

2012 and 2013 Trends in Cannabimimetic Urine Drug Testing



Erin L. Karschner, Rebecca Heltsley, Timothy Robert, David L. Black

Aegis Sciences Corporation, 515 Great Circle Road, Nashville, TN, USA

INTRODUCTION

- ❖ Cannabimimetic use in the U.S. was first documented in 2008, when these chemicals were detected on plant materials packaged and sold in gas stations, smoke shops and on the internet.
- ❖ Several cannabimimetic agents were placed into Schedule I of the Controlled Substances Act beginning March 11, 2011.
- ❖ Our laboratory reported a 4.5% positivity rate for JWH-073 and JWH-018 metabolites in samples collected from athletes in 2011.
- ❖ Few data are available describing recent cannabimimetic use trends and specific metabolites detected in urine.

OBJECTIVE

To characterize urinary cannabimimetic trends in 2012 and 2013 in a diverse client population.

METHODS

- ❖ This study complied with approved external IRB policies.
- ❖ Samples were received from pain management, sports and workplace clients (included drug-free workplace, healthcare professionals, criminal justice and probation and parole testing).
- ❖ **Urine cannabimimetic extraction and analysis (Screen)**
 - 400 µL urine + potassium acetate buffer pH 5 + β-glucuronidase; heat at 60°C for 2h
 - Extract analytes with mixed-mode SPE and elute with dichloromethane
 - Reconstitute with 1 mg/mL ethylenediaminetetraacetic acid in acetonitrile/water/ammonium hydroxide (75:24:1)
 - Spot 2 µL onto Laz Well plates
 - Analyze samples with Phytronix laser diode thermal desorption (LDTD)-MS/MS (1 ng/mL LOQ; 2 ng/mL for UR-144 pentanoic acid)

- ❖ **Urine cannabimimetic extraction and analysis (Confirmation)**
 - ❖ 1 mL urine + potassium acetate buffer pH 5 + β-glucuronidase; heat at 60°C for 2h
 - ❖ Extract analytes with mixed-mode SPE and elute with isopropanol/ethyl acetate/ammonium hydroxide (60:35:5)
 - ❖ Reconstitute with mobile phase
 - ❖ Analyze samples with LC-MS/MS (1 ng/mL LOQ)
 - ❖ Mobile Phases: 10 mM ammonium acetate and 0.1% formic acid in water and 0.1% formic acid in acetonitrile.
 - ❖ September 2012 method modifications:
 - ❖ 0.1% formic acid in methanol as organic mobile phase
 - ❖ Scheduled multiple reaction monitoring (sMRM)
 - ❖ Addition of metabolites of 9 cannabimimetics

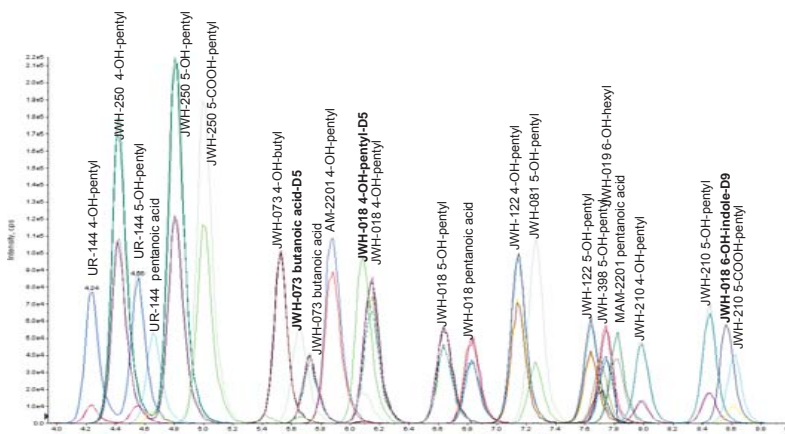


Figure 1. MRM chromatogram of a 10 ng/mL urine calibrator

RESULTS and DISCUSSION

Table 1: Cannabimimetic Positive Urine Samples in 2012 and 2013

	JWH-073	JWH-018	AM-2201	UR-144	JWH-122	JWH-210	JWH-250	JWH-081	JWH-019	JWH-398	MAM-2201													
	4-hydroxybutyl Butanoic acid	4-hydroxypentyl* 5-hydroxypentyl Pentanoic acid	4-hydroxypentyl* Pentanoic acid	4-hydroxypentyl* 5-hydroxypentyl* Pentanoic acid*	4-hydroxypentyl* 5-hydroxypentyl* Pentanoic acid*	4-hydroxypentyl* 5-hydroxypentyl* Pentanoic acid*	4-hydroxypentyl* 5-hydroxypentyl* Pentanoic acid*	4-hydroxypentyl* 5-hydroxypentyl* Pentanoic acid*	5-hydroxypentyl* 6-hydroxyhexyl*	5-hydroxypentyl*	Pentanoic acid*													
2012																								
# confirmed	12	148	2	363	401	3	4	45	65	1	2	0	0	0	0	0	0	0	0	0	0	0		
% of total	2.4	29.2	0.4	71.6	79.1	0.6	0.8	8.9	12.8	0.2	0.4	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	
2013																								
# confirmed	1	7	5	30	26	6	10	235	329	3	3	1	0	0	0	0	0	0	0	0	0	0	0	
% of total	0.3	1.8	1.3	7.8	6.8	1.6	2.6	61.4	85.9	0.8	0.8	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Analytes added to the assay September 2012

