetamine



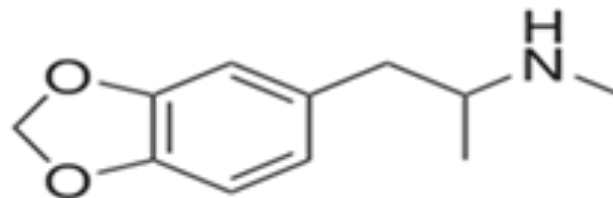
Methcathinone



Methamphetamine



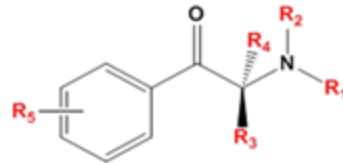
Methylone



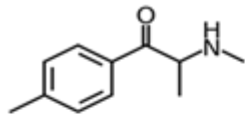
MDMA

Other synthetic amines

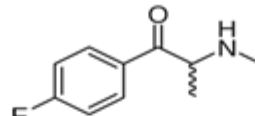
A. Parent structure of synthetic cathinones



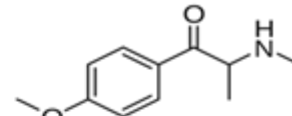
B. Ring-substituted cathinones



Mephedrone

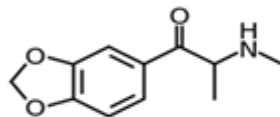


Flephedrone

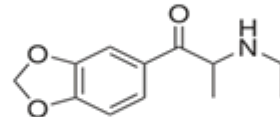


Methedrone

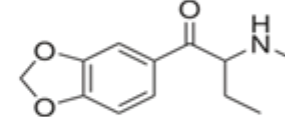
C. Methylenedioxy-substituted cathinones



Methytlone

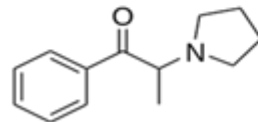


Ethylone

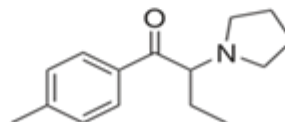


Butylone

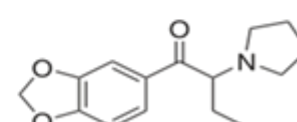
D. Pyrrolidinyl-substituted cathinones



α -PPP



Pyrovalerone



MDPV

“Rave”

A 25-y old male attends a Rave party. The arena is dark, warm and a strobe light flashes the kids while dancing. He is in the mosh pit bumping and grinding with others. During a break someone gives him a free pill. 45 min later, he passes out and taken to the hospital in a coma.

My post doc is called and comes into the lab to do the analysis. Police think it is tainted Ecstasy.







IN RD

Rave or adulterant? Labs- Capsule analysis

Fifteen capsules/pills were submitted by the DCPD for analysis

CASE 34A- XZ- White Capsule (135mg)

CASE 34B- VT- Light Yellow Capsule (275mg)

