Synthetic Cannabinoid Testing in Military Urine Sample

MAJ Marisol S. Castaneto, MS, USA
NIDA, NIH, Baltimore, MD

Disclaimer

The opinions or assertions herein are those of the authors & do not necessarily reflect the views of the National Institutes of Health, Department of Defense, Army, Navy, or Air Force.
Illicit Drug Use in the U.S. (2012)

- 23.9 M Americans (12 or older) used illicit drugs in the past month (9.2% of population)
- Cannabis use most prevalent (7.2%)
- 18 to 25 years had highest rate (21.3%)
  - 18.7% cannabis use
- 2.9 M Americans 1st time illicit drug users, cannabis (65.6%) most preferred

Illicit Substance Use Active Duty Military (2012)

<table>
<thead>
<tr>
<th>Drug</th>
<th>All Service (N=39,877, 3.2% total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lifetime</td>
</tr>
<tr>
<td>Cannabis</td>
<td>27.1 ± 0.3</td>
</tr>
<tr>
<td>Cocaine (including crack)</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>LSD</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>PCP</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>MDMA (“Ecstasy”)</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Other hallucinogens</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>Heroin</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>GHB/GBL</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Any Illicit Drug Use</td>
<td>27.5 ± 0.3</td>
</tr>
<tr>
<td>Inhalants</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Synthetic cannabinoids</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>Overall (excluding prescription)</td>
<td>28.2 ± 0.4</td>
</tr>
</tbody>
</table>
Novel Psychoactive Substances (NPS)

- **Description**
  - Structurally similar or completely different from natural or synthetic controlled drugs
  - Agonists at controlled drug's receptors
  - Synthesized to avoid drug regulations
  - Elicit adverse effects
  - Not detected by routine drug testing methods

- **United Nations Office on Drugs and Crime (UNODC)**
  - Reported 348 NPS by December 2013, which increased from 251 from July 2012
  - Synthetic cannabinoids is the currently the dominant NPS

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**Drug Enforcement Administration Classification**

- Designer Drugs
  - Pyrrolidinophenone derivatives
  - Phenylalkylamines
  - Phenylpiperazines
  - Monomethoxy derivatives
    - Dimethoxyphenylpropanamine
    - 2C-series dimethoxyethanamine
  - Piperazines
  - Benzylpiperazines
  - Phenylpiperazines
  - Dimethoxy derivatives
  - Trimethoxy derivatives
  - 4-MTA homologs
  - **Synthetic Cannabinoids**
    - *including eicosanoids (anandamide & oleamide)*
  - Anabolic agents
    - Steroids & Selective androgen receptor modulators (SARMs)
  - Fluoro-containing compounds
  - Others (e.g. MDMA)
Drug Testing Programs

- Illicit drugs & non-medical intake of prescription medications
- Clinical settings, criminal investigations, driving under the influence of drug, performance enhancement, workplace & military drug testing
- Qualitative screen & quantitative confirmation
- 75 FR 71858 (Guidelines for Federal Workplace Drug Testing Program), 49 CFR Part 40 (DOT rule), DOD Directive 1010.1

Drug Demand Reduction Initiative

- Interagency agreement between DOD & NIDA, NIH
- Feasibility study to identify & address gaps for synthetic cannabinoids drug testing
- Evaluate best analytical approaches for synthetic cannabinoids identification in urine
- Determine synthetic cannabinoids intake prevalence in the military
Scope (Research Objectives)

- Comprehensive literature review that covered synthetic cannabinoids epidemiology, pharmacology, chemical synthesis & structure, identification in herbal products & biological matrices, receptor binding affinity, legal status
- Evaluate a biochip array technology immunoassay for screening of 20,017 urine service members urine specimens for synthetic cannabinoids
- Determine positivity rate of synthetic cannabinoids by confirming all presumptive positive specimens and randomly selected presumptive negative specimen

METHODS

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Literature Review

- Bibliographic databases (PubMed, Embase, Web of Science, Scopus, Cochrane, Biological Abstracts) & 2 chemical structure databases (Chemical Abstracts, SciFinder) & Web searches (Google, Google Scholar, drug forums, etc.)
- Date: 30 Nov 2011 – complete DOD report
- Expanded search up to 31 Dec 2013 (synthetic cannabinoids epidemiology, pharmacology, receptor binding)
- Expanded search up to 30 Sep 2014 (synthetic cannabinoids pharmacokinetics & detection in biological matrices)

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Synthetic Cannabinoids: Literature Review

