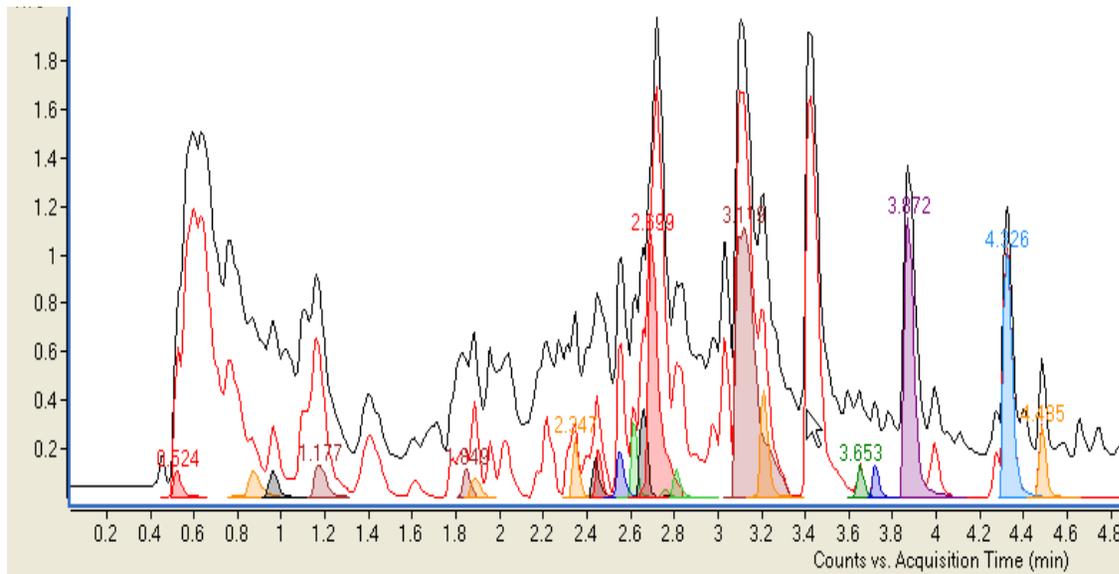


Solving Poison Control Center intoxication cases using Time-of-Flight Mass Spectrometry

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University of California, San Francisco*



Outline

Toxic Substance Identification

Time-of-Flight Mass Spectrometry

**Targeted and Non-targeted Drug Screening
Using TOF LC-MS**

Cases

**(Potential Applications to Environmental
Biomonitoring)**

Emergency Intoxications

Annual ED visits: 124 M

Emergency intoxications: 0.9 M

Toxicology Screens ordered: 3.7 M

Drugs Most Commonly Reported

Ibuprofen (1- 17M)

Acetaminophen/ Hydrocodone (2- 14.5M)

Morphine (6- 7M)

Hydromorphone (8- 5.7M)

Acetaminophen/ Oxycodone (9- 5.3M)

Diphenhydramine (13- 3.6M)

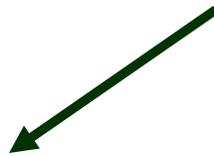
Drugs Commonly Detected: Ethyl alcohol, Cocaine, THC, Heroin, Stimulants, PCP, MDMA

Identifying Toxic Substance in Patients

Toxidromes (T, BP, HR, RR, mental status)



Initial Diagnosis



Therapeutic Intervention



Order Tests (e.g. DAU)



Confirm/ Revise Diagnosis



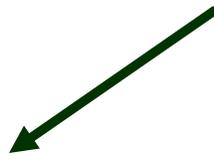
Patient Disposition

Identifying Toxic Substance in Patients

Toxidromes



Initial Diagnosis



Therapeutic Intervention



Order Tests (e.g. DAU)



Confirm/ Revise Diagnosis



Patient Disposition

Identifying Toxic Substance in Patients

Challenges in Emergency Intoxication Testing

- ❖ lack of serum comprehensive drug tests for emergency testing
- ❖ long turnaround times of analysis (1-2 days)
- ❖ lack of information on the clinical utility of laboratory test results
- ❖ some emergency cases are quite complex that targeted screening may not be sufficient

Most Common Methods Used in Toxic Substance Identification

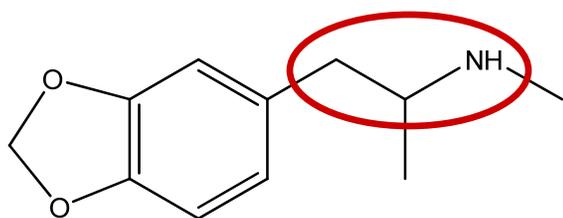
Immunoassay

Chromatography- HPLC, GC

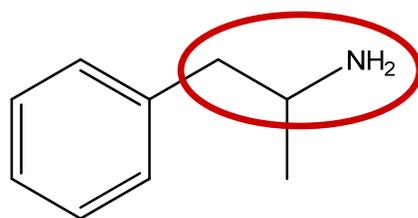
LC-Mass Spectrometry/ GC-Mass Spectrometry

Immunoassay

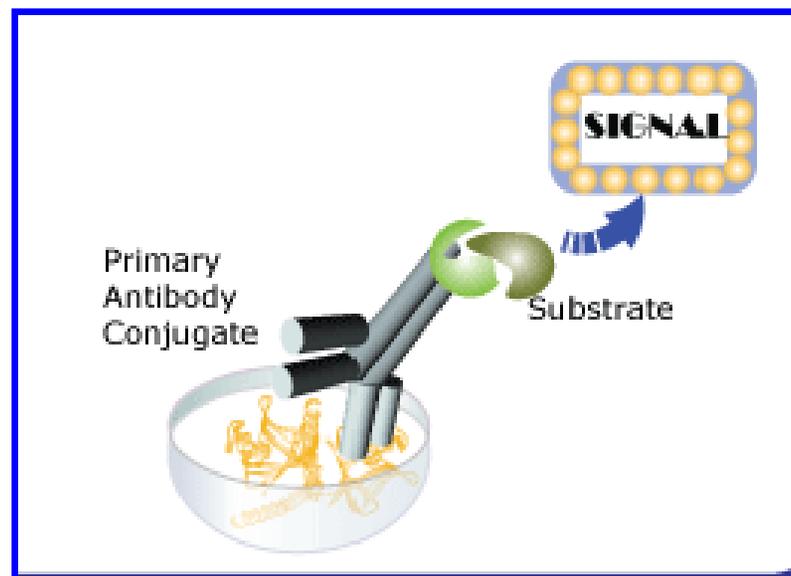
Use of antibody to identify a structural motif in a compound or class of compounds



MDMA (Ecstasy)



Amphetamine



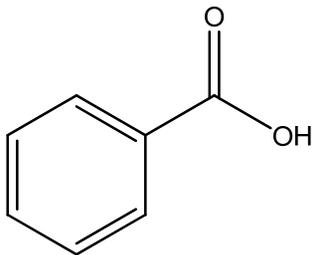
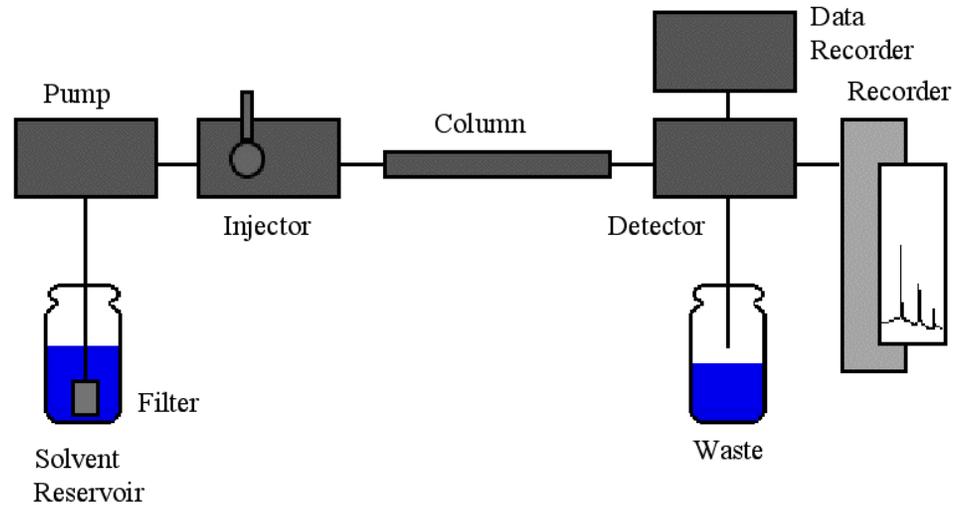
Targeted screening- antibody is raised against a specific compound or class of compounds

Specificity is not always good; cross-reactivity of compounds with similar structural motif to the targeted compound

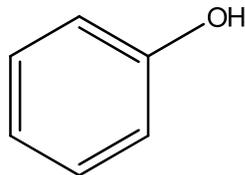
Chromatography

Separation of compounds according to differences in polarity

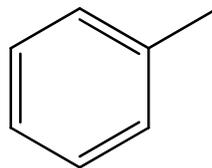
Compounds are detected according to their chemical property (e.g. UV absorbance)