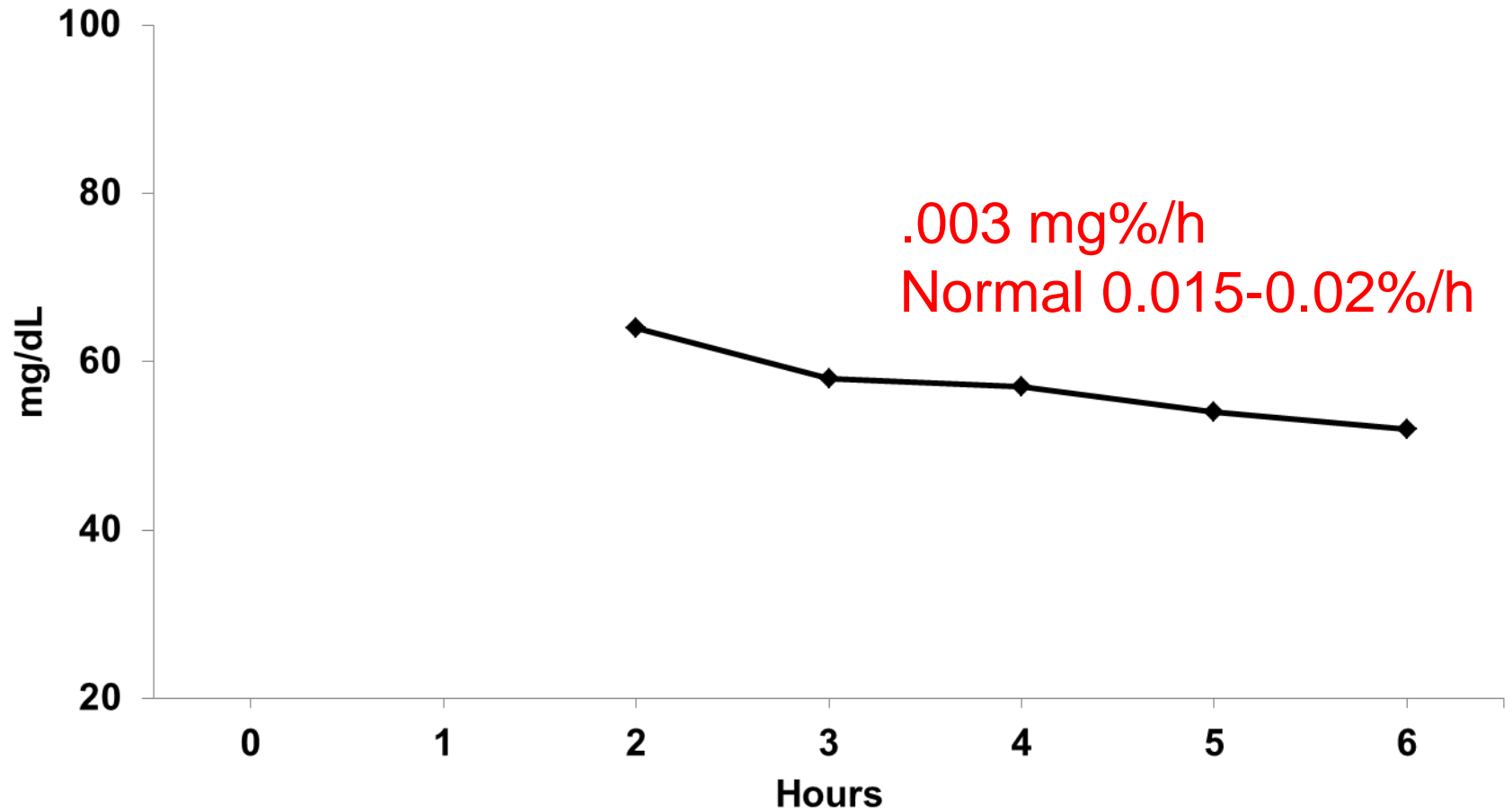
Dosage of Regular Ecstasy in the Black Market: 80-120mg/ pill
Ecstasy is regularly sold as a pill **NOT AS A CAPSULE**; apparently, the two samples above are **suspiciously just being given away instead of being sold.**

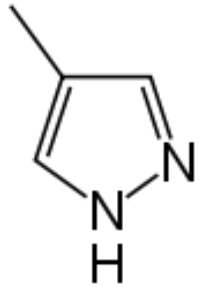
“Sloe gin”

- Physician tests positive for alcohol, license suspended by CA medical board.
- Had 3 glasses of wine the night before. Claims he is a slow metabolizer, was not drinking on the job.
- Experiment conducted: drank 3 glasses, blood collected and tested hourly for ethanol.
- Normal metabolic rate: 15-20 mg/dL. Physician: 3 mg/dL.
- But did he cheat?

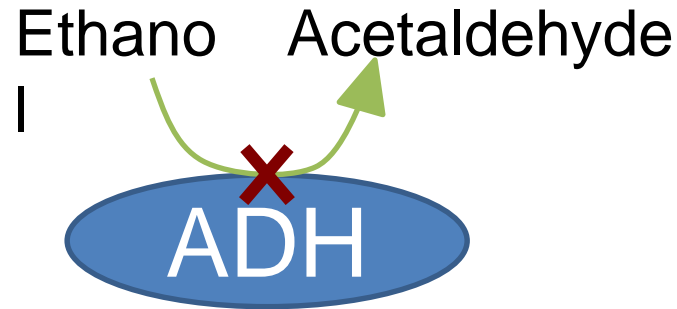


Blood Alcohol Concentrations





Fomepizole
MW 82 Da



Can we identify
Fomepizole in subject's
serum?

“Blood beer making”

