Drug Testing: the moving target

Kara Lynch, PhD, DABCC
kara.lynch@ucsf.edu
University of California San Francisco
San Francisco, CA

Learning Objectives

• Understand recent trends in designer drug use
• Describe challenges the laboratory faces in keeping up with the detection of novel psychoactive substances and new pharmaceutical agents
• Develop a laboratory based approach for detecting novel psychoactive substances and new pharmaceutical agents in biological samples

Emergence of Designer Drugs / Novel Psychoactive Substances (NPS)

- 1960s: DOM
- 1970s: PCP analogues, ALD-52
- 1980s: Methcathinone, methylamphetamines, Novel anabolic steroids, First synthetic cannabinoids sold on internet, "research chemicals" for "scientific research"
- 2000s: MDMA, Fentanyl/meperidine analogues, Internet Sales
- 2010s: 2012: Synthetic Drug Abuse Prevention Act
Traditional Drug Screening Approach

- Screening for classes of abused or prescribed drugs (Immunoassay, ELISA) – rapid, minimal labor
- Limitations: 1) Assays not available for all drugs of clinical interest, 2) prone to false positives and false negatives

- Targeted confirmation testing (GC/MS or LC-MS/MS)
- Limitations: 1) Testing not always available in-house, 2) Long turn around times, 3) not designed to detect new/novel pharmaceutical and illicit drugs

Drug Screening Panels by Immunoassay

<table>
<thead>
<tr>
<th>Drugs commonly in “Drug of Abuse” and/or “Pain Management” Panels</th>
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<tbody>
<tr>
<td>Amphetamines</td>
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<td>Opiates</td>
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<td>Phencyclidine (PCP)</td>
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<td>Marijuana (THC)</td>
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<td>Diphenoxylate</td>
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Other assays: Fentanyl, Buprenorphine, 6-acetylmorphine
IA Drug Screening Panels do not include all possible toxicological exposures

Challenges in NPS testing

- Mostly legal alternatives to street drugs
- New compounds always emerging
- High geographic variability

Difficult to stay on top of NPS trends using targeted screening approach
Overview, Trends, Detection Methods, Cases...

- Designer Amines
- Synthetic Cannabinoids
- Opioid Therapeutics / Designer Opioids
- Other Rx Drugs
- Novel drugs of abuse

NPS: Designer Amines

- “Bath salts” first appeared in US in 2010
- Ivory Wave, Cloud 9, Vanilla Sky
- Cathinone derivative and/or amphetamine-like stimulant
- Effects can include:
  - Psychosis
  - Hallucinations
  - Agitation
  - Tachycardia
  - Hypertension
  - Hyperthermia

"Phenethylamines I Have Known And Loved"
"Tryptamines I Have Known And Loved"
NPS: Designer Amines

National Forensic Laboratory Information System (NFLIS)

<table>
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<tr>
<th>Compound</th>
<th>Concentration Tested (mg/mL)</th>
<th>% Cross-reactivity</th>
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Randox EUSA kits
MDPV (Bath Salts)
Mephedrone / Methcathinone (Bath Salts)

NPS: Cross-reactivity with Immunoassay?

Petrie, MS et al. Clinical Toxicology 2013
Cross-reactivity with Amphetamine IAs

Detection by Mass Spectrometry

Detection by High Resolution Mass Spectrometry
Case 1: MDMA cardiotoxicity?

- A 18 y/o male presented to an outside ED with vomiting, shortness of breath, chest tightness and altered mental status
- 5 hours prior he purchased and ingested what he thought was MDMA
- 5 hours after presentation he developed cardiogenic shock requiring intraaortic balloon pump, inotropic and ventilatory support
- Admission UTOX + for amphetamines, opiates and THC.

Case 1: MDMA cardiotoxicity

- Poison control medical toxicologist suspects novel psychoactive substance ingestion and requested extended toxicological screening by LC-HRMS
- Samples sent to SFGH from outside hospital

Data analysis

Targeted

naltrexone
naproxen
fluoxetine
Trazodone

Suspect

4-fluoroamphetamine

Untargeted

335.8251

D1

Pharmacology

Case 1: 4-fluoroamphetamine

- No cases reported to date in the literature
- Substituted amphetamine, phenethylamine
- Stimulant causing euphoria and mood elevation
- Acute side effects include: nausea, headaches, increased heart rate and insomnia
- Case Outcome: Patient’s cardiac function improved by HD3, discharged home on HD14
Case 2: LSD and seizures?