1



2



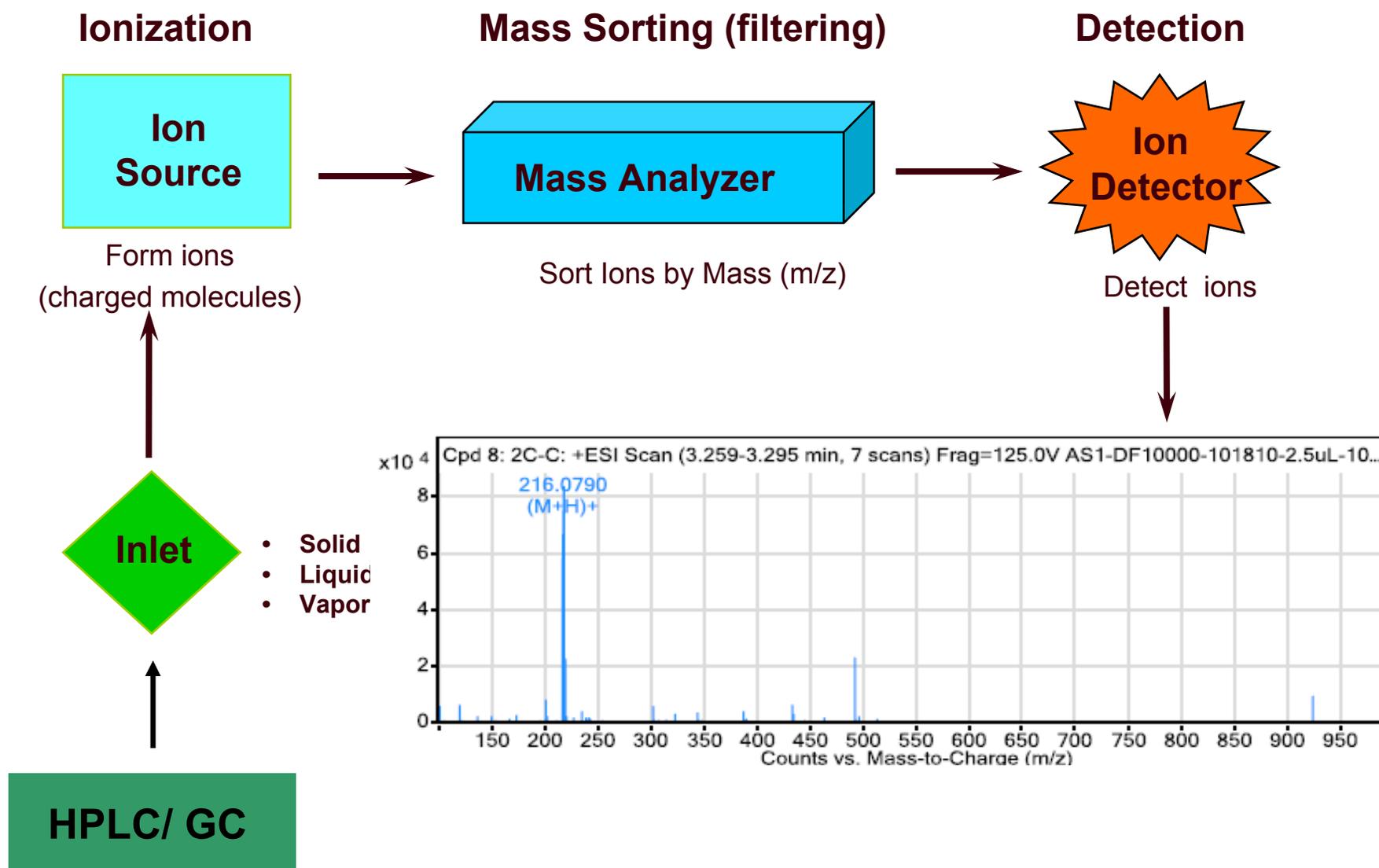
3

Order of elution in a NON-POLAR stationary phase:

1 > 2 > 3

Limited specificity in complex biological matrices- urine and serum

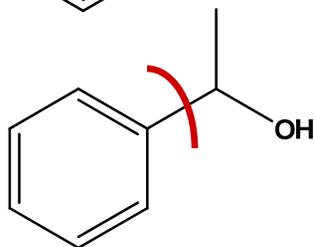
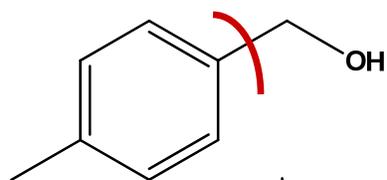
LC (GC)- Mass Spectrometry



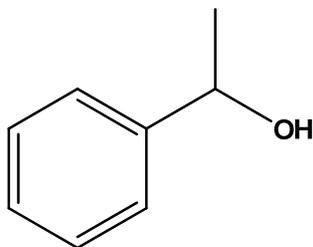
LC (GC)- Mass Spectrometry

Modes by which MS identify molecules

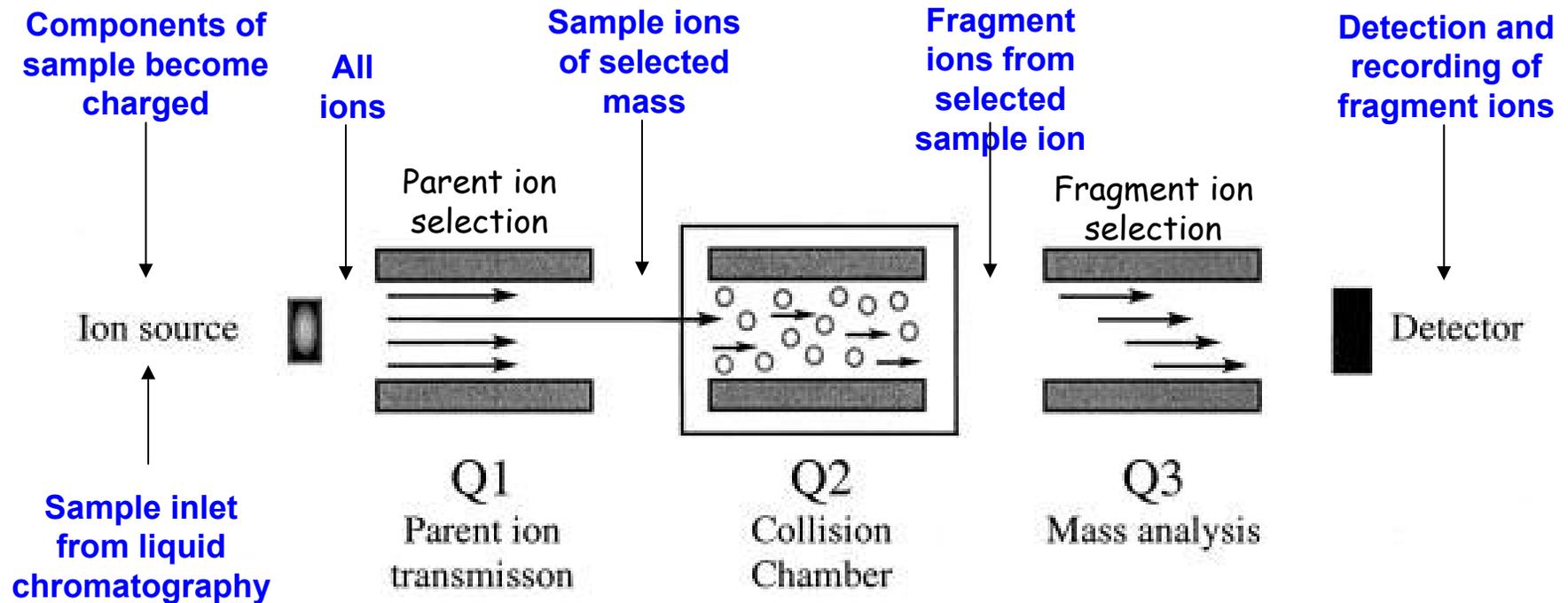
1. Unique fragmentation pattern



2. Accurate Mass



LC-MS/MS Mass Analyzer



- Q1. Ions of interest are selected (parent ions)**
- Q2. Fragmented into smaller product ions**
- Q3. Product ions separated by mass (m) to charge (z) ratio (m/z)**

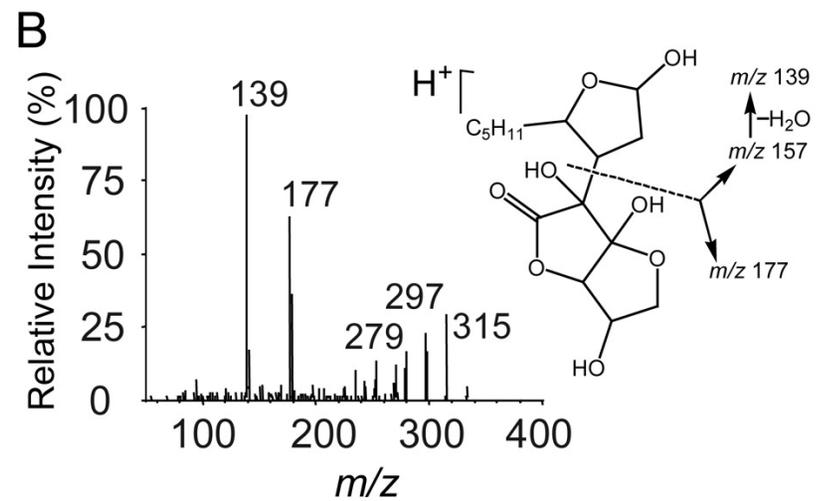
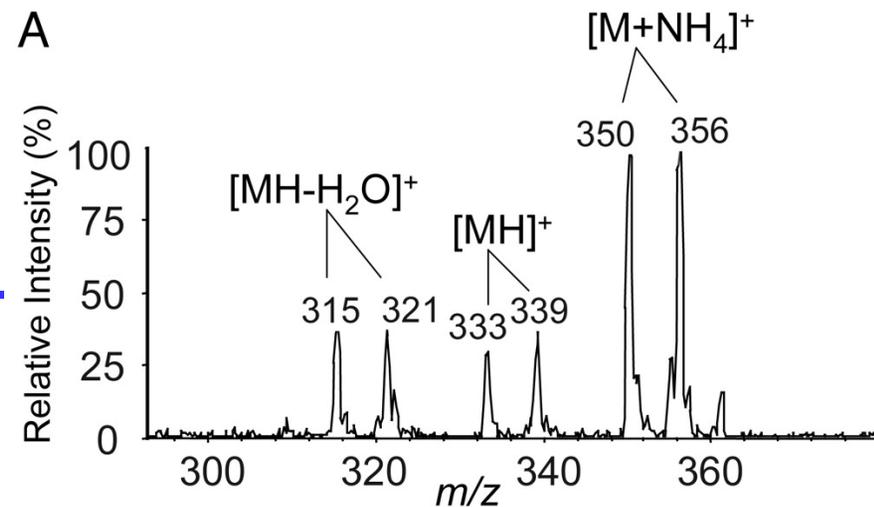
Liquid Chromatography- Mass Spectrometry

Compounds are characterized by their specific fragmentation patterns (specific mass transitions)- e.g. $339 \rightarrow 177$ and $339 \rightarrow 139$