RESULTS
Synthetic cannabinoids

- Agonists at cannabinoid receptors 1 & 2 (CB₁ & CB₂)
- First developed in research laboratories to study the endocannabinoid system and THC mechanisms of action
- Emerged as NPS in mid-2000s (Europe), 2009 in the US
  - Sold as “legal highs” alternative to cannabis
  - Sprayed on dried plant materials, packaging labeled “not for human consumption”
  - Produced by clandestine laboratories
  - Do not cross-react with cannabinoid immunoassays

Synthetic cannabinoids: Legal Status

- Synthetic Drug Abuse Prevention Act (SDAPA) 2012 placed 15 under Schedule I
  - AM2201, AM694, CP47,497, CP47,497(C8), JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-250, JWH-398, RCS-4, RCS-8
- DEA Emergency Scheduling I
  - AKB-48, XLR-11, UR-144 (May 2013)
  - AB-FUBINACA, ADB-PINACA, PB-22, 5F-PB-22 (Feb 2014)
  - AB-PINACA, THJ-2201, AB-CHMINACA (Dec 2014)
- Banned by World Anti-Doping Agency, controlled in most countries
- Clandestine laboratories modify structures, producing new compounds not covered by CSA
Synthetic Cannabinoids: Structure Classification

- Dibenzopyrans
- Indole carboxamide
- Naphthoylindole
- Naphthoylindazole
- Aminoalkylindoles
- Adamantoylindoles
- Benzoylindoles
- Cyclohexylphenols

<table>
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<th>Compound</th>
<th>Structure</th>
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<tbody>
<tr>
<td>WIN55,212-2</td>
<td><img src="image1" alt="WIN55,212-2" /></td>
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<tr>
<td>AB-001</td>
<td><img src="image2" alt="AB-001" /></td>
</tr>
<tr>
<td>AM694</td>
<td><img src="image3" alt="AM694" /></td>
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<tr>
<td>CP47,497</td>
<td><img src="image4" alt="CP47,497" /></td>
</tr>
<tr>
<td>HU-210</td>
<td><img src="image5" alt="HU-210" /></td>
</tr>
<tr>
<td>ADB-PINACA</td>
<td><img src="image6" alt="ADB-PINACA" /></td>
</tr>
<tr>
<td>JWH-018</td>
<td><img src="image7" alt="JWH-018" /></td>
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<tr>
<td>THJ-018</td>
<td><img src="image8" alt="THJ-018" /></td>
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</tbody>
</table>

AND MANY MORE!!!

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Synthetic cannabinoids pharmacology

- **Animal:** catalepsy, hypomotility, hypothermia, analgesia, immunosuppressant, anti-inflammatory, anti-epileptic, substituted for THC (drug-discrimination studies)
- **Human:** nausea, abdominal pain, hypertension, tachycardia, chest pain, myocardial infarction, stroke, acute kidney injury, depressed breathing, psychosis/hallucinations, withdrawal symptoms similar to cannabis (chronic users), death (rare)
- Human pharmacokinetics studies limited to self-experiment (only one IRB-approved)
- Earlier pharmacokinetic studies conducted in animal, also limited.
- Extensive biotransformation (Phase I) and most phase II metabolites were glucuronidated (sulfate- & cysteine-conjugates uncommon)

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Synthetic Cannabinoids: Receptor Affinity

*THC CB₁ $K_i = 41$ nM (Compton et al.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB₁ $K_i$ (nM)</th>
<th>CB₂ $K_i$ (nM)</th>
<th>THC*SC CB₁ $K_i$</th>
</tr>
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<tbody>
<tr>
<td>AB-FUBINACA</td>
<td>0.9</td>
<td>--</td>
<td>45.6</td>
</tr>
<tr>
<td>ADB-FUBINACA</td>
<td>0.4</td>
<td>--</td>
<td>103</td>
</tr>
<tr>
<td>AM694</td>
<td>0.1</td>
<td>1.4</td>
<td>410</td>
</tr>
<tr>
<td>AM2201</td>
<td>1.0</td>
<td>2.6</td>
<td>41</td>
</tr>
<tr>
<td>CP47,497(C8)</td>
<td>0.8</td>
<td>--</td>
<td>51.3</td>
</tr>
<tr>
<td>HU-210</td>
<td>0.2</td>
<td>0.4</td>
<td>205</td>
</tr>
<tr>
<td>JWH-018</td>
<td>9.0</td>
<td>2.9</td>
<td>4.6</td>
</tr>
<tr>
<td>JWH-073</td>
<td>8.9</td>
<td>--</td>
<td>4.6</td>
</tr>
<tr>
<td>JWH-122</td>
<td>0.7</td>
<td>1.2</td>
<td>58.6</td>
</tr>
<tr>
<td>JWH-200</td>
<td>42.0</td>
<td>--</td>
<td>1.0</td>
</tr>
<tr>
<td>JWH-210</td>
<td>0.5</td>
<td>0.7</td>
<td>82</td>
</tr>
<tr>
<td>UR-144</td>
<td>29.0</td>
<td>4.5</td>
<td>1.4</td>
</tr>
<tr>
<td>XLR-11</td>
<td>24.0</td>
<td>2.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

