LC/MS Tandem and TOF MS: A New Paradigm for Emergency Department Drug Testing

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Clinical toxicology

• Fast or accurate?
OF IMMUNOASSAYS FOR DRUGS OF ABUSE
Performance of immunoassays

THE GOOD

• Cocaine (no conf. for clin tox)
• Marijuana (no acute pathology)
• Monoclonal amphetamines: separates d from l-isomers (workplace use)
• Methadone (opiate therapy mainly)
• Propoxyphene (little use today)
• Barbiturates: detects most class members fairly equally (reduced utilization as a sedative).
Performance of immunoassays

THE BAD

• Amphetamines: variable cross-reactivity towards MDMA (Ecstasy)
• Opiates: little cross-reactivity with oxycodone (need oxycodone assay)
• Benzodiazepines: poor or no cross-reactivity with many benzo metabolites.
Performance of immunoassays

**THE UGLY**

- Tricyclic antidepressants: cross-reacts with diphenhydramine, cyclobenzaprine, and others
- Phencyclidine: cross-reacts with diphenhydramine, dextromethorphan and venlafaxine.

<table>
<thead>
<tr>
<th></th>
<th>Nonusers</th>
<th>Abusers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>True negative</strong></td>
<td>9800</td>
<td>10</td>
</tr>
<tr>
<td><strong>False positive</strong></td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td><strong>False negative</strong></td>
<td>9,990</td>
<td>10</td>
</tr>
</tbody>
</table>

10,000 subjects

Prevalence: 0.1%
Test accuracy: 99%
Unexpected cross-reactivities for DOA immunoassays

oxaprozin  oxazepam
Performance of immunoassays

THE NON-EXISTENT

- Fentanyl and analogs
- Ketamine
- $\gamma$-hydroxybutyrate
- Therapeutic drugs
  - $\beta$-blockers, Ca-channel blockers,
  - hypoglycemics, novel anticonvulsants,
  - hypnotics, etc.
Why use LC-tandem MS for drug testing?

1. Polar and charged and heat labile drugs that require derivatization with GC or are not compatible with GC
2. Drugs that don’t ionize well with EI or are “over-fragmented” with EI
3. Low-dose drugs (sub-ng/mL)
4. Reduce labor required for specimen prep
   a. direct inject
   b. on-line extraction
   c. eliminate derivatization
   d. may be able to eliminate hydrolysis
Why use LC-tandem MS for drug testing?

Discontinuance of the Bio-Rad Remedi System (2007):
EI GCMS Spectra

Amitriptyline

MASS SPECTRUM

58 m/z

NI ST Chemistry WebBook (http://webbook.nist.gov/chemistry)
El GCMS Spectra

Diphenhydramine Hydrochloride
MASS SPECTRUM

58 m/z

NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)
El GCMS Spectra

Methamphetamine
MASS SPECTRUM

58 m/z

NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)
LC-MSMS (EPI) Spectra

Amitriptyline from A3200
SFGH Amphetamine confirmation

• 12 amines (plus 4 deuterated internal standards)
• more designer amines to follow
• 2 antidepressants (source of false positive screens)

Faces of meth
Crystal meth use among SF homeless

%}


Female
Male
MSM
XIC of +MRM (19 pairs): Exp 1, 199.0/164.8 amu from Sample 1 (Sample003) of amp final 10 16 06 2.wiff (Turbo Spray) Max. 8.2e4 cps.

Time, min

0.0 2.0e4 4.0e4 6.0e4 8.0e4 1.0e5 1.2e5 1.4e5 1.6e5 1.8e5 2.0e5 2.2e5 2.4e5 2.6e5 2.8e5 3.0e5

ephedrine
phenylpropanolamine
D8-methamphetamine & methamphetamine
D5-MDA & MDA
PMA
MDE
buproprion
isomethamphetamine
venlafaxine
fenfluramine
Clinical Case

• 39 year-old female presents to ED
  – Diminishing mental status, agitation, hypothermia, severe hypertension, vasoconstriction
• History of ingestion of MDMA, cocaine & 2C-I
  – Sudden onset of severe headache, nausea and vomiting
• Toxicology screen ordered
What is 2C-I?