- 18 y/o male partying with friends using LSD and marijuana started seizing
- EMS noted: unresponsive, with left gaze deviation, dilated pupils, abnormal movement of extremities
- BP 132/66, HR 125, Temp 37.3, RR 24, SpO2 95%
- Given 2.5 mg of midazolam with stopped seizures only briefly, second dose had no effect
- Upon presentation to ED: tonic head turning and gaze deviation
- Sedated, paralyzed and intubated
- Labs: anion gap metabolic lactic acidosis, profound leukocytosis, early rhabdomyolysis, hyperglycemia, normal electrolytes, negative troponin
- Toxicology: positive for opioids and cannabinoids
- Neither Marijuana or LSD are typically associated with seizures

Case 2: LSD and seizures?

- Treating physician at UCSF suspects novel psychoactive substance ingestion and requested extended toxicological screening by LC-HRMS
- Samples sent to SFGH from UCSF medical center

- Data analysis

Case 2: DOC

- Psychedelic drug originally described by Shulgin
- Very potent hallucinogen
- Sold as blotter paper or as tabs
- Similar compounds – DOB, DOM, DOI – all have similar effects and potency
- Seizures and metabolic acidosis have been reported with DOB (2005)
- DEA report (2007) – “LSD blotter acid mimics containing DOC in Concord, CA
Case 2: Final Results and Outcome

- DOC confirmation and quantitation (3 ng/mL) with standard
- Rhabdo, leukocytosis improved with supportive care
- Extubated on HD2
- Slow response times, but otherwise normal neurological exam
- Discharged on HD3, with close outpatient follow-up
- Discharge diagnosis: seizures due to ingestion of DOC

NPS: Synthetic Cannabinoids

- Activate the endocannabinoid system in the brain (CB1 and CB2 receptors)
- First identified (2009) were originally "research cannabinoid compounds"
- Severe effects include: seizures, sympathomimetic toxidrome, organ specific toxicity

![Chemical structures of synthetic cannabinoids](image)

**Figure 1:** Structures of 
A. **Δ9-THC**
B. **Δ8-THC**
C. **Δ9-THC acid**
D. **Nabilone**
E. **Nabilone-acid**
F. **Nabiximols**
G. **Nabilone-10-acetate**
H. **Nabilone-10-acetate**
I. **Nabilone-10-acetate**
J. **Cannabinoids of uncertain structure**
NPS commercial methods

**Randox ELISA kits**
- MDPV (Bath Salts)
- Mephedrone / Methcathinone (Bath Salts)
- Synthetic Cannabinoids (JWH-018 / AM-2201)
- Synthetic Cannabinoids (UR-144 / XLR-11)
- Synthetic Cannabinoids (JWH-250 / RCS-8)
- Neogen Corporation Synthetic Cannabinoids ELISAs
  - JWH-18
  - JWH-250
  - UR-144, XLR-11
- Immunoassays Synthetic Cannabinoids ELISA – JWH, HU, AM, CP
- Cayman Chemicals JWH Metabolite ELISA
- NMS Synthetic Cannabinoid ELISA – JWH-18 and JWH-73

**LC-MS/MS, LC-TOF, LC-QTOF, SWATH...**

The use of ultra high performance liquid chromatography with time of flight detection for the identification of synthetic cannabinoids in seized drugs

Forensic Science International
Opioid Therapeutics

• Estimated 30% suffer from chronic pain in US
• Opioid prescribing has increased significantly over the last decade
• Not all commonly prescribed pain medications are detected by opiate immunoassays

Opioid / Chronic Pain Therapeutics

• Opiates
  – Codeine
  – Morphine
• Semi-synthetic opioids
  – Dextropropoxyphene
  – Hydromorphone
  – Tyloxapine
• Muscle Relaxers
  – Cyclobenzaprine
  – Carisoprodol
  – Metaxalone
  – Methocarbamol
• Synthetic opioids
  – Fentanyl
  – Methadone
  – Meperidine
  – Tramadol
  – Propoxyphene
  – Levoephonol
  – Tapental
• Anticonvulsant
  – Gabapentin
  – Pregabalin
  – Oxcarbazepine
  – Carbamezepine
  – Topiramate

Opiates: Immunoassay cross-reactivity

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Opioid Therapeutics - Methods