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Synthetic Cannabinoids: Detection

- Biological Matrices
  - Parent: blood, urine (uncommon), oral fluid (OF), hair
  - In exposed animals, parent analyte measured in adipose & brain tissues
  - Metabolites: blood, hair, urine
  - Windows of detection:
    - Blood: 24-48 h after acute exposure (longer if taken orally); 30 days after last use in serum (chronic intake)
    - Urine: few days (acute)
    - OF: up to 24 h (acute)
    - Hair: >weeks
Synthetic Cannabinoids: Epidemiology

- 1 in 10 college students reported intake in the past 12 months (Hu et al. 2010)
- 1 in 12 US high school students reported intake in the past 12 months (Monitoring the Future survey, 2012)
- 1.4% military personnel reported intake in the past 12 months (Health Related Behavior survey, 2012)
- 70.2% of 1,635 service members’ urine analyzed by AFMES confirmed for synthetic cannabinoids (probable cause or command directed urinalysis)

American Association Poison Control Calls Related to Synthetic Cannabinoids

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Synthetic Cannabinoids:  
Immunoassay Evaluation & Validation  
Confirmation in Urine

METHODS

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Biochip Array Evaluation

- Randox Drugs of Abuse V biochip array technology
  - Biochip labeled with 4 antibodies for synthetic cannabinoids (SCI, SCII, SCIII & SCIV)
  - Manufacturer’s recommended cutoffs:
    > SCI 10 µg/L
    > SCII 20 µg/L
    > SCIII & SCIV 5 µg/L
    > SCI-III 100% cross-reactivity with JWH-018
    > SCIV 100% cross-reactivity with JWH-250
- Biochip paired with Evidence analyzer
- 20,017 US service members’ urine specimens collected between July 2011 & June 2012
  - Previously screened negative for other illicit drugs

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Competitive Binding on a Biochip

Biochip Array Method Validation

- Limits of detection (LOD)
- Accuracy & imprecision
- Cross-reactivity (75 synthetic cannabinoids)
- Interference (endogenous & exogenous)
- Performance around cutoff
- Sensitivity, specificity & efficiency
  - True positive (TP): screened & confirmed positive
  - True negative (TN): screened & confirmed negative
  - False positive (FP): screened positive, but confirmed negative
  - False negative (FN): screened negative, but confirmed positive
  - Sensitivity = \( \frac{100 \times TP}{TP+FN} \); specificity = \( \frac{100 \times TN}{TN+FP} \); efficiency = \( \frac{TP + TN}{\text{total specimens}} \)
Qualitative LC-MS/MS Confirmation

- AB SCIEX Triple Quad™ 5500
- Wohlfarth et al. 2013, *Anal Chem*
- 20 OH-alkyl, OH-indole & carboxy metabolites
  - JWH-018, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, AM2201 & RCS-4
- 9 parent compounds:
  - JWH-018, JWH-073, JWH-081, JWH-122, JWH-210, JWH-250, AM2201, RCS-4 & MAM-2201
- LODs: 0.5 – 10.0 µg/L

Quantitative LC-MS/MS Confirmation

- Scheidweiler K, Huestis M. 2014 *J Chrom A*
- 33 OH-alkyl, OH-indole, & carboxy metabolites
  - JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, AM2201, MAM2201, RCS-4, UR-144, CP47,497-C7, CP47,497-C8
- 20 parent compounds:
- Limits of quantification: 0.1 – 1.0 µg/L
Synthetic Cannabinoids: Immunoassay Evaluation & Validation
Military Prevalence

RESULTS

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Results: Flowchart

1,432 Presumptive Positive

20,017

18,585 Presumptive Negative

Qualitative LC-MS/MS (N=2,501)

285 TP

1147 FP

1064 TN

5 FN

1,069

Quantitative LC-MS/MS (N=777)

163 FP + 324 TN

290 Positive

1.4% Positivity rate

TP: True positive
FP: False positive
TN: True negative
FN: False negative
Biochip Array Method Validation

• LOD (µg/L): 5 SCI, 11 SCII, 3 SCIII, 1.5 SCIV

• %Bias: -80.8 to -28.0% target

• Imprecision:
  • Fortified QCs 15, 30, 60 & 90 µg/L
  • <13.1% intra, <37.7% inter-assay
  • Inter-assay <15.6% with authentic urine samples (SCI, II, & III only)