- Hallucinogenic designer amine derived from phenethylamine
- 2C drug family developed by Alexander Shulgin (PiHKAL)
  - 2C-I appeared around 2003
- Little is known about the toxicological properties of the 2C drugs
Initial drug screen results

2C-I does not cross-react with the amphetamine screen

<table>
<thead>
<tr>
<th>Time</th>
<th>2007 01 Jan 14:02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval-HRS</td>
<td>RANDOM</td>
</tr>
<tr>
<td>Volume</td>
<td>25.00</td>
</tr>
<tr>
<td>AMP/METH/MDMA</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>Benzo/Metab</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td><strong>Cocaine Metab</strong></td>
<td><strong>NEGATIVE</strong></td>
</tr>
<tr>
<td>Opiates/Metab</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>Methadone Metab</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>Oxycodone,Urine</td>
<td>NEGATIVE</td>
</tr>
</tbody>
</table>

**Preliminary Positive** - Confirmation will be performed. 74% of preliminary positive screens are confirmed positive for amphetamine, methamphetamine, MDMA or MDA.
Amphetamine confirmation: HPLC/scanning UV results

<table>
<thead>
<tr>
<th>IDENTITY</th>
<th>NOTES</th>
<th>PEAK#</th>
<th>RT</th>
<th>L-MAX</th>
<th>PEAK-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA [Methylenedioxyamphetamine]</td>
<td>W4</td>
<td>10</td>
<td>4.17</td>
<td>239</td>
<td>OverRange</td>
</tr>
</tbody>
</table>

MDA?
Over range, poor match
New amphetamine confirmation method using LC/MS/MS

- Detects designer amines and other drugs that can cause false positive screens
MDA and 2C-I detected in patient urine

- 2C-I’s spectrum
- MDA’s spectrum
- 2C-I’s spectrum

- MDA
- D5-MDA
- D8-Methamphetamine
- D6-Amphetamine
- D5-MDMA

MDA and 2C-I detected in patient urine
Quantification

- **MDA = 5.56 mg/L**
- **2C-I = 0.311 mg/L**
- **No MDMA detected**
  - First time MDA detected in absence of MDMA in our lab
- **Recorded cases:**
  - Fatal MDMA overdose: 171 mg/L MDMA, 4.0 mg/L MDA in urine
  - Average urine concentration in 12 MDA fatalities was 108 mg/L MDA (range: 2-175)

Diagnosis

• Symptoms: altered mental status, agitation, hypothermia, severe hypertension, vasoconstriction
  – Extensor posturing
• Head CT: massive hemorrhagic stroke
• Head angiocentesis: Moyamoya
  – Underlying condition
Moyamoya

- Obstruction of two main brain vessels
- Network of new vessel formation
  - Compensation for decreased blood flow
- At risk for hemorrhagic strokes (69%)

“Puff of smoke”
Patient Outcome

• Extended stay in ICU
• One year later:
  – Quadriplegia
  – Modest improvement of mental status
  – Requires extensive care
Case presentation

- A 46 year-old man with a history of diabetes, hypertension, and ethanol abuse was brought to the emergency department after being found with decreased responsiveness. He had become increasingly despondent after losing his job. His medications were unknown. His blood pressure was 85/62 mm Hg, and the heart rate was 55 beats/min.
What drugs/drug classes do you suspect?

- Beta blockers
- Calcium channel blockers
- Tricyclic antidepressants
- Digoxin
- alpha-2 adrenergic agonists (clonidine, methyldopa)
- Opiates
- Benzodiazepines
Next generation urine drug screen

• Oral hypoglycemic agents (sulfonylureas metformin)
• Antipsychotics (risperidone, olanzapine, quetiapine, ziprasidone, clozapine, aripiprazole, haloperidol, fluphenazine, perphenazine, chlorpromazine, thioridazine)
• Low-dose opiates (buprenorphine, fentanyls)
• “Orphan” drugs of abuse (carisoprodol, dextromethorphan, ketamine, zolpidem)
• β-blockers & Ca channel blockers (atenolol, metoprolol, labetalol, propranolol, carvedilol, diltiazem, verapamil, nifedipine, amlodipine, felodipine, isradipine, nicardipine)
Antipsychotics by LC/MS
A seizure panel using LCMS to help diagnose drug-related seizures
Drug-related seizures

- SFGH sees 2-3 seizing patients in the ED every day
  - Many are unexplained
- 6% of new onset seizures are estimated to be drug-related
- No way to quickly determine if a seizure is drug-related
- A seizure panel could improve patient care and cut costs