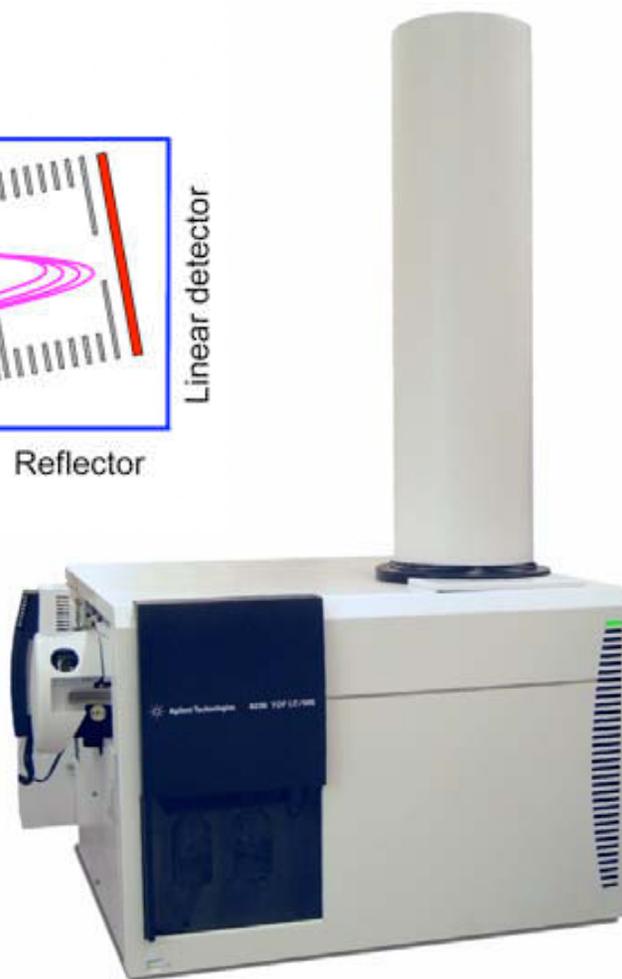
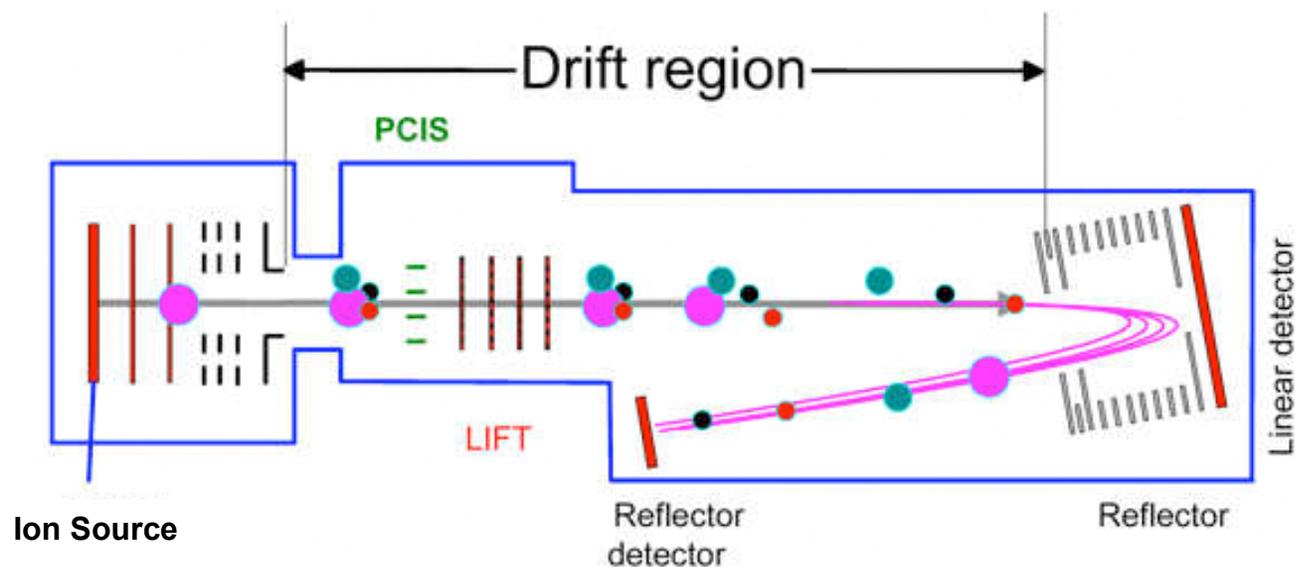
Compounds are identified by building a database of specific transitions

Method has high specificity and sensitivity

Cannot be used for non-targeted screening



Time-of-Flight Mass Spectrometry (MS-TOF)

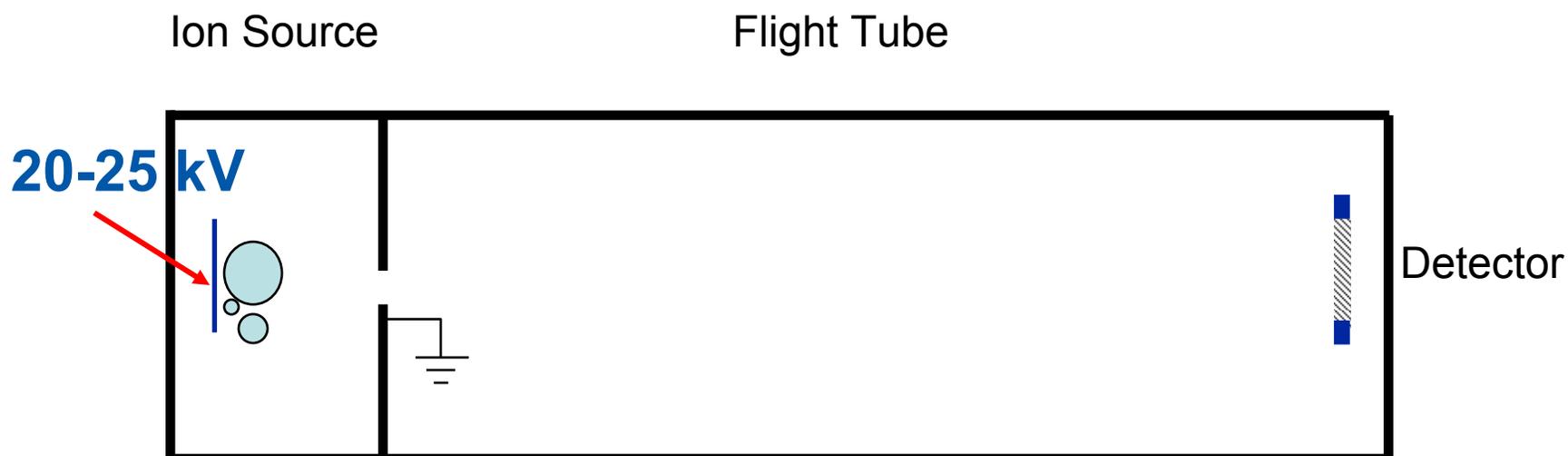


Time of flight of ions measured

TOF of ion is proportional to its m/z

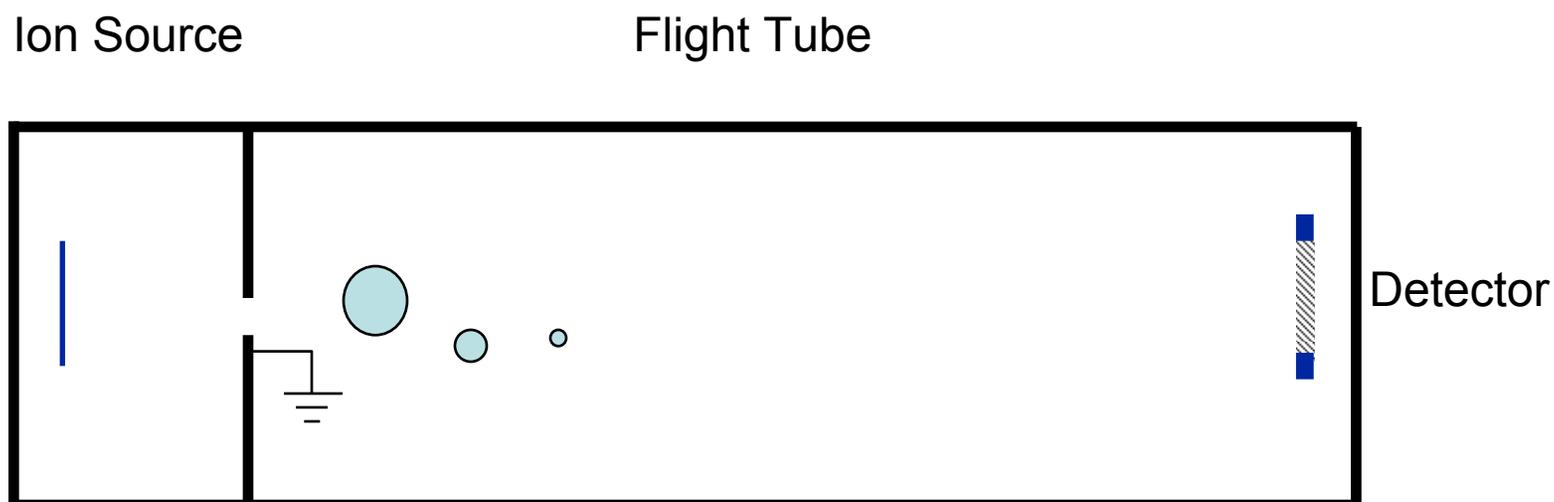
High mass accuracy in the sub-2 ppm

Time-of-flight mass analyzer



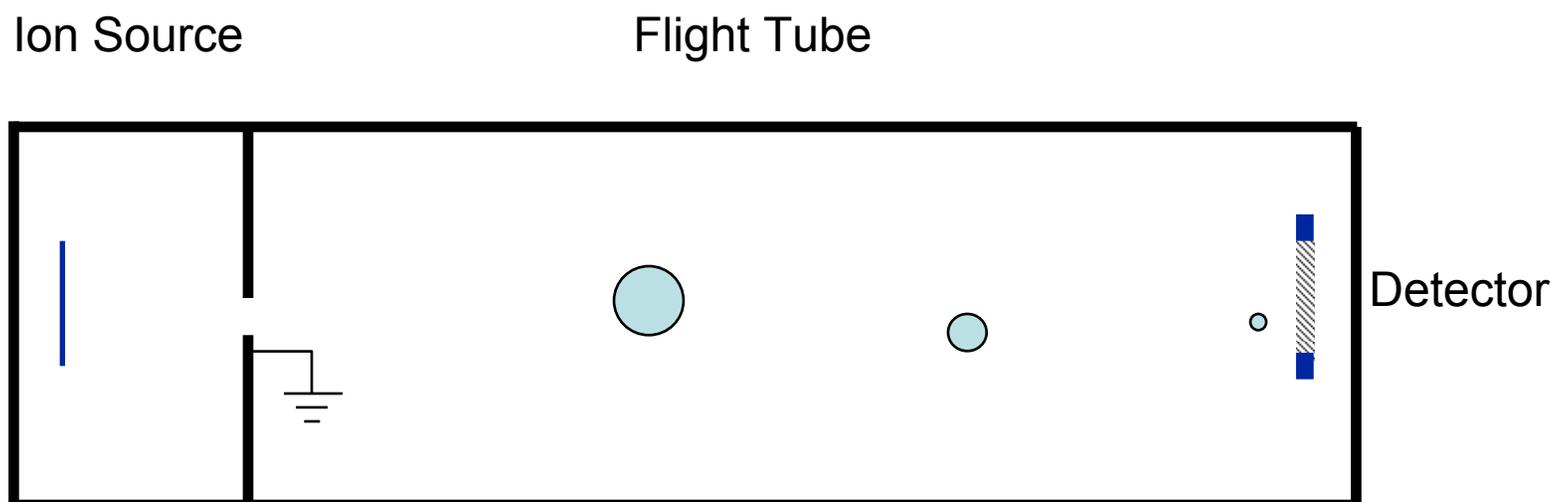
Principle: If ions are accelerated with the same potential at a fixed point and a fixed initial time and are allowed to drift, the ions will separate according to their mass to charge ratios.