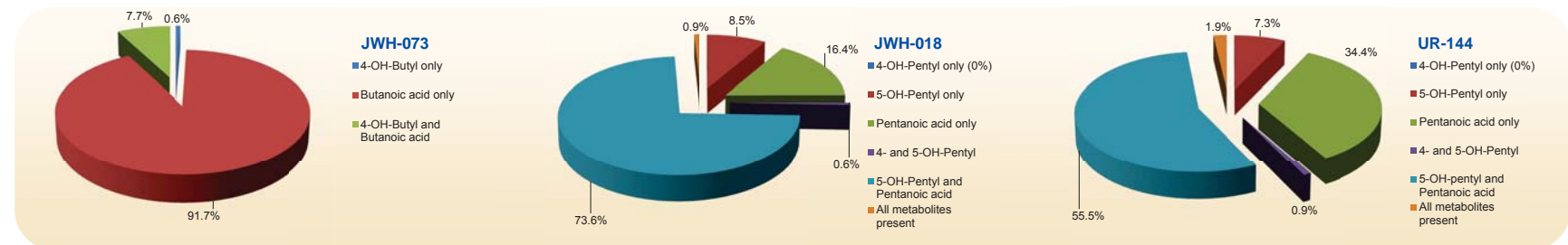


Figure 2. Metabolic Patterns Observed in Urinary Cannabimimetic Positive Samples

How many positive samples would my laboratory miss if it only monitored hydroxylated metabolites?

- 91.7% of JWH-073 positives
- 16.4% of JWH-018 positives
- 34.4% of UR-144 positives

Should my laboratory only monitor acidic metabolites?

- 99.4% of JWH-073 metabolite positive samples included the butanoic acid metabolite.
- 90.9 and 91.8% of JWH-018 and UR-144 metabolite positive samples, respectively, included the pentanoic acid metabolite.

Should my laboratory continue to monitor JWH-018 and UR-144 4-OH-pentyl?

- 0% of samples contained only JWH-018 4-OH-pentyl or UR-144 4-OH-pentyl
- 2.8% of UR-144 metabolite positive samples contained 4-OH-pentyl

Co-positivity with THCCOOH

- Although cannabis is the most widely abused illicit drug, it was once believed that cannabimimetics could be smoked as a "legal" substitute to cannabis due to detection difficulties and lack of legal ramifications.
- 890 samples were confirmed positive for cannabimimetic metabolites between 2012 and 2013.
- 789 of the 890 samples were screened for 11-nor-Δ⁹-tetrahydrocannabinol (THCCOOH) and 263 (33.3%) confirmed positive by GC/MS (LOQ 2 ng/mL).

Table 1. Cannabimimetic Positivity Rate in Varying Market Segments

2012				2013			
Total Samples Screened	Confirmed Positive Samples	Market Segment	Positivity Rate	Total Samples Screened	Confirmed Positive Samples	Market Segment	Positivity Rate
89,762	436	Pain Compliance	0.49%	133,392	349	Pain Compliance	0.26%
13,203	40	Sports	0.30%	15,028	10	Sports	0.07%
5,982	31	Workplace	0.52%	17,396	24	Workplace	0.14%
108,947	507	TOTAL	0.47%	165,816	383	TOTAL	0.23%

CONCLUSIONS

- ❖ Between 2012 and 2013, JWH-073 and JWH-018 metabolite prevalence substantially declined.
 - ❖ Legislative efforts, combined with increased public health warnings, may be one explanation for the decrease in positive samples in 2013.
 - ❖ A second explanation involves the primary assay limitation, which targets only those metabolites with available reference standards at validation. Thus, it is likely that the emergence of additional third and fourth generation cannabimimetics in 2012 and the absence of available reference standards for urinary metabolites prevented detection.
- ❖ UR-144 pentanoic acid was the analyte most commonly observed in positive samples in 2013.
- ❖ One-third of cannabimimetic positive samples screened for THCCOOH confirmed positive, indicating that cannabimimetics may be used in conjunction with, and not only as a substitute for cannabis.
- ❖ These data are valuable to laboratories monitoring urinary cannabimimetics. If laboratories are only able to monitor a single metabolite per parent cannabimimetic, it is recommended that they monitor the acid metabolite for JWH-073, JWH-018 and UR-144.
- ❖ Monitoring cannabimimetic metabolites in urine remains a challenge. It is important for laboratories to report trends, particularly in raw products and blood/plasma, as parent analyte reference standards are more readily available. Once parent analytes are identified, metabolic studies may be conducted to determine appropriate urinary metabolites.