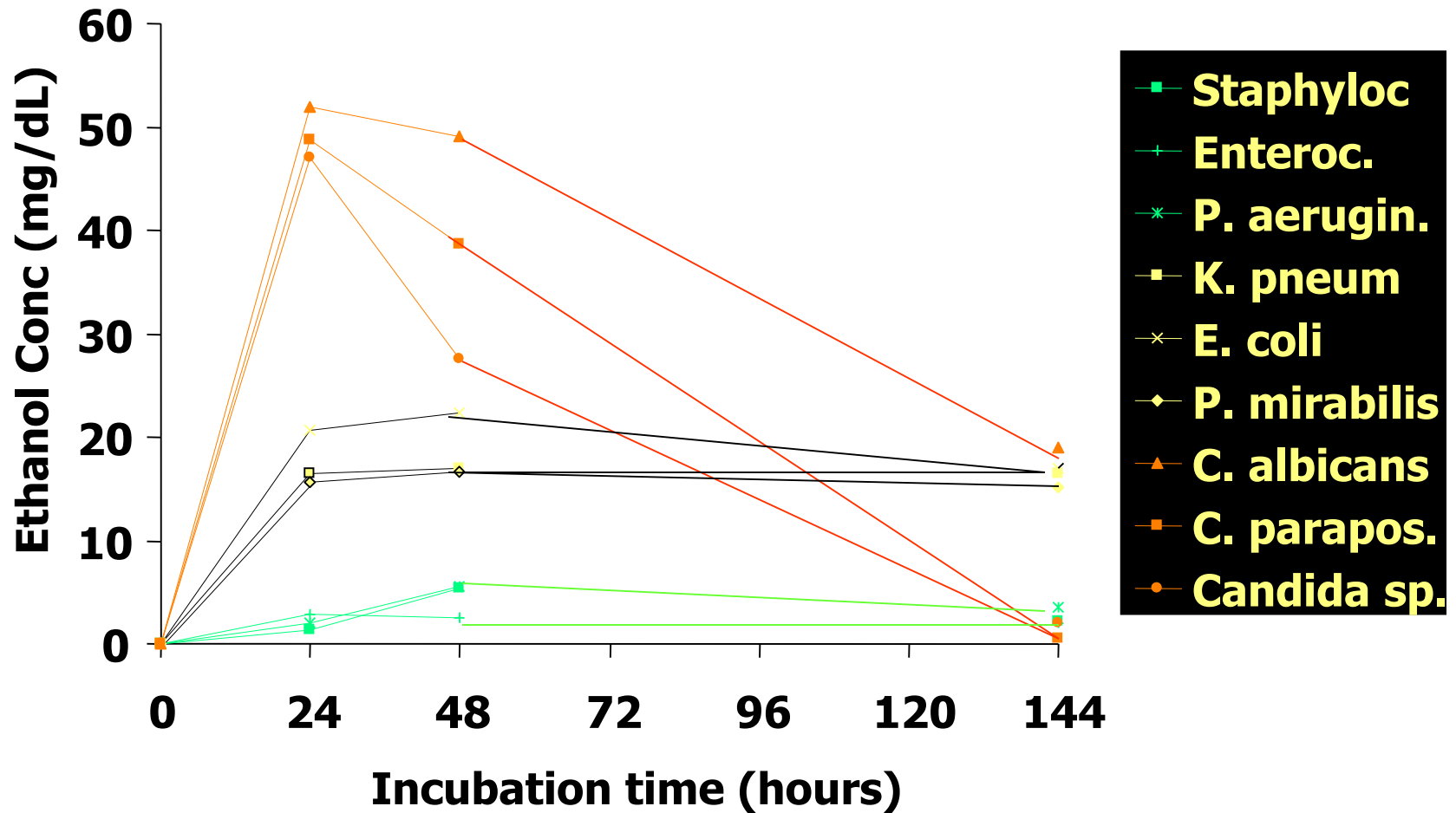
A driver is injured in a traffic accident. His passenger is killed. Blood test at the hospital is positive for alcohol. After his hospital discharge, driver is arrested for DUI and involuntary manslaughter.

In court, defense attorney states that he is a diabetic and that his blood alcohol was from fermentation.