Time-of-flight mass analyzer



The ions enter the flight tube with the lighter ions travelling faster than the heavier ions to the detector

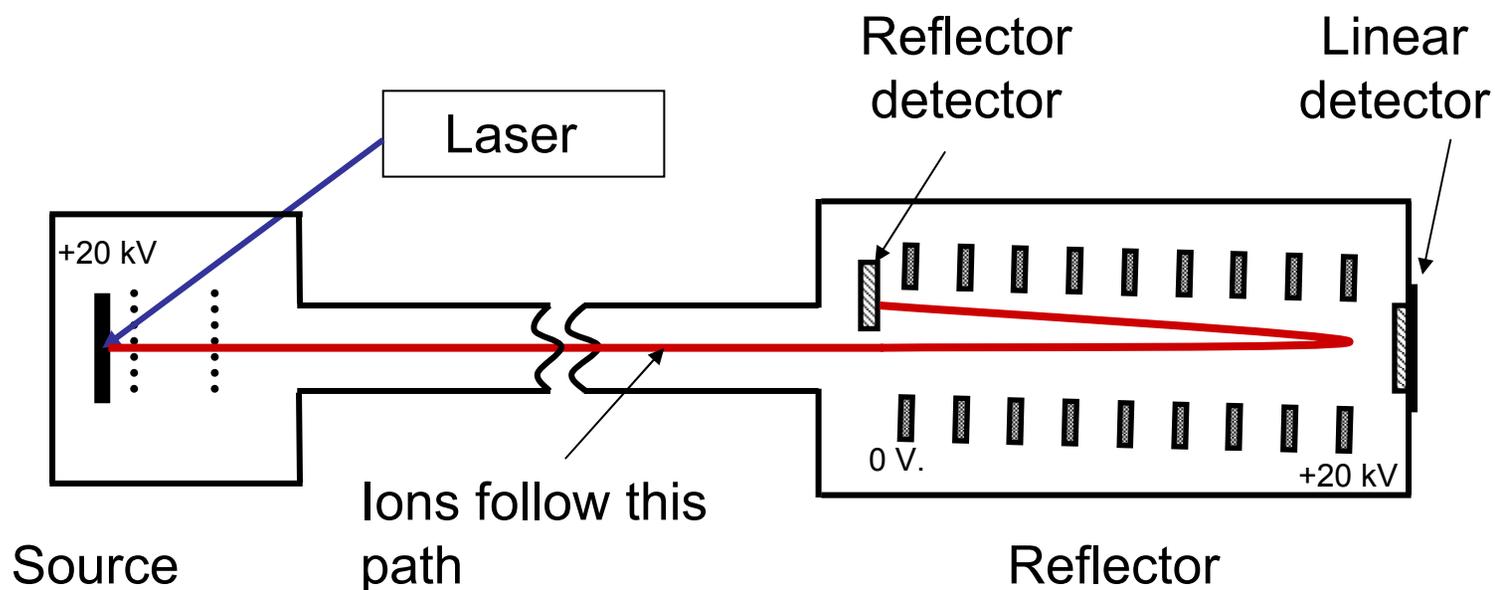
Time-of-flight mass analyzer



The lighter ions strike the detector before the heavier ions. This “time of flight” (TOF) can be converted to mass

TOF reflectron allows for higher accuracy

Ions are sent back down part of the flight tube.
This device corrects for ions of same mass but
different energies



Mass Accuracy

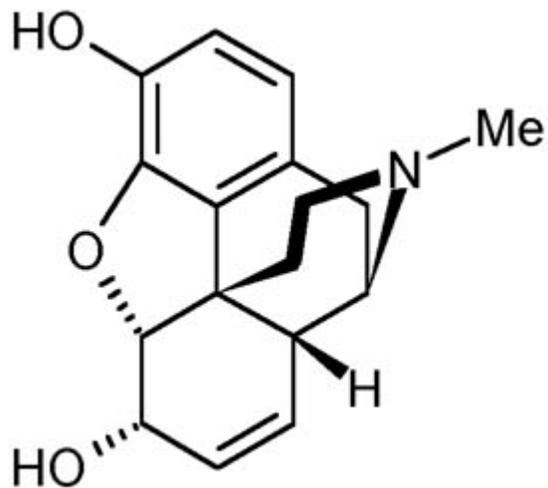
Accuracy is measured as mass error (in ppm)

$$\begin{aligned}\text{Mass error (ppm)} &= \frac{|MW_{\text{meas}} - MW_{\text{theo}}|}{MW_{\text{theo}}} \cdot 1 \times 10^6 \\ &= \frac{|250.0005 - 250.0000|}{250.0000} \cdot 1 \times 10^6 \\ &= \mathbf{2 \text{ ppm}}\end{aligned}$$

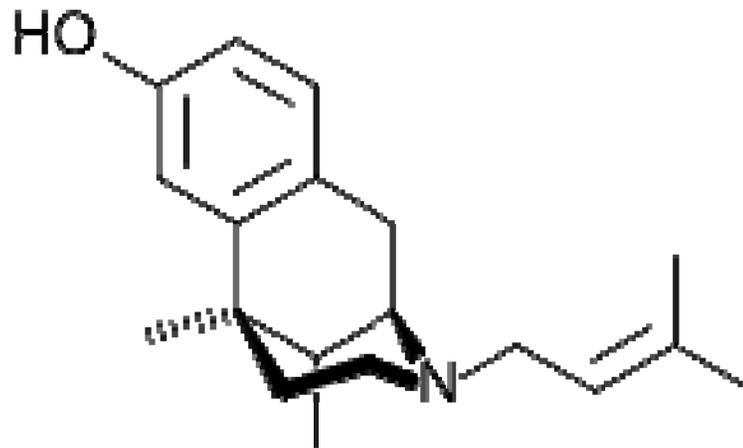
2 ppm accuracy translates to:

MW	amu error
100	0.0002
200	0.0004
300	0.0006
400	0.0008

Mass Accuracy



Morphine: $C_{17}H_{18}NO_3$
285.1365



Pentazocine: $C_{19}H_{27}NO$
285.2093

For $M/Z = 285.1365$

At 10ppm accuracy

5 possible formula

At 5ppm accuracy

$C_{17}H_{18}NO_3$, $C_{15}H_{26}N_4O_2$, $C_{20}H_{16}N_2$

At 3ppm accuracy

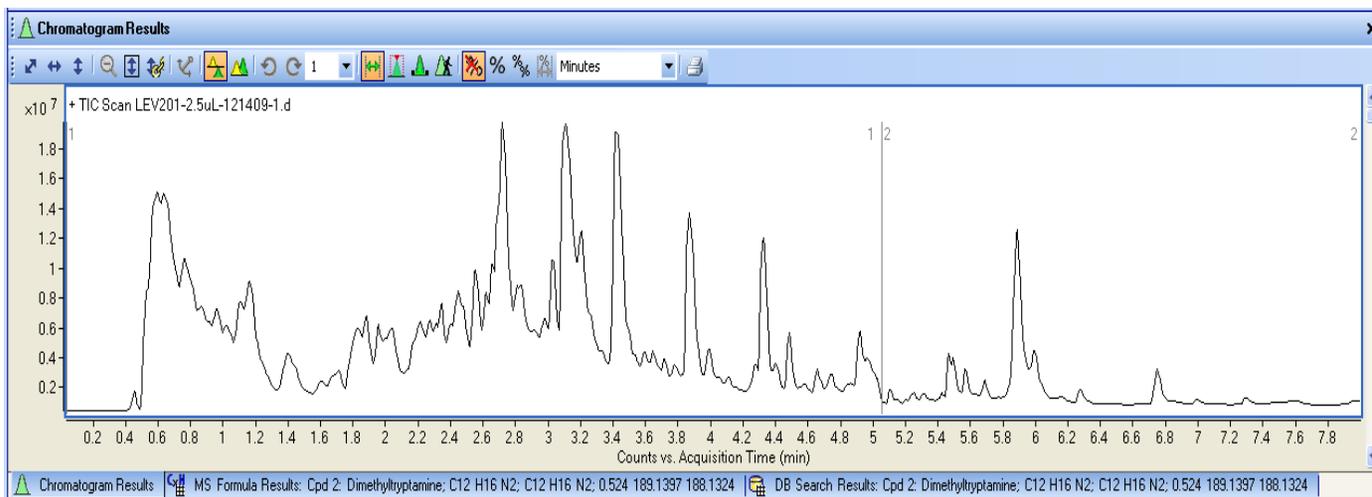
$C_{17}H_{18}NO_3$

Qualitative Analysis

Agilent 6230 TOF



Total Ion Chromatogram



Targeted Screening

- Database search
- Formula search

Non-Targeted Analysis

Targeted Screening by Database Search

Library of Compounds with established RT is created (**DATABASE**)
Database is used as reference in searching for unknowns in a sample
Search TIC for formula and RT time matches in the library

RT match within 0.15 min

Mass accuracy < 10ppm

Target Score ≥ 70

Name	RT	RT (Tgt)	RT Diff (Tgt)	Mass	Diff (Tgt, ppm)	Formula (Tgt)	Score (Tgt)	Area
Amphetamine	2.597	2.643	-0.046	135.1058	7.39	C9H13N	87.73	189303
Benzoyllecgonine	3.065	3.12	-0.055	289.1311	-1.18	C16H19NO4	87.45	870459
Benzylpiperazine	2.27	2.275	-0.005	176.1329	8.67	C11H16N2	89.27	1164833
Buprenorphine-d4	4.093	4.184	-0.091	471.3273	-2.91	C29H37D4NO4	73.11	751809
Carisoprodol	4.493	4.582	-0.089	260.173	-2.48	C12H24N2O4	78.43	362501
Cocaine	3.174	3.249	-0.075	303.1469	-0.53	C17H21NO4	82.05	1963967

Panels Available for Targeted Screening

Seizure Panel (44 drugs, serum)

Drugs-of-Abuse Panel (213 drugs, serum)

Phenylethylamines (41), Stimulants (9), Psychotropic alkaloids (10), Opioids (28), Benzodiazepines (32), Barbiturates (16), Antidepressants (24), Sedatives/Hypnotics (10), Anesthetics (10), Antihistamines (14), Analgesics (12), Muscle Relaxants (7)

Comprehensive Drug Screen (319, serum)

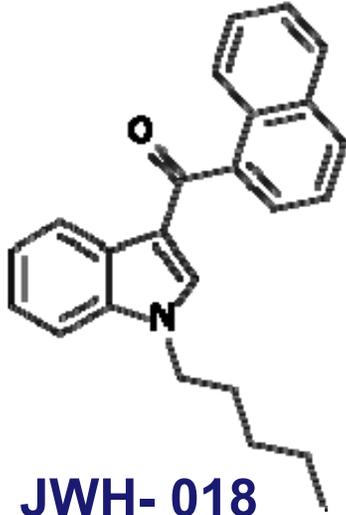
DOA panel plus Anticonvulsants (23), Antipsychotics (14), Cardiovascular (44), Respiratory (8), Antidiabetics (8), Anorectics (9)

Synthetic Cannabinoids (33 compds, serum, urine, pill)

Herbal Bioactive Markers (serum, urine, pill)