- Specific immunoassays – opiates, oxycodone, 6-acetylmorphine, fentanyl, methadone/EDDP, buprenorphine, tramadol, gabapentin, pregabalin, carisoprodol, tapentadol, zolpidem
- Mass spectrometry based methods – GC-MS, LC-MS/MS, LC-HRMS (TOF, QTOF, Orbitrap)
- Not all immunoassays are equal
- Not all MS assays are equal – targeted vs. untargeted

Example: Buprenorphine IA

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Designer Opioids - Krokodil

- Thioeryl chloride
- Codeine → e-Chlorocodeine → Desocodeine
- HEROIN → KROKODIL
- THE FLESH EATING ZOMBIE 1995

Designer Opioids – Fentanyl analogues

- Acetylfentanyl, methylfentanyl, acrylfentanyl, butyrfentanyl, 4-fluoro-butyrfentanyl, parafluorofentanyl, 4-fluorofentanyl, etc.
- Acetylfentanyl is 5 x more potent than heroin
- Responsible for >60 fatalities in RI, PA, NC and LA
- Detected by fentanyl IAs

Designer Opioids - Others

- AH-7921, desmethylprodine (MPPP), desmethyltramadol, MT-45, U-47700, W-15, W18, etc.

Case 3: Opioid Intoxication?

- 18-month male
- Diarrhea, vomiting, somnolence, lethargic, pinpoint pupils
- Blood pressure: 125/77 mmHg
- Heart rate: 70/min
- ECG: bradycardia with first degree heart block
- Temperature: 36.5°C
- Respiratory rate: 25/min
- Blood glucose: 101 mg/dL
- UTOX - negative

Case 3: Tetrahydrozoline Ingestion

- Imidazolines with direct activity on peripheral alpha-1 adrenoreceptors
- Decrease neurotransmitter release in CNS, leading to sedative effect, muscle relaxation, and analgesia
- Common symptoms: altered mental status, bradycardia, respiratory depression, pinpoint pupil, emesis, hypothermia, transient hypertension
- Management: supportive care and close observation of neurological and hemodynamic status

Case 3: Opioid Intoxication?

- Samples sent to SFGH laboratory for LC-TOF-MS/MS testing

Data analysis

Targeted

Suspect

Untargeted

Tetrahydrozoline Urine: 2430 ng/ml
Serum: 21.56 ng/ml
Milk: 2995.4 ng/ml

335.0270

C15H12ClN03

Imidazolines Direct雄厚

30

10

5
Case 3: Tetrahydrozoline Ingestion

On further questioning, it was discovered that child ingested milk that had been adulterated with Visine™ eye drops active ingredient – tetrahydrozoline

Case 3: Tetrahydrozoline Ingestion

- After 20 hours, the boy had a heart rate of 90/min, blood pressure of 90/50 mmHg
- Mental status was back to baseline
- No treatment was given
- Child protective services and the police department were involved in the investigation of the case

Conclusions: Advantages of Mass Spectrometry

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<td>Development of compound dependent parameters for all compounds of interest, build method, establish RTs</td>
<td>Run an analytical standards to establish RTs</td>
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<td>Acquisition Method</td>
<td>Targeted – SRM/product ion scan</td>
<td>Full Scan – Full TOF MS scan with triggered collection of product ion scans</td>
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<td>Resolution</td>
<td>Nominal Mass</td>
<td>Exact Mass (~2ppm)</td>
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Acknowledgements

- Katie Thoren (DOC Case)
- Jennifer Colby (4-fluoroamphetamine case)
- Sarina Yang (tetrahydrozoline case)
- Ryk Sheppard

Self-Assessment Questions

1. Which of the following is not a class of amphetamine/cathinone like novel psychoactive substances?
   a) piperazines
   b) phenethylamines
   c) naphthoylfimbines
   d) tryptamines

2. Which of the following is not a characteristic of a high resolution mass spectrometry method?
   a) Provides accurate mass, retention time, isotope pattern and fragmentation information for drugs and metabolites in a biological sample
   b) Acquires data in an untargeted manner
   c) Method development is complex due to the need to establish multiple method parameters
   d) Data can be analyzed using targeted, suspect or untargeted data analysis

3. Which of the following compounds is not detected by most opiate immunoassays?
   a) Morphine
   b) Codeine
   c) Hydrocodone
   d) Oxycodone
   e) Fentanyl
   f) Both d and e