Ethanol production by fermentation

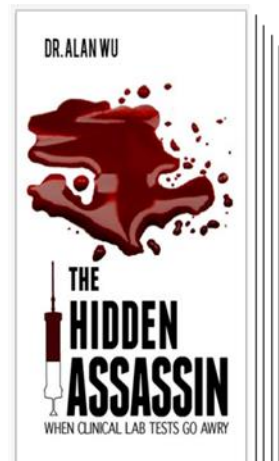
Glucose substrate



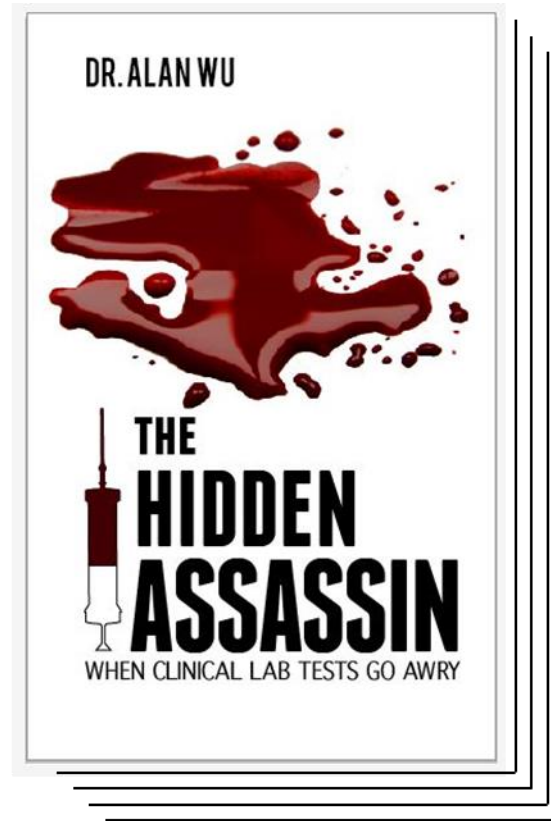
“Blood beer making”

In vivo fermentation requirements:

1. Bacterial source. No evidence of systemic infection.
2. Hyperglycemia. No evidence of uncontrolled diabetes
3. Incubation time (days required). Lab testing occurred immediately
4. In vivo fermentation occurs more frequently in urine and postmortem blood (due to putrefication).



Paperbacks with real stories of personalized medicine

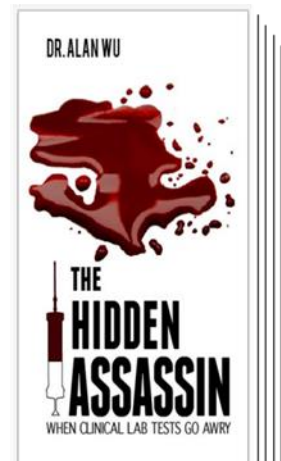


*Clin lab tests that solved cases
or caused harm*

“Explosive Blood”

A chemist works in his father’s rug factory. He is playing soccer when he collapses on the field. At ZSFG he is cyanotic. Blood gas analysis reveals an O₂ desaturation of 74% (normal 95-100%).

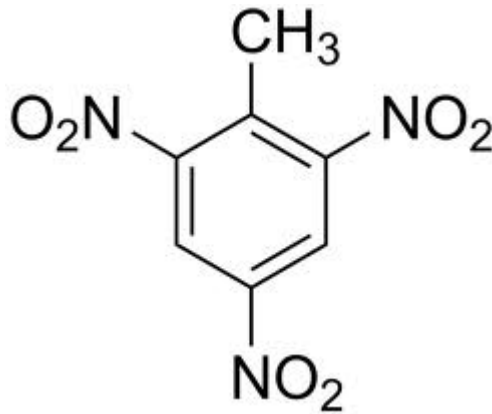
Toxicological analysis was conducted to determine the source of the methemoglobinemia.



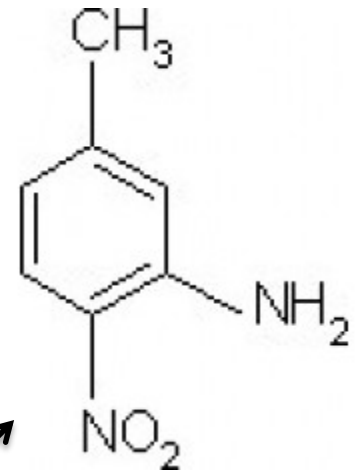
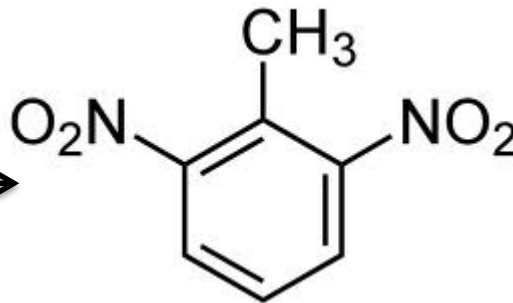


“Explosive Blood”

MS-TOF findings:



TNT



Homeland security was called, who searched the house. Chemicals used to make bombs were found.

Knife Attack

A man was jailed for stabbing a 41-yo woman in the neck at a park.

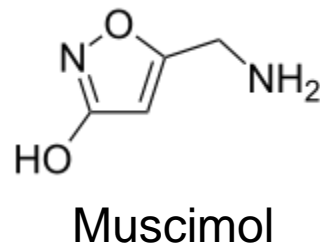
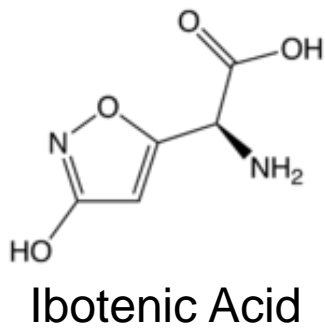
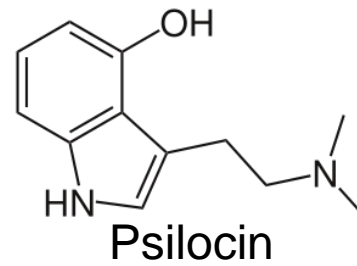
Several passing motorists witnessed the incident and stopped to render aid until emergency personnel arrived.