22 compounds from 20 of the most commonly used herbal medications

Targeted Screening by Formula Search



JWH- 018
C₂₄H₂₃NO

JWH-018 is a synthetic cannabinoid commonly used to lace herbal incense

JWH-018 is metabolized rapidly and usually not detected in serum and urine

Its metabolites have been detected in urine

Predicted metabolites

Hydroxylation: C₂₄H₂₄NO₂

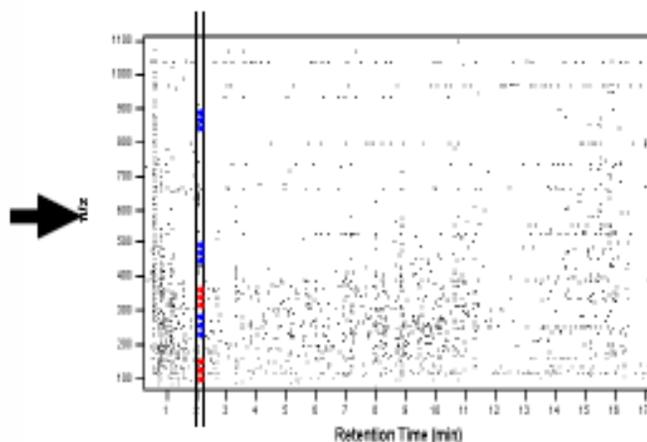
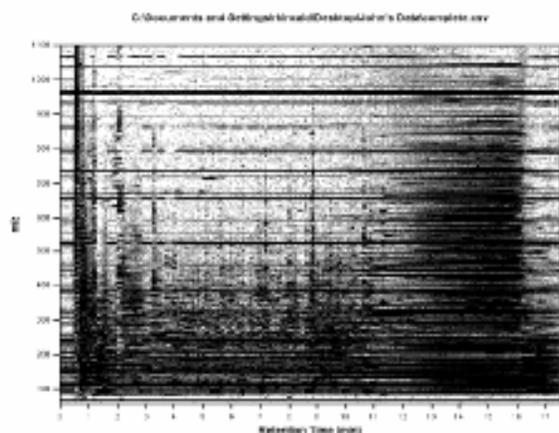
N-desalkylation: C₁₉H₁₂NO

Hydroxylation/N-desalkylation: C₁₉H₁₂NO₂

Non- Targeted Analysis: Molecular Feature Extractor

How MFE works-

- Map signal in the 3-dim. space in time and mass at the MS level
- Remove areas which only contain noise and NO signals
- Identify all mass signals with a common RT (narrow time window)
- Combine mass signals with common RT and chemical relation (isotope adduct, dimer, higher charge state) → **Molecular Feature or Compound**



Compound list or Molecular feature list

RT	m/z	Abund
...
...
2.11	195.1745	21000
2.11	257.2568	33550
2.34	224.2134	11784
...
...

Non-Targeted Analysis: Qualitative Analysis

Compound List										
Show/Hide	Cpd	Name	Score (MFG)	RT	Mass	Diff (MFG, ppm)	Formula (MFG)	Ions	Height	
▶	<input checked="" type="checkbox"/>	1		0.515	150.1403			3	1108906	

Compound List										
Show/Hide	Cpd	Name	Score (MFG)	RT	Mass	Diff (MFG, ppm)	Formula (MFG)	Ions	Height	
▶	<input checked="" type="checkbox"/>	1	88.42	0.515	150.1403	3.49	C11 H18	3	1108906	

Compound List										
Show/Hide	Cpd	Name	Score (MFG)	RT	Mass	Diff (MFG, ppm)	Formula (MFG)	Ions	Height	
▶	<input checked="" type="checkbox"/>	19	Pyrrolidinopropiophenone	86.75	0.876	203.1298	6.16	C13 H17 N O	3	995274
	<input checked="" type="checkbox"/>	83	Oxycodone	93.05	3.653	315.149	-6.07	C18 H21 N O4	4	1087365
	<input checked="" type="checkbox"/>	26	Orthocaine	97.77	1.177	167.0582	0.18	C8 H9 N O3	4	1242958
	<input checked="" type="checkbox"/>	84	Nor-triptyline	94.44	3.721	263.1689	-5.78	C19 H21 N	3	1071428
	<input checked="" type="checkbox"/>	57	m-Hydroxybenzoylecgonine	90.76	2.616	305.1278	-4.91	C16 H19 N O5	4	2396293
	<input checked="" type="checkbox"/>	21	Meturedopa	99.65	0.963	275.1402	-1.05	C11 H22 N3 O3 P	3	960282
	<input checked="" type="checkbox"/>	88	Methadone	90.78	4.326	309.2115	-7.16	C21 H27 N O	4	6496722
	<input checked="" type="checkbox"/>	51	Levamisole	97.15	2.439	204.073	-4.31	C11 H12 N2 S	4	1392675
	<input checked="" type="checkbox"/>	89	Isamfazona	79.71	4.485	361.1765	6.94	C22 H23 N3 O2	4	2286808

What can be analyzed using the TOF?

Targeted and Non-Targeted Screening of Small Molecules

Small Molecules: 75- 3000 amu (100-1000 amu)

Most Organic Drugs and metabolites, Herbal and Food Supplements' bioactive compounds and adulterants, Small molecule biomarkers, Non- proteinaceous hormones, Small peptides, Steroids, Fatty acids, Fat and Cholesterol derivatives, Nucleotides and derivatives, Sugars and small oligosaccharides (~10mer), Pesticides, Small Organic Environmental Pollutants

Samples: Serum, Urine, CSF, Breast milk, Other Body Fluids (Saliva, Sweat etc.), Tissue extracts, Plant extracts, Cell lysates, Pills

Qualitative Data, Semi-quantitative, Quantitative

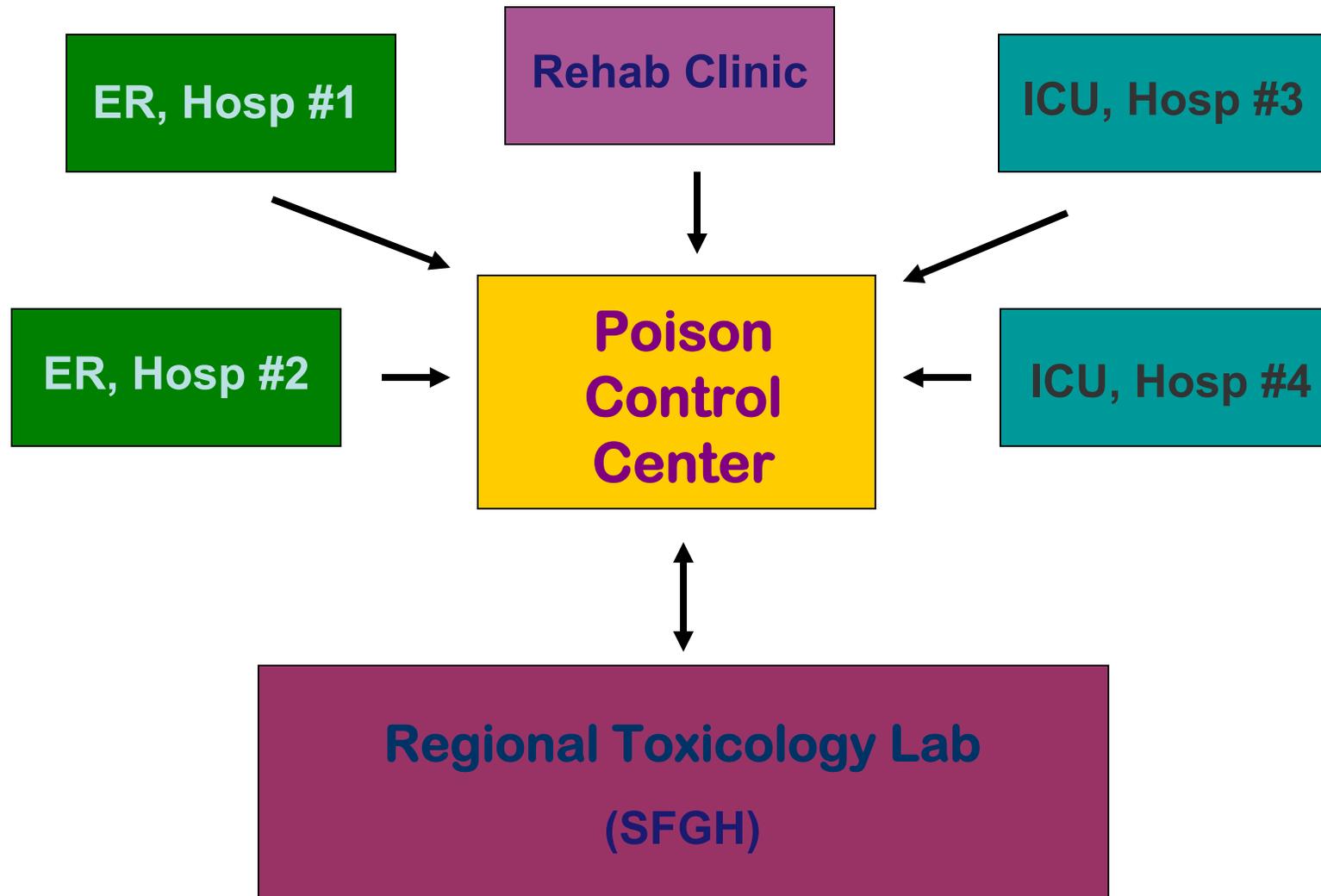
TAT for LC-MS/TOF run: 8-12 min Sample Prep: 30-60min

We can develop methods of analysis for specific groups of compds

We can't analyze proteins, nucleic acids, polysaccharides, polymers, metals, gaseous compds, inorganic salts

CASES

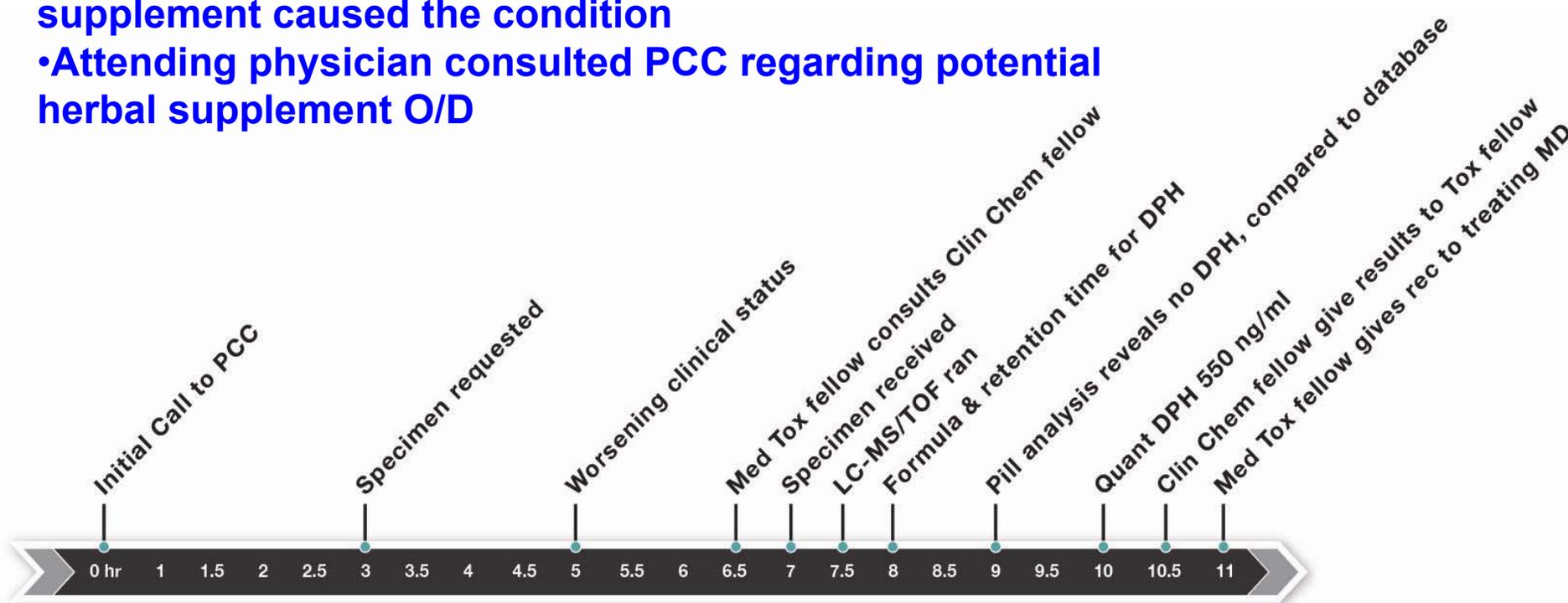
PCC-centric Operation of a Regional Toxicology Lab: The NACB Paradigm



Red Herring

CASE

- 45yo F with multiple seizures, later became delirious
- Family claims patient has been taking an herbal sleep aid supplement and was suspicious that something in the herbal supplement caused the condition
- Attending physician consulted PCC regarding potential herbal supplement O/D



Nyquil-induced seizure?