2003 CA Poison Control Study

Analyzed 386 drug-related seizures

- 3.6% status epilepticus
- 66% involved suicide attempts
- 15% resulted from drug abuse
- 7 deaths

➢ Identified the most common drugs and drug classes that caused the seizures

Breakdown of seizures by drug type

- Buproprion 23.0%
- TCAs 7.7%
- Venlafaxine 5.9%
- Antipsychotics 4.7%
- Cocaine 4.9%
- Amphetamines 6.9%
- Diphenhydramine 8.4%
- MDMA 3.4%
- Isoniazid 5.9%
- Other 12.4%
- Antidepressants-other 9.3%
Chromatography of seizure panel
Library search results

Unknown spectra

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Molecular weight (amu)</th>
<th>Fit</th>
<th>RevFit</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid positive</td>
<td>C6-H7-N3-O</td>
<td>137.1399</td>
<td>98.780</td>
<td>98.255</td>
<td>97.056</td>
</tr>
<tr>
<td>p-nitrophenol negative</td>
<td>C6-H5-N-O3</td>
<td>139.1100</td>
<td>80.092</td>
<td>26.769</td>
<td>26.769</td>
</tr>
<tr>
<td>Parathion negative</td>
<td>C10-H14-N-O5-P-S</td>
<td>291.2600</td>
<td>81.541</td>
<td>26.165</td>
<td>25.692</td>
</tr>
</tbody>
</table>
Drugs found in seizing patients

- 128 patient samples tested
  - 13 positive samples (10%)
Clinically relevant cases

• Isoniazid (sub-therapeutic level)
  – Rx started 4 days before seizure
  – No history of seizures

• Quetiapine (8x therapeutic)
  – Psychotic patient
  – History of drug abuse

• Diphenhydramine (9x therapeutic)
  – Suicide gesture with 48 “sleeping pills”
  – Seizure and car accident
Expansion of the serum seizure panel

Widening the QRS interval by ECG:

Normal range QRS < 100 msec
QT < 440 msec
Differential of widening QRS interval

- Right or left bundle branch block
- Hyperkalemia
- Intraventricular conduction delay (myocardial infarction, cardiomyopathy with ventricular fibrosis, chamber enlargement)
- Drug-induced (particularly class IC antiarrhythmics): encainide, flecainide, moricizine, pilsicainide, and propafenone.
Class I antiarrhythmic drugs

74 yo female, on propafenone. QRS = 300 ms, QT = 780 msec
Class I antiarrhythmic drugs

Cause: co-administration of fluoxetine and risperidone, inhibitors and competitors of P450 2D6
LC/MS-TOF for toxicology analysis

- Measurement of mass to 4 decimal places.
- Distinguishes compounds with the same nominal mass but different exact mass.
- Soft ionization to produce molecular ion
- Development of protocols for all intended drugs by LC/MS/MS will be labor intensive.
- **TOF may enables detection of compounds without prior experience by the testing lab**
Clinical vs. forensic tox needs

• Clinical toxicology needs are less stringent than forensic toxicology needs. Exact assignment of unknown intoxicant may be unnecessary.
• Turnaround time for reporting of results is more important than exact drug determination.
• Reporting of a list of candidate compounds is acceptable, i.e., clinical history and toxicology knowledge is used by clinicians to cull down list.
• Presence of isomers of compounds without exact identification of unknown is acceptable, i.e., clinical management approach is unchanged.
• Serum will be examined as a specimen of choice for potential impairment assessment
TOF-MS

Time measured from ionization to detector
Example for MS-TOF

Morphine, $\text{C}_{17}\text{H}_{19}\text{NO}_3$
$\text{MW} = 285.3377$

7-aminoclonazepam
$\text{C}_{15}\text{H}_{12}\text{ClNO}_3$
$\text{MW} = 285.7283$
Time of flight of ions measured
TOF of ion is proportional to its m/z
Measurement of mass to four decimal places
Clinical Applications of LC/MS-TOF

- Adjunct to Poison Control Center Consults
- Investigational tool in monitoring intoxication
- Analysis of herbal supplements (dopants, toxic compounds)
- Development of clinical diagnostic tests
CASE 1
27yo F, Hx of chronic abdominal pain and vomiting, chronic use of APAP. pH= 7.12, AG= 22, APAP= 3µg/mL. Lactate, ethanol, methanol, ethylene glycol= neg