Was the man impaired? Man's home was searched.



A. gemmata found 50'
from subject's front door

Hallucinogenic mushroom toxins

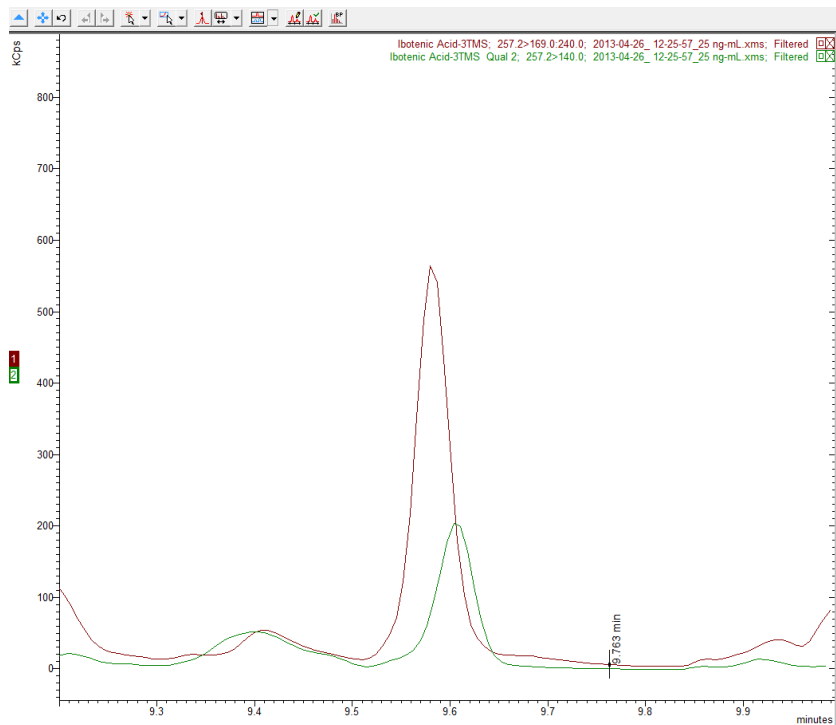


Hallucinogenic mushroom toxins

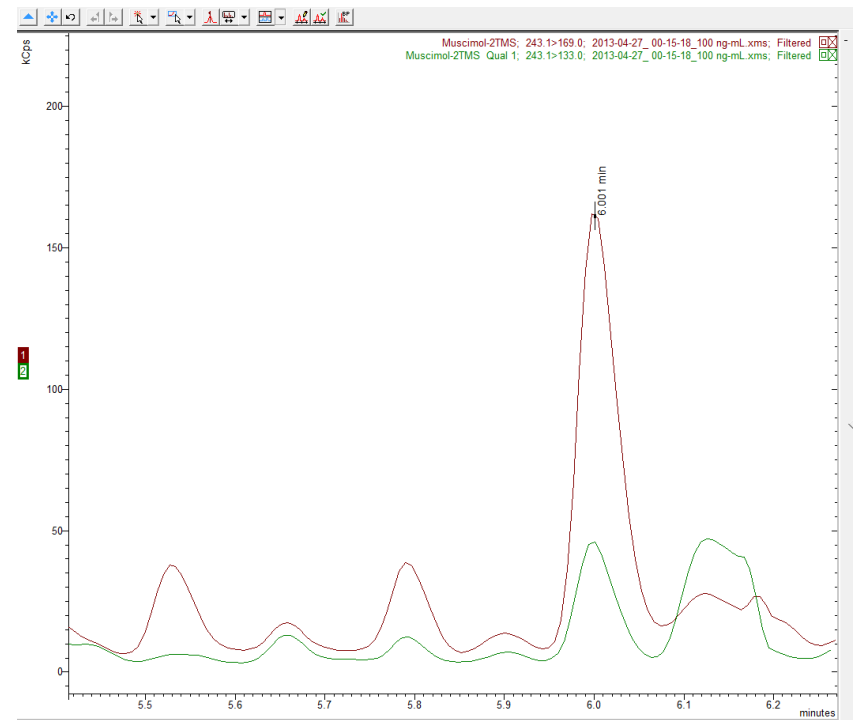
- Extracting active compounds from dried mushroom material needed
- Molecules of interest:
 - Muscimol (*Amanita* species)
 - Ibotenic Acid (*Amanita* species)
 - Psilocybin (*Psilocybe* species)
 - Psilocin (*Psilocybe* species)