Case 1

27yo F with hx of EtOH abuse (9 days s/p cessation)

Reported taking 6 tabs generic Nyquil with BF and had seizure 2 hrs post-ingestion (witnessed BF seizing first); tachycardic, diaphoretic

Negative APAP (at 14th hr), amphetamines, cocaine, benzos, opiates

Received IVF and discharged 16hrs post-ingestion

Case 2

31yo M with hx of EtOH abuse (9 days s/p cessation)

Reported taking 6 tabs generic Nyquil with GF and had seizure 2 hrs post-ingestion; tachycardic, diaphoretic

Negative APAP (at 14th hr), amphetamines, cocaine, benzos, opiates

Received IVF and discharged 16hrs post-ingestion

Nyquil-induced seizure?

Seizure Panel Drugs (44)

Anesthetic- Lidocaine, Phencyclidine

Anticonvulsant- Carbamazepine, Topiramate

Antidepressant- Amitriptyline, Desipramine, Doxepin, Imipramine, Nardoxepin, Nortriptyline, Protriptyline, Trimipramine

Bupropion, Citalopram, Fluoxetine, Paroxetine, Sertraline, Venlafaxine

Antihistamine- Diphenhydramine, Doxylamine, Hydroxyzine

Antipsychotic- Clozapine, Lamotrigine, Olanzapine, Quetiapine

Cardiovascular- Propranolol

Muscle Relaxant- Carisoprodol

Opioid Analgesic- Meperidine, Propoxyphene, Tramadol

Psychotropic Alkaloid- Benzoyllecgonine, Cocaine

Sedative/Hypnotic- Dextromethorphan

Stimulant- Amphetamine, Benzylpiperazine, Caffeine, MDA, MDE, MDMA, Methamphetamine, Methylphenidate, PMA, Trifluoromethylphenylpiperazine

Tuberculostatic- Isoniazid

Drugs chosen according to three surveys conducted by the California Poison Control Center on drugs commonly reported to cause drug-induced seizures in the San Francisco Bay Area

Nyquil-induced seizure?

Serum and urine samples were run on seizure panel; searches for pseudoephedrine, phenylephrine and acetaminophen (reported Nyquil components) were also done.

Lab Results

No matches for dextromethorphan, pseudoephedrine, phenylephrine, doxylamine and acetaminophen found in the urine and serum sample
BUT

Both patients have tramadol in their serum and urine

Patient 1 Serum Tramadol= 2974 ng/mL

Patient 2 Serum Tramadol= 1676 ng/mL

Serum Hydroxyzine= 91.25 ng/mL

	Tramadol	Hydroxyzine
Therapeutic level	100-800 ng/mL	50-90 ng/mL
Toxic level	1000 ng/mL	100 ng/mL
Lethal level	2000 ng/mL	

Unknown Green Pill

Case

- 15 yo M with vfib arrest after smoking marijuana and ingestion of unknown green pill with puma imprint on it.
- Patient was shocked out of vfib and was intubated and sedated but had very labile blood pressure, intermittently requiring pressors and antihypertensive meds.
- On HD 4, he was extubated, off pressors, and was mentally oriented

- Patient's serum and urine samples, green pill and two carrying tubes were sent to SFGH for analysis.

Lab Consult

Identify active ingredient of unknown green pill, compounds on tubes and match them with serum and urine samples



Unknown Green Pill

Sample Analysis

Sample	MDMA (Ecstasy)	MDA	MDE
Serum, 2/20	87 ng/mL	18.82 ng/mL	61 ng/mL
Serum, 2/26	ND	ND	ND
Urine, 2/22	395 ng/mL	123.3 ng/mL	46 ng/mL
Green Pill	114 mg/125mg	ND	4.38 mg/125mg
Tube 1	ND	ND	ND
Tube 2	474 ng/mL	ND	ND

1 Ecstasy Pill = 80-120 mg MDMA

MDMA Recreational Level (Serum): 100-250ng/mL

Unknown **Green Pill**

Sample Analysis

Sample	Potential Drugs
Serum, 2/20	Trimethoxyamphetamine
Urine, 2/22	Trimethoxyamphetamine Trimethoxymethamphetamine
Green Pill	Trimethoxyamphetamine Trimethoxymethamphetamine Methylenedioxy-2-methoxyamphetamine
Tube 1	Codeine Trimethoxyamphetamine Trimethoxymethamphetamine
Tube 2	Trimethoxyamphetamine

Case of Mistaken Identity

Case One

57yo non-diabetic male with hx of benzodiazepine abuse.

Fingerstick glucose = 41mg/dL

Patient was administered with naloxone and continuous glucose infusion.

Admission glucose= 33 mg/dL

Octeotride and glucagon given, ED doctors were suspicious of sulfonylurea intoxication

On HD2, patient's glucose rebounded to normal range without supplemental glucose, extubated and his mental status became clear.

Case Two

48yo F found unresponsive by son at home; GCS=5, glucose was undetectable as per paramedics report. Administration of one ampule of glucose, patient's GCS improved to 7.

Upon ED arrival, patient became alert upon administration of second ampule of glucose and admitted she ingested two pills of "street valium"

Patient recovered on HD 2; glucose level rebounded to normal.

Case of Mistaken Identity II

Serum analysis revealed formula match for **GLYBURIDE**. Confirmed by retention time match to reference standard.

Patient	Serum level (ng/mL)
#1 T ₀	1198
T ₀ + 4.5 hours	693
T ₀ + 8 hours	590
T ₀ + 12.5 hours	undetectable
#2 T ₀	647

Therapeutic dose: 30-350 ng/mL

Toxic: > 600ng/mL

Glyburide

Diazepam



SPICE

Case

Two 17yo were brought in by ambulance after smoking Spike Maxx

Patient 1 - F, vomiting, slightly confused, T=36.3, HR=110, RR=20, K=3.1 mmol/L, no urine drug screen

Patient 2 - M, vomiting, very altered/confused, dilated pupils, cool skin, excess salivation, HR= 120, K= 3.0 mmol/L, Lactate= 4.4 mmol/L, **NEGATIVE** urine drug screen

Both patients were treated with antiemetics, IV fluid and were discharged 3, and 6hrs post-presentation

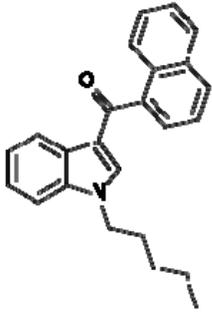
SPICE

- Most popular brand of **HERBAL INCENSE**
- Herbal incense- herbal blends originally intended as room deodorizers but are laced with synthetic cannabinoids
- Synthetic cannabinoids- class of compounds that mimic the effect of THC, the active component of cannabis
- THC binds to two types of receptors- CB1 and CB2
- SCs are originally synthesized to produce drugs with selective analgesic property
- Some SCs are more potent CB1 agonists



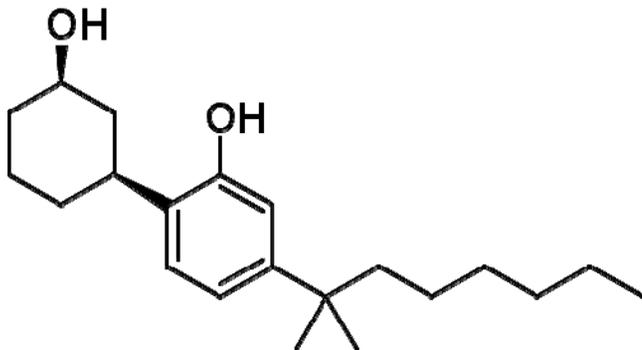
	CB1 K _i (nM)	CB2 K _i (nM)
THC	40.7	36.6
HU- 210	0.234	
CP 47,497	2.1	
JWH-018	9	2.9
JWH-073	8.9	3.8
JWH-051	1.3	0.03

Spice... A little History



1990's Synthetic cannabinoids are synthesized in the lab of John W Huffman (JWH) at Clemson University
JWH-018 (1994)

- 2002** Spice first appeared in Asia as herbal incense
- 2004** Spice became available in Europe
- 2006** First incidences of Spice intoxication called in to Poison Control Centers in Europe
- 2008** Japan and Germany started analyzing herbal incense samples bought from headshops/ internet



- Feb, 2009** First published articles on SPICE being adulterated with synthetic cannabinoids
- Apr, 2009** German lab quantified adulterants in SPICE; more JWH compds found
- April, 2010** JWH-018 metabolites identified

SPICE

LEGAL STATUS OF SPICE IN THE US

Illegal

Kansas (02/2010)

North Dakota (02/25/2010)

Mississippi (04/2010)

Kentucky (04/13/2010)

Alabama (04/25/2010)

Tennessee (07/01/2010)

Louisiana ((07/18/2010)

Illinois (07/2010)

Hawaii (08/01/2010)

Georgia (08/15/2010)

Missouri (08/28/2010)

Michigan (10/2010)

Temporary Ban/ Legislation Proposed

Arkansas

Florida

Iowa

Maryland

New York

Oklahoma

Utah

Municipal Restriction

Indiana

Minnesota

Texas

Wisconsin

FDA Schedule I Drugs (March 1, 2011)

JWH-018, 073, 200, CP-47,497, Cannabicyclohexanol

SPICE

Spike Maxx

Analysis suggests the presence of

JWH – 007

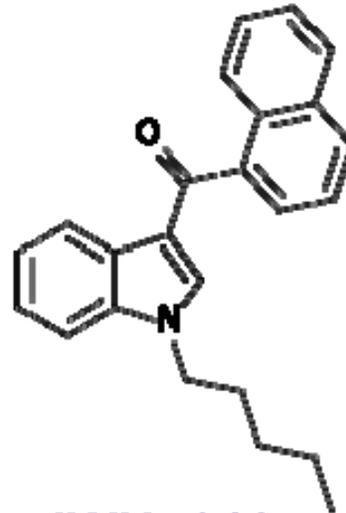
JWH – 073

JWH – 398

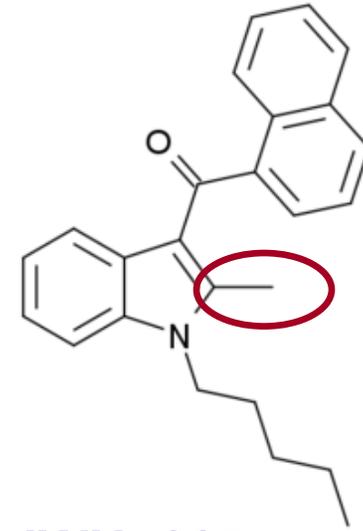
Blood sample results

Both patients have 11-OH-THC

Girl's serum has a formula match
for JWH-018 (007 demethylates to
018)



JWH- 018



JWH- 007

We've developed pill, urine and serum synthetic cannabinoids assays.