CASE 2
38yo F, Hx of depression and chronic pain, possible O/D of Vicodin
pH= 6.9, AG= 33, Osm gap= 24. APAP= 371mcg/mL, ALT= 8045U/L, AST=8026 U/L, lactate, ethanol, methanol, ethylene glycol = neg

Cause of acidosis?
• Chronic ingestion and acute intoxication of APAP depletes the body of glutathione → loss of negative feedback to \(\gamma\)-glutamylcysteine synthetase

• Accumulation of \(\gamma\)-glutamylcysteine and overproduction of 5-oxoproline (pyroglutamic acid)
**LC-TOF for oxoproline**

<table>
<thead>
<tr>
<th>Name</th>
<th>Score (MFG)</th>
<th>RT</th>
<th>Mass</th>
<th>Formula (MFG)</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-oxoproline</td>
<td>83.91</td>
<td>0.674</td>
<td>129.0433</td>
<td>C5 H7 N O3</td>
<td>431995</td>
</tr>
<tr>
<td>5-oxoproline</td>
<td>97.98</td>
<td>0.688</td>
<td>129.0425</td>
<td>C5 H7 N O3</td>
<td>1778980</td>
</tr>
<tr>
<td>5-oxoproline</td>
<td>97.03</td>
<td>0.68</td>
<td>129.0432</td>
<td>C5 H7 N O3</td>
<td>3215050</td>
</tr>
</tbody>
</table>

**Patient 1:** Serum 5-OP= 35.8 mmol/L  
Urine 5-OP= 0.65 mmol/mmol Cr  
**Patient 2:** Urine 5-OP= 0.1 mmol/mmol Cr
Valproic acid toxicity

CASE
50 yo male O/D with valproic acid. Pt was comatose, hypernatremic, hypocalcemic, initial AG= 18

Monitor metabolites, pathways, and therapy relevant in VPA toxicity
VPA intoxication

• Metabolism of VPA leads to toxic metabolites, 2-/4-en-VPA
• VPA consumed carnitine leading to deficiency
• Cells cannot transport long-chain fatty acids into the mitochondrion for their metabolism through β-oxidation, shift to ω-oxidation to metabolize LCFAs→↑in adipic and suberic acids.
VPA intoxication

Therapeutic approach: carnitine supplementation
Analysis of herbal supplements

Herbal medicine use in the US

• CDC Survey: about 18.9% of adult US population
• Herbals are regulated as dietary supplements by the FDA
• Manufacturers of products falling into this category are not required to prove the safety or efficacy, but the FDA may withdraw a product if harmful
• Structural analogues as dopants present in herbal medicines produced in Asia has concerned US distributors

AIM: Evaluate the presence of synthetic chemical drugs and their analogues in herbal medications for distribution
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, Cornus, Alpinia, Lycium, Cherokee Rose fruits, Black walnut seed, Cimicifuga rhizome, Cordyceps mycelium, Poria sclerotium, and Glycyrrhiza, Panax Ginseng, and Rehmania roots

MS-TOF Qualitative Analysis

• Methanol extract of Herbal X contains very high formula matches for

  • acetildenafil (structural analogue of Viagra)
  
  • hydroxyhomosildenafil (derivative of Cialis)

• Both have been reported as dopants in traditional Chinese medicine
Analysis of herbal supplements

<table>
<thead>
<tr>
<th>Name</th>
<th>Score (MFG)</th>
<th>RT</th>
<th>Mass</th>
<th>Diff (MFG, ppm)</th>
<th>Formula (MFG)</th>
<th>Ions</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetildenafil</td>
<td>84.05</td>
<td>4.147</td>
<td>466.2700</td>
<td>-1.7</td>
<td>C25 H34 N6 O3</td>
<td>4</td>
<td>1548390</td>
</tr>
<tr>
<td>Hydroxyhomosildenafil</td>
<td>91.08</td>
<td>4.602</td>
<td>488.2208</td>
<td>-0.53</td>
<td>C23 H32 N6 O4 S</td>
<td>7</td>
<td>1118384</td>
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</tbody>
</table>

Sildenafil: R=H  
Homosildenafil: R=CH₃  
Acetildenafil: R=CH₃  
Hydroxyhomosildenafil: R= CH₂OH
## Development of seizure panel