LOQs extracted from mushroom tissue

Ibotenic Acid: 25 ng/mL



Muscimol: 100 ng/mL

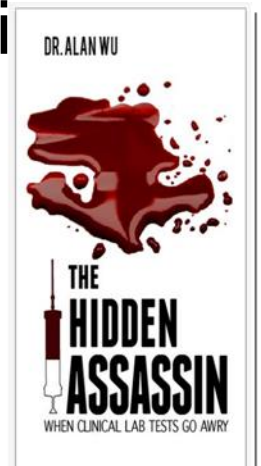


Did he eat the mushrooms?

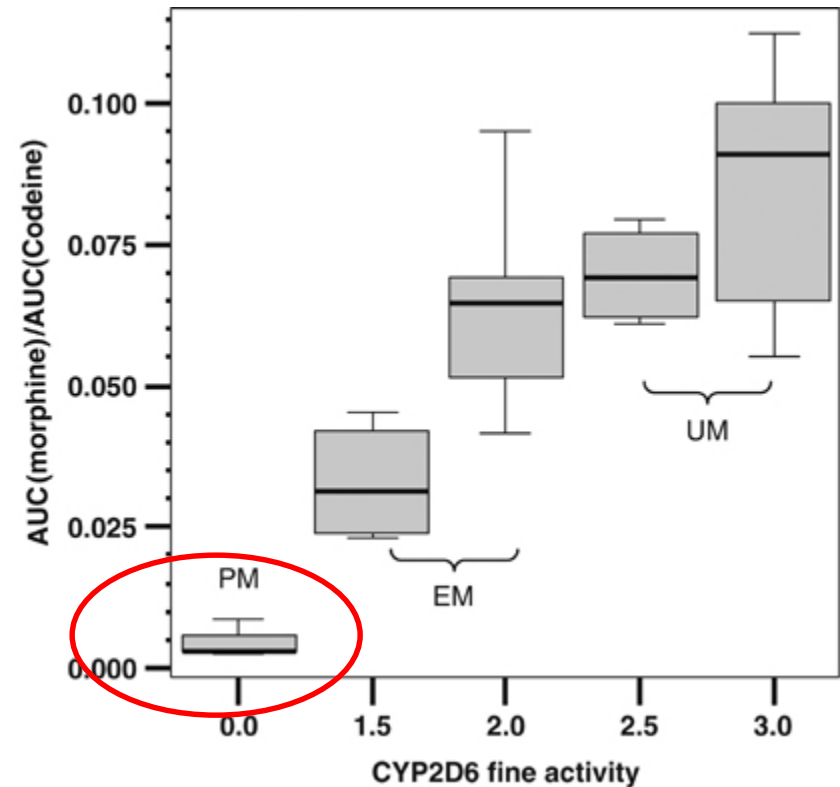
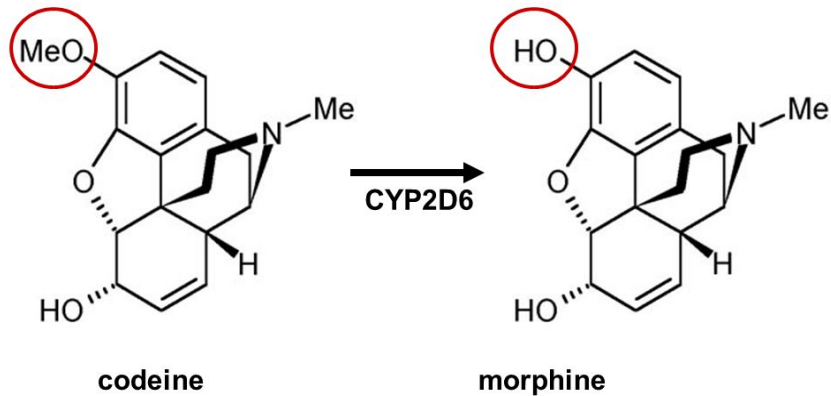
Sample	Ibotenic Acid (ng/mg)	Muscimol (ng/mg)
1	492 ± 96	1194 ± 137
2	1548 ± 198	1628 ± 131

“Lesphine”

- Self medicates himself on codeine
- Gets into a car accident, seriously injures pedestrian
- Convicted and sentenced for DUI
- Court overturned with pharmacogenomics new evidence: he is a CYP 2D6 poor metabolizer
- Judge rules he was not impaired by morphi



Prodrug codeine metabolizes to morphine by liver enzyme CYP 2D6

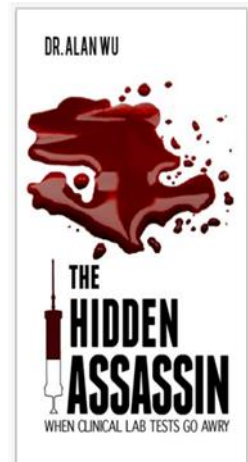


“Not Mine”

A 25-y old female legal aide undergoes mandatory drug testing and comes up positive for THC.