PCC Cases

Cases Referred (2010): 55

Cases Resolved: 43 (78%)

Case	No.	Drugs Involved
Unexplained Seizure	9	Venlafaxine, Tramadol, Diphenhydramine, Methadone, Dextromethorphan, Hydroxyzine, Quetiapine, Bupropion
Therapeutic Drug O/D	10	VPA, Atenolol, Sertraline, Trazodone, Ibuprofen, Retinoic acid, Propranolol, Verapamil,
Illicit Drug O/D	15	MDMA, Methadone, Propoxyphene, Clonazepam, Fentanyl, Dextromethorphan
Designer Drug	3	JWH compounds
Misrepresented Drug	4	Diazepam (Glyburide), Amphetamine (Zolpidem), Clomifene (Clenbuterol)
Adverse Drug Reaction	2	MDMA, acetaminophen

Non-Targeted Analysis of Environmental Toxins

New frontiers in expanding our understanding of chemicals in pregnant women (T Woodruff, A Zota, R Gerona)

Hypothesis: There are many more chemicals present in people's bodies than currently identified through biomonitoring studies

Specific Aims

- To develop a comprehensive database of environmental chemical formulas
- To identify previously unmeasured candidate chemicals in the serum of pregnant women using unbiased interrogation methods
- To quantify the levels of 10-15 most commonly detected chemicals which have previously been unmeasured or underreported chemicals

Approach: Non-targeted analysis of environmental toxins in second trimester pregnant women using TOF LC-MS

Non-Targeted Analysis of Environmental Toxins

Sample Source and Study Population

- Serum samples from patients prior to second trimester pregnancy termination procedures at the San Francisco General Hospital Women's Option Center (WOC)
- Patients are ethnically diverse and predominantly low income
 - 38% Black
 - 21% Hispanic
 - 21% Caucasian
 - 10% Asian
- Initially analyze stored serum samples for 20 pregnant women using a non-targeted approach for multiple environmental chemicals
- Analyze 30 prospectively collected serum samples using a targeted approach (qualitative and quantitative)

Non-Targeted Analysis of Environmental Toxins

Method

Sample Extraction: Protein precipitation/ SPE

Chromatography: C18 column with gradient elution (run time=8 min; re-equilibration= 2min)

Mass Spectrometry: TOF/MS using ESI positive, ESI negative, APCI positive, APCI negative (full scan between 100-1000 amu)

Output: Detected MWs, Mass accuracy, Target Score, Area under the curve

Criteria for Positive Hit: Mass accuracy \leq 10ppm; Target Score \geq 70; AUC \geq 1000

Positive hits will be ranked according to detection frequency across 20 retrospective samples

Select top 10-15 positive hits for targeted quantitative analysis (LC-MS/MS) in 30 prospective samples

Preliminary Results

Potential environmental toxins found in 3 patient serum samples by non-targeted analysis

Compound	Class	Patient		
		1	2	3
Bisphenol A	Phenolic	+		+
Bisphenol S	Phenolic			+
BDE 47/66	PBDE		+	+
Monomethyl phthalate	Phthalate	+		+
Monoisononyl phthalate	Phthalate	+	+	+
Anabasine	Insecticide	+	+	+
DEET	Insecticide	+	+	+
DNOC	Insecticide	+	+	+
Ethoxyquin	Herbicide	+	+	+
Malathion	Insceticide	+	+	+
Zectran	Insceticide	+	+	+
Glyphosate	Herbicide		+	+

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**Dr. Tracey Woddruff, Dr. Ami Zota, Carrie
Dr. Deborah French, Jean Branch, Dr. Kathy
Chen**

Dr. Richard Ko

Additional Slides

Analysis of Herbal Supplements

Herbal X

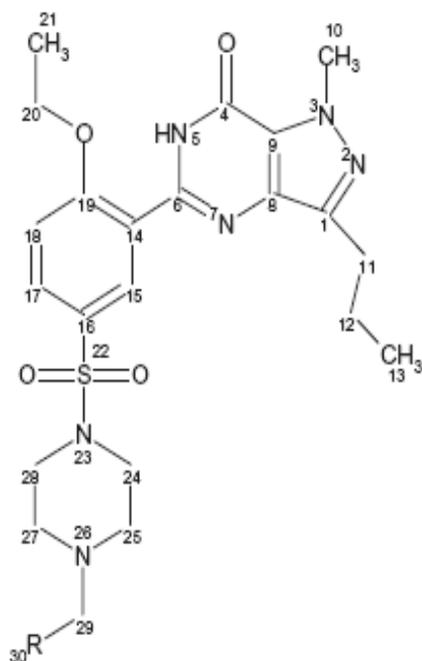
- Claims to increase libido
- Most ingredients have been studied for their role in supporting healthy sexual performance and increased libido
- Listed ingredients- Acorus rhizome, Alpinia fruit, Black walnut seed, Cherokee Rose fruit, Cimicifuga rhizome, Cordyceps mycelium, Cornus fruit, Glycyrrhiza root, Lycium fruit, Panax Ginseng root, Poria sclerotium, Rehmania root

MS-TOF Qualitative Analysis

- Methanol extract of Herbal X contains very high formula matches for
 - acetildenafil (structural analogue of Viagra)
 - hydroxyhomosildenafil (derivative of Levitra)
- Both compounds have been reported as dopants used in herbal supplements for erectile dysfunction.

Analysis of Herbal Supplements

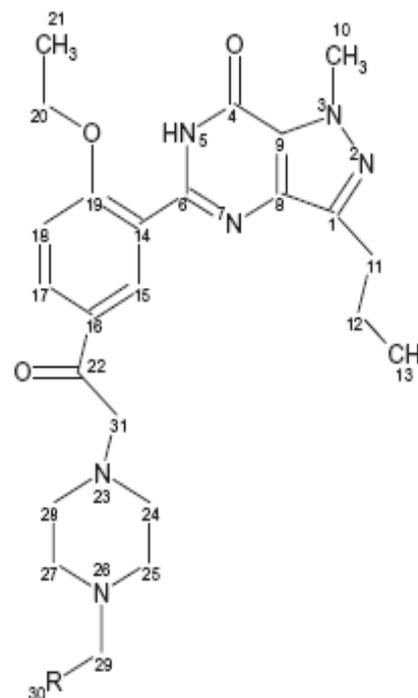
Name	Score (MFG)	RT	Mass	Diff (MFG, ppm)	Formula (MFG)	Ions	Height	Area	Abund	File
Acetildenafil	84.05	4.147	466.27	-1.7	C ₂₅ H ₃₄ N ₆ O ₃	4	1548390	4476039	483578	HerbalX-1.d
Hydroxyhomosildenafil	91.08	4.602	488.2208	-0.53	C ₂₃ H ₃₂ N ₆ O ₄ S	7	1118384	2318706	298117	HerbalX-1.d



Sildenafil: R=H

Homosildenafil: R=CH₃

Hydroxyhomosildenafil: R= CH₂OH



Acetildenafil

Solving a puzzle

Case

54 yo M with complex medical history had a suicide attempt. In the ER, his mental status precipitously diminished leading to intubation with need for sedation/ paralysis.

Patient had a heart attack; while being resuscitated, patient developed **Torsades de Pointes** which resolved after CPR, IV MG, and IV NaHCO₃. EKG showed a prolonged QT >600msec

List of Medications

Aspirin

Atorvastatin

Buspirone

Fluoxetine

Levothyroxine

Metformin

Metoprolol

Trazodone

Which medication did he overdose on that may have caused torsades?

Solving a puzzle

Drugs with formula and RT matches in patient's serum:

Aspirin, Fluoxetine, Metformin, Metoprolol, Trazodone

Drugs not detected in patient's serum:

Atorvastatin, Bupirone, Levothyroxine

Sample	Trazodone (mcg/mL)	Metformin (ng/mL)	Fluoxetine (ng/mL)	Norfluoxetine (ng/mL)	Metoprolol (ng/mL)
02/08 1215H	4.06	159.78	20.11	11.0	10.9
02/08 2120H	4.09	101.69	34.07	23.7	7.1
02/10 0420H	2.74	16.97	39.86	31.4	15.5
02/11 0505H	2.47	9.41	40.97	32.5	7.7
02/16 0445H	0.0276	4.76	22.50	13.8	8.9
Therapeutic level	0.5-2.5	1000-4000	150-500		100-600
Toxic level	4.0	5000	>1300		650

Studying the Progress of VPA Intoxication

CASE

22 yo F O/D with valproic acid

Comatose

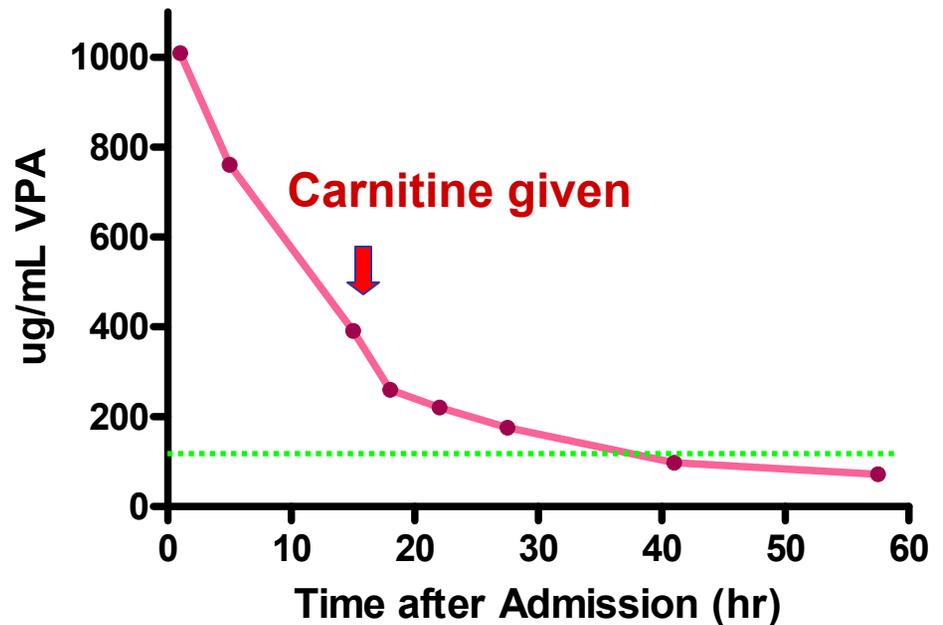
Hypernatremic, hypocalcemic,
initial AG= 18