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Mass</th>
<th>RT</th>
<th>Accuracy (ppm)</th>
<th>%CV</th>
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<tbody>
<tr>
<td>Bupropion</td>
<td>239.1092</td>
<td>3.512</td>
<td>2.75</td>
<td>0.50</td>
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<tr>
<td>Citalopram</td>
<td>324.1638</td>
<td>3.780</td>
<td>4.00</td>
<td>2.50</td>
</tr>
<tr>
<td>Cocaine</td>
<td>303.1471</td>
<td>3.162</td>
<td>4.75</td>
<td>2.20</td>
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<tr>
<td>Diphenhydramine</td>
<td>255.1623</td>
<td>3.842</td>
<td>2.00</td>
<td>0.75</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>137.0589</td>
<td>0.755</td>
<td>4.50</td>
<td>1.30</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>255.0079</td>
<td>3.194</td>
<td>3.00</td>
<td>1.50</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>149.1205</td>
<td>2.631</td>
<td>1.75</td>
<td>0.20</td>
</tr>
<tr>
<td>MDMA</td>
<td>193.1103</td>
<td>2.643</td>
<td>4.25</td>
<td>2.25</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>383.1690</td>
<td>4.003</td>
<td>5.00</td>
<td>2.20</td>
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<tr>
<td>Tramadol</td>
<td>263.1885</td>
<td>3.172</td>
<td>5.00</td>
<td>2.10</td>
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<tr>
<td>Venlafaxine</td>
<td>277.2042</td>
<td>3.689</td>
<td>4.00</td>
<td>1.20</td>
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</table>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Medication</th>
<th>LC/MS-TOF Detection</th>
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<tbody>
<tr>
<td>SP1- ED</td>
<td>Quetiapine</td>
<td>No</td>
</tr>
<tr>
<td>SP2- DM</td>
<td>Citalopram</td>
<td>Yes</td>
</tr>
<tr>
<td>SP3- LP</td>
<td>Citalopram</td>
<td>Yes</td>
</tr>
<tr>
<td>SP4- RA</td>
<td>Isoniazid</td>
<td>Yes</td>
</tr>
<tr>
<td>SP5-JH</td>
<td>Isoniazid</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Diphenhydramine</td>
<td>Yes</td>
</tr>
<tr>
<td>SP6- LV</td>
<td>Lamotrigine</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Seizure panel case

Case: ED patient w/ multiple seizures and was comatose. Family claims pt took herbal supplement that caused condition. At 2:30 pm, tox fellow called SFGH for seizure panel serum and herbal capsule to rule out possible intoxication from supplement.

Analysis: Samples arrived at 3PM. By 4PM the following results were obtained:

**Formula and retention time matches for diphenhydramine**

**Patient Serum Level ~ 550ng/mL** (therapeutic range 20-50 ng/mL)

Methanol and acetonitrile extracts of the herbal supplement

**No formula matches for seizure-causing drugs and 7000 drugs/pesticides on Agilent’s database on herbal supplement extracts**

Tox confers that the patient’s clinical presentation is consistent w/ diphenhydramine intoxication. Pt was managed accordingly and recovered
Current model for clinical toxicology: ED

Symptoms suggestive of drug toxicity or overdose.
“Drug screen” order placed.

↓

Immunoassay screening (1 h TAT)
cocaine, opiates, THC, barbs, benzos
PCP, amphet, methadone, etc.

↓

GC/MS confirmation analysis, if available (1-7 d TAT)

What about toxicity to other compounds, e.g., therapeutic drugs?
Current model for clinical toxicology: ICU

Unexplained comatose, renal, liver, pulmonary, or muscle disease
Comprehensive drug screen order placed

Sample received from ICU, no clinical information provided

Sent to reference lab for “blinded” comprehensive drug screen, TAT 5-7 d

Results received too late for real-time therapeutic implementation. Results used for documentation purposes only
New model for clinical toxicology: ICU

Unexplained comatose, renal, liver, pulmonary, or muscle disease
Comprehensive drug screen order placed

Consultation to Poison Center

Case referred to regional toxicology laboratory
Info on suspected intoxicants supplied.

“Targeted” on-site LC/MS/MS analysis, 8-16 h TAT

Real-time therapeutic management decisions made
PCC-centric operation for regional toxicology lab

ER, hosp. #1
Rehab clinic
ICU, hosp. #1
ER, hosp. #2
ICU, hosp. #2
Regional tox lab
Clin tox testing strategy?

Definitive and slow (24-48 h) vs. Presumptive and fast (1 h)?