Biochip Array Method Validation

• No interference from other DOA & OTC

• pH>8 increased immunoassay value, but < cutoff

• 10% bleach or peroxide increased results
  ~ 10-27%

• Cross-reactivity: 52 of 75 SC analytes >10%
  • 31 potential metabolites, 21 parent analytes
  • <1% RCS-8, STS-135, UR-144 pentanoic acid
    & XLR11
Biochip vs. LC-MS/MS

<table>
<thead>
<tr>
<th></th>
<th>SCI-IV</th>
</tr>
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<tbody>
<tr>
<td>N = 2501</td>
<td>SCI-IV</td>
</tr>
<tr>
<td>True Positive</td>
<td>285</td>
</tr>
<tr>
<td>True Negative</td>
<td>1064</td>
</tr>
<tr>
<td>False Positive</td>
<td>1147</td>
</tr>
<tr>
<td>False Negative</td>
<td>5</td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>98.3</td>
</tr>
<tr>
<td>Specificity %</td>
<td>48.1</td>
</tr>
<tr>
<td>Efficiency %</td>
<td>53.9</td>
</tr>
</tbody>
</table>

LC-MS/MS: Positive 290  Negative 2211

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Randox Biochip Cutoff Evaluation

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LC-MS/MS Qualitative Confirmation

- 1432 (screen positive) & 1069 (screen negative) by Randox
  - 285 TP & 5 FN
- Identified 15 synthetic cannabinoids metabolites & 1 parent
  - JWH-018 N-hydroxypentyl, JWH-018 5/6-hydroxyindoles
  - JWH-018 pentanoic acid
  - JWH-073 N-hydroxybutyl, JWH-073 butanoic acid
  - JWH-122 N-hydroxypentyl, JWH-200 6-hydroxyindole
  - JWH-210 N-hydroxypentyl, JWH-210 5-hydroxyindole
  - JWH-250 N-hydroxypentyl, JWH-250 5-hydroxyindole
  - JWH-250 pentanoic acid
  - AM2201, AM2201 N-hydroxypentyl, AM2201 6-hydroxyindole

Quantitative LC-MS/MS Confirmation

- Confirmed 290 positive & 487 negative
- No parent synthetic cannabinoid identified
- Identified metabolites:
  - JWH-018 N-hydroxypentyl, JWH-018 pentanoic acid, JWH-018 5 & 6-hydroxyindoles
  - JWH-073 N-hydroxybutyl, JWH-073 butanoic acid
  - JWH-081 N-hydroxypentyl
  - JWH-122 N-hydroxypentyl, JWH-122 pentanoic acid
  - JWH-210 N-hydroxypentyl, JWH-210 5-hydroxyindole
  - JWH-250 N-hydroxypentyl JWH-250 pentanoic acid JWH-250 5-hydroxyindole
  - AM2201 N-hydroxypentyl, AM2201 6-hydroxyindole
Quantitative LC-MS/MS Confirmation

- Identified metabolites:
  - MAM2201 N-hydroxypentyl
  - RCS-4 pentanoic acid, RCS-4 M9
  - UR-144 N-hydroxypentyl, UR-144 N-pentanoic acid
- Concentration ranges: 0.1 – 2,464 µg/L
- Top 5 predominant metabolites:
  - JWH-018 N-pentanoic acid (N=270)
  - JWH-018 N-hydroxypentyl (N=243)
  - AM2201 N-hydroxypentyl (N=201)
  - JWH-073 N-butanoic acid (N=199)
  - JWH-122 N-hydroxypentyl (N=129)
Converging Metabolic Pathways

AM2201 N-5-hydroxypentyl, JWH-018 pentanoic acid & hydroxypentyl, JWH-018 N-pentanoic acid, JWH-073 N-butanoic acid, JWH-018 N-4-hydroxybutyl

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Analytical Challenges

• Vast array of target analytes
• Changing target analyte availability
• Lack of information about human urinary metabolites of new cannabimimetics
• Highly potent compounds produce lower metabolite concentrations
• Common metabolites for different targets

Summary

• NPS will continue to pose public health & safety concerns
• Synthetic cannabinoids dominate the market, appealing to recreational drug users
• Military prevalence: 1.4% synthetic cannabinoids between July 2011 and June 2012
• To address analytical challenges, *in vitro* metabolite profiling studies by high resolution mass spectrometry can be a viable option in lieu human controlled administration studies
• Analytical approaches in this study can be applied to other synthetic drugs
• DOD expanded synthetic cannabinoids urine drug testing effective 1 December 2013
Acknowledgments

• National Institute on Drug Abuse, NIH
• Department of Defense Drug Testing Program
• Society of American Federal Medical Laboratory Scientists
• Clinical Laboratory Management Association

References

• Castaneto et al. Bioanalysis 2014, 6 (21), 2917-2928.