“A mistake has been made. I don’t use marijuana. The lab must have had a sample mix-up.”

She agrees to have DNA analysis of the blood, fresh urine, and archived urine positive for THC



“Not Mine”

My lab offers DNA identity analysis for urine.
She comes to my lab with government photo ID.

Wu: “do you have a twin sister?” No

“Had a kidney transplant?” No

“Do you have any identifying tatoos?” Why?

“If the suspect urine does not match your fresh urine,
I have to prove the urine came from you.”

“Yes.” A photo is taken for my records.



DQA-PM1: A perfect identity match

Fresh serum



Fresh urine

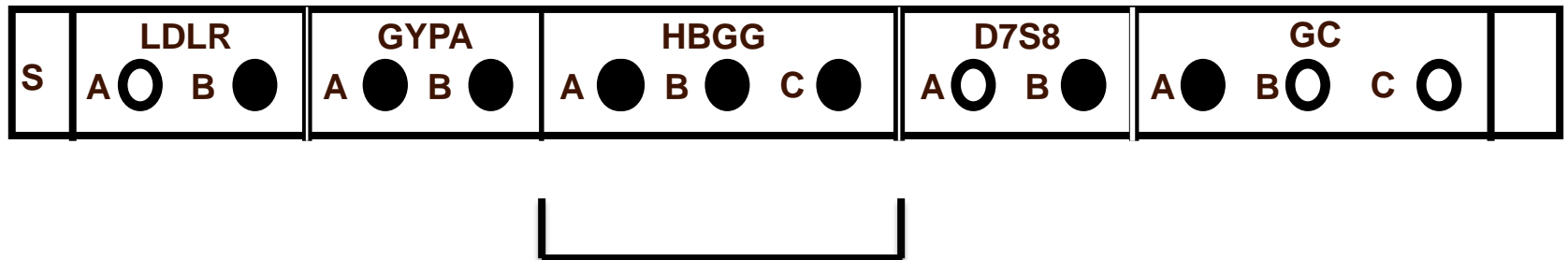


Toxicology sample, drug positive



Likely contaminated specimen: 3 different alleles

Toxicology sample, drug positive



Kidney transplant recipient

Fresh serum



Fresh urine



Toxicology sample, drug positive



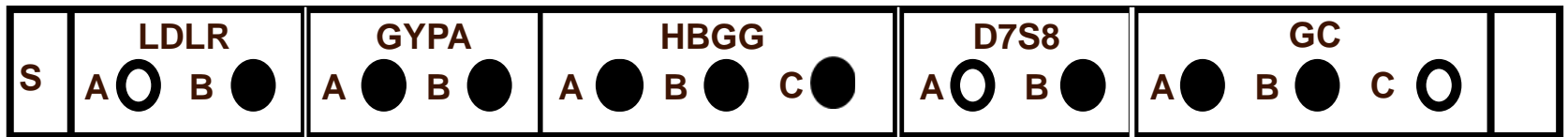
Same contamination at different sites and on different days?

“Not Mine”