AIM

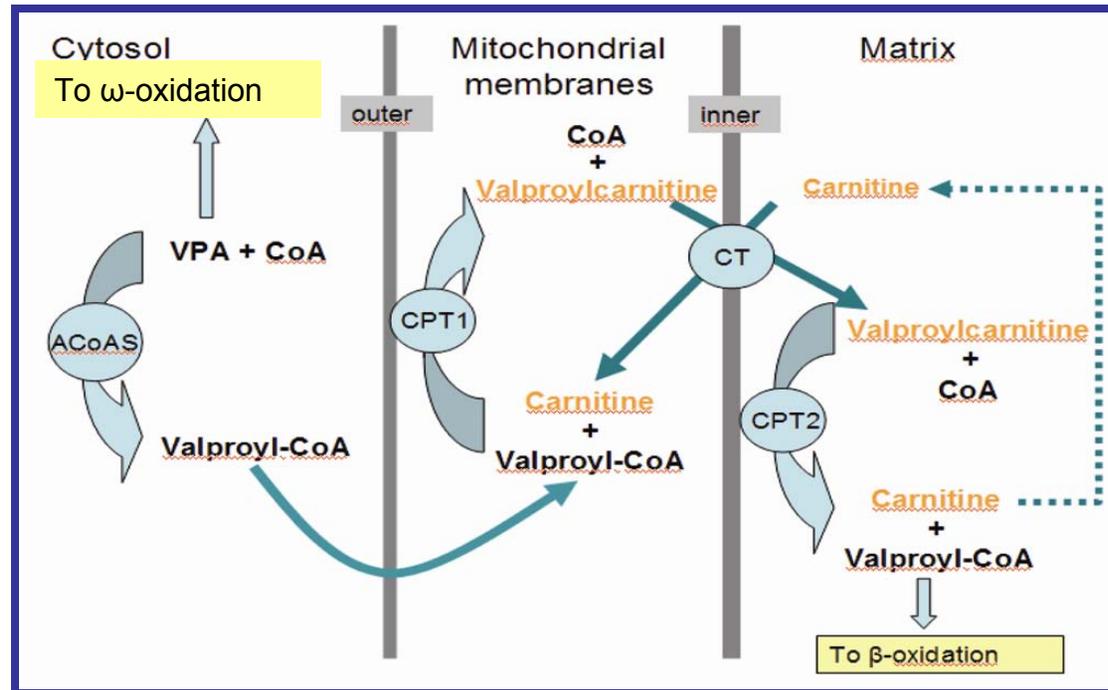
Monitor metabolites relevant
in VPA toxicity

USE of MS-TOF

Simultaneous analysis of
metabolites in patient
serum samples drawn
intermittently at the ICU

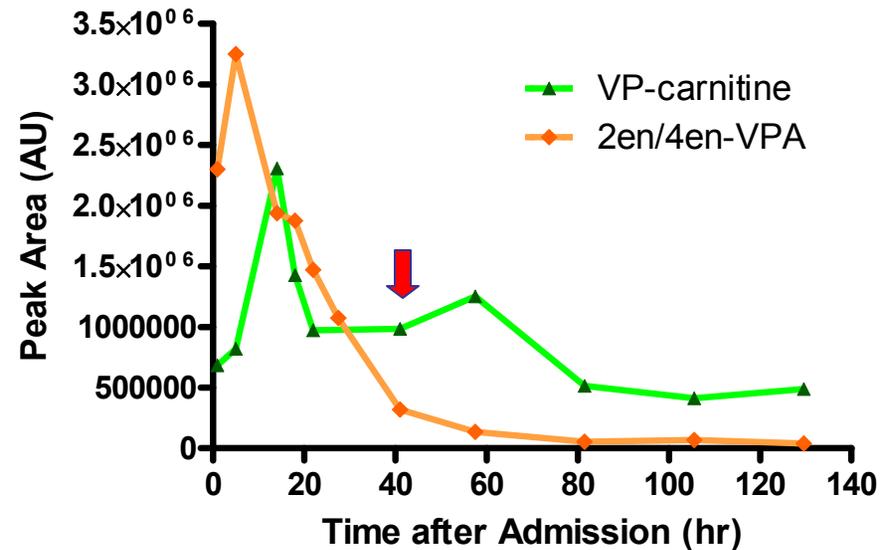
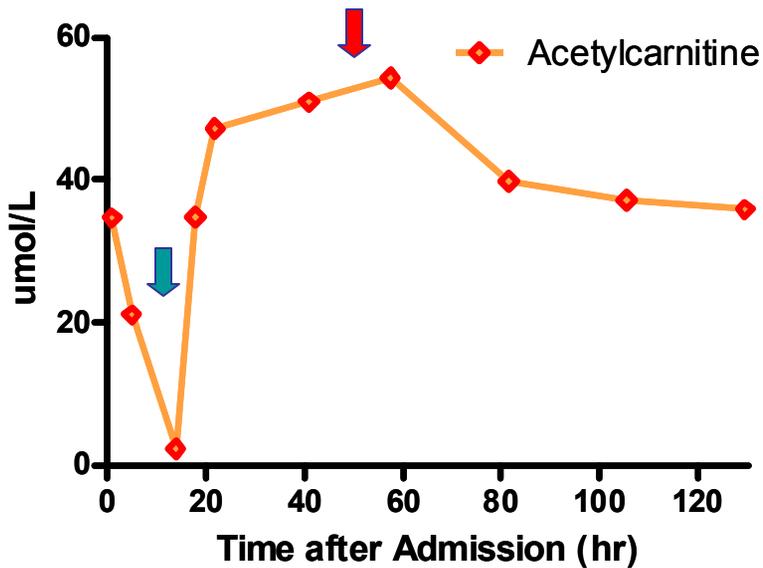
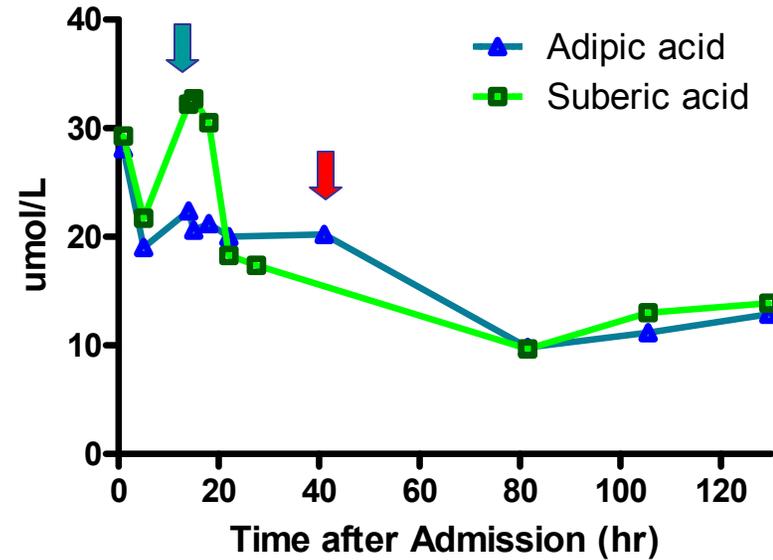
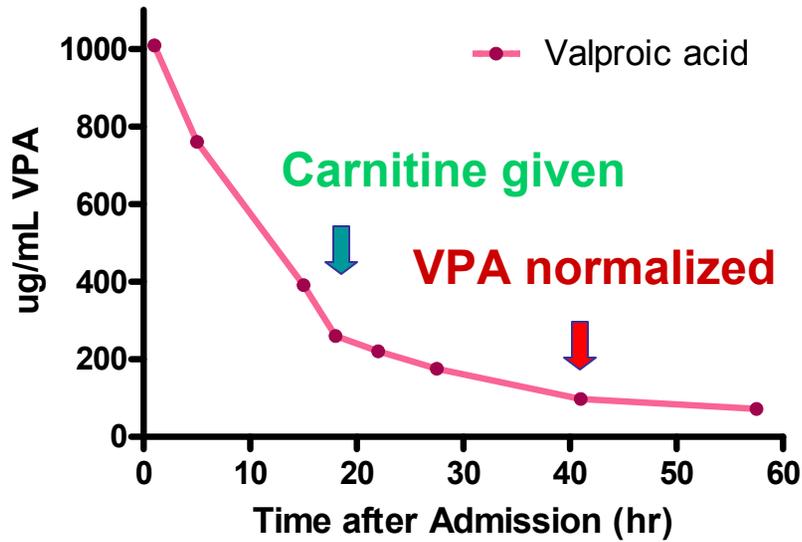


Studying Progress of VPA Intoxication



- Valproic acid uses up carnitine leading to carnitine deficiency
- Cells cannot transport long chain fatty acids into the mitochondrion for their metabolism through β -oxidation
- Cells shift to ω -oxidation to metabolize long chain fatty acids- increase in adipic and suberic acids, metabolites of ω -oxidation
- Metabolism of valproic acid leads to production of its toxic metabolites, 2-/4-en-VPA

VPA Intoxication



A Case of Mistaken Identity

Case

Patient presented to the ED after taking 0.5mL Clomifene citrate (obtained from the black market) sublingually. Patient had chest discomfort, palpitations, tremor and anxiety Patient claimed that he felt his heart rate suddenly racing 5 minutes after taking the drug. EKG confirmed narrow complex tachycardia with the rate of 150.

Patient was taking testosterone obtained illicitly for body building. He self-prescribed Clomifene to counteract the feminizing effects of testosterone intake.

A sample of Clomifene purchased by the patient was sent to SFGH for analysis. Attending was suspicious that the bottle actually contains clenbuterol.

Sample Analysis

Sample contains **CLENBUTEROL** at 28.45mg/mL. **NO CLOMIFENE** was detected.

Recommended dosage: <120mcg/day

Patient Intake with 0.5mL= 14.2mg



Chemical Exposures During the Second Trimester of Pregnancy

(Project in collaboration with Dr. Tracey Woodruff)

Specific Aims:

- 1. To describe the fetal exposure to BPA during the second trimester and explore the feasibility and accuracy of using maternal biological monitoring results to predict fetal exposure**
- 2. To investigate predictors of maternal and fetal exposures to BPA during second trimester of pregnancy**
- 3. To characterize hepatic metabolism of BPA in the fetal and adult liver**

Study will recruit 100 women undergoing elective pregnancy terminations between 14 and 23 weeks gestation.

Develop method to measure BPA, BPA glucuronide and BPA sulfate in maternal urine, maternal serum, umbilical cord blood, placenta and amniotic fluid