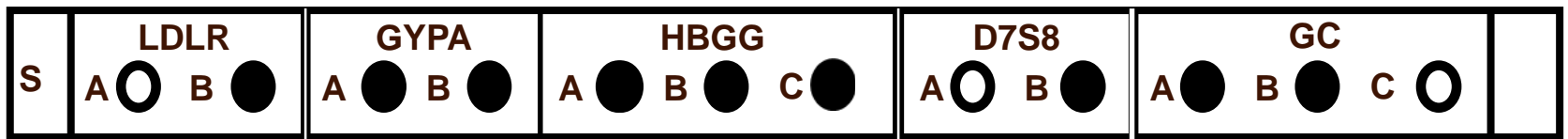
Fresh serum



Fresh urine



Toxicology sample, drug positive

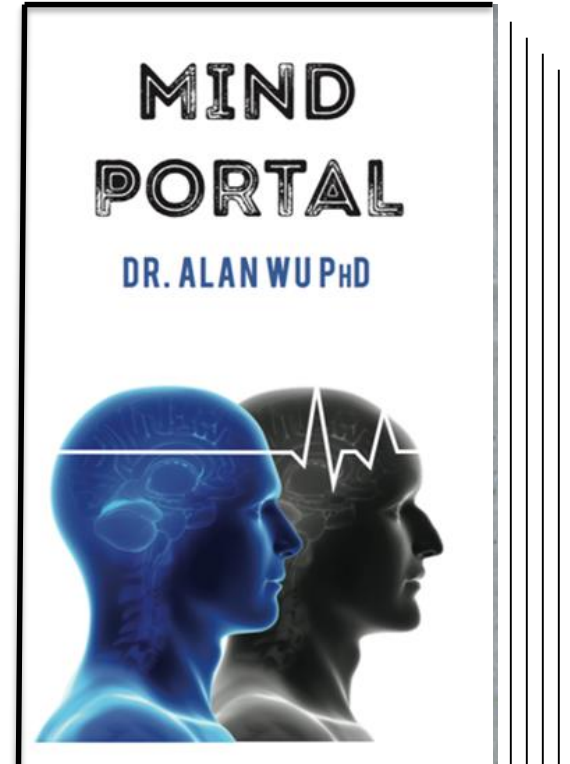
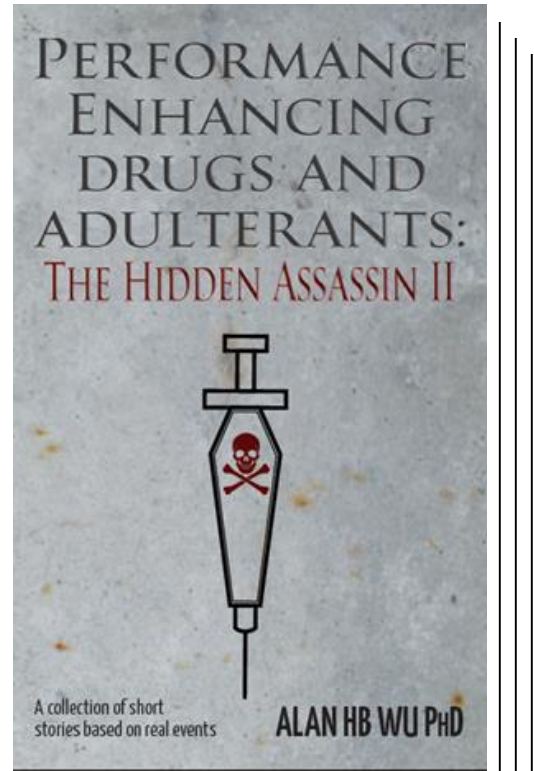
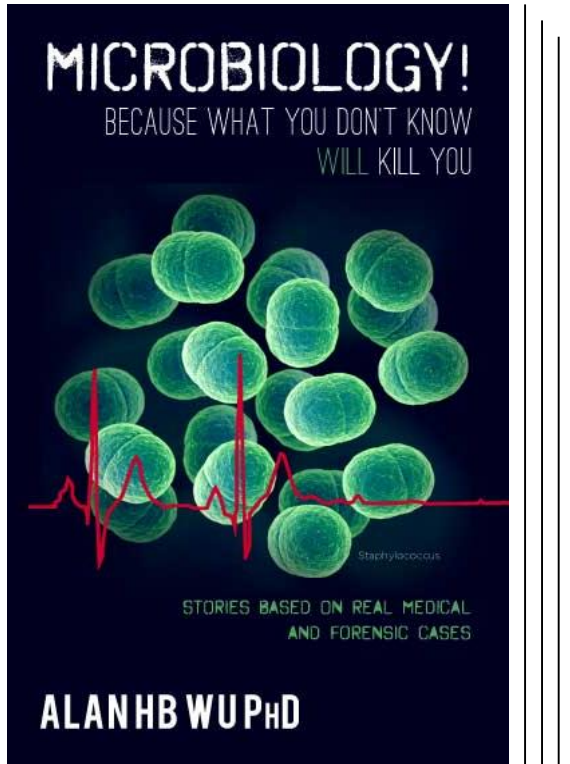


“Not Mine”: resolution

- Legal aide has sexual intercourse with the same man on the night before urine donation (on both occasions).
- Spermatozoa DNA present in vulvular region during donor void.
- THC comes from female donor, i.e, no urine contamination by male partner



Other Wu Paperbacks



SFGH's clinical and forensic toxicology lab

- Medico-legal cases; expert witness testimony
- Biological fluids analysis:
 - Postmortem
 - Clinical toxicology supporting CA Poison Control Center
- Drugs, powders, pills
- DNA identity analysis
- Molecular analysis