Development of a Quantitation Method for Synthetic Cannabinoid Metabolites in Urine Using LC-MS/MS

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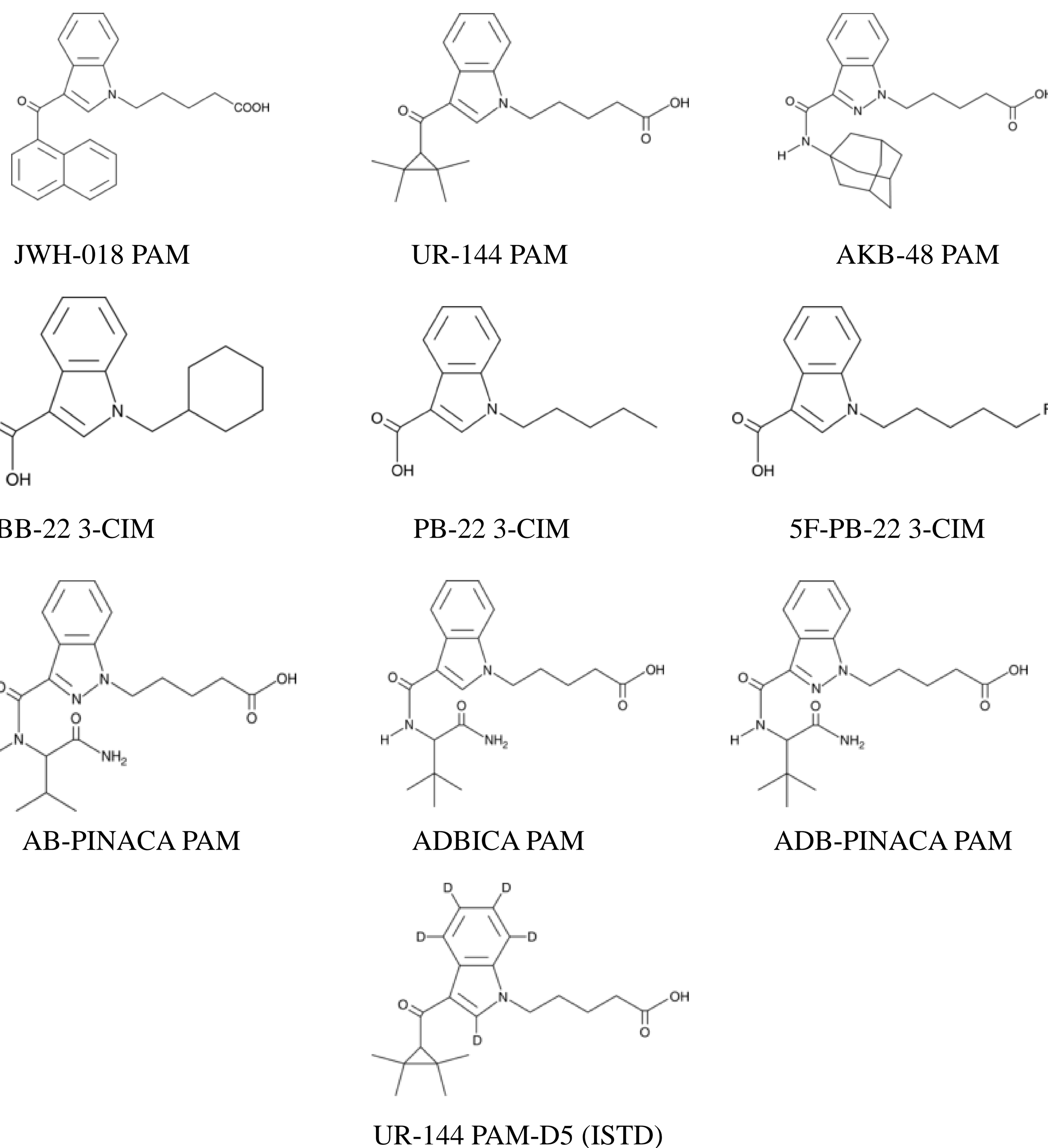
Introduction

Over the past few years, synthetic cannabinoids have become increasingly popular and prevalent in an effort from drug users to bypass current legislation and achieve a "legal" high. At the federal level in the United States, most are Schedule I substances by the Drug Enforcement Administration (DEA) as they have a high potential for abuse and no medical purpose, so in an attempt to escape legal consequences, "manufacturers" produce compounds that are structurally different from currently scheduled drugs but still give similar effects to achieve that high. JWH-018, UR-144, ADB-PINACA, AKB-48, PB-22, and 5F-PB-22 have been scheduled by the DEA; while AB-PINACA, ADBICA, and BB-22 are currently not scheduled but have recently been identified as components in synthetic cannabis blends in Japan and the United States. Since some are scheduled and since newly emerged compounds may not register a positive result in common drug screening procedures, it was essential to develop an assay to detect these compounds. Because the matrix that was focused on was urine, the method that was developed in this research focused on the pentanoic acid metabolites (PAM) or the 3-carboxyindole metabolites (3-CIM) of those nine compounds. Three different β-glucuronidases were evaluated for their efficacy in converting the phase two glucuronide conjugate back to the phase one carboxylic acid metabolite. The three that were evaluated and compared were β-glucuronidase from *Helix pomatia* (Sigma Aldrich; St. Louis, MO), *E. coli* (Sigma Aldrich), and red abalone (UCT; Bristol, PA). The method has been developed and is now in the process of being validated following Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines and will be applied to authentic urine samples once validation is complete.

Structures



Methods

Optimization

1. Optimized the cone voltage and collision gas energies for each compound
2. Acquired a daughter scan of the parent to daughter ion transitions
3. Optimized extraction solvent for optimal recovery of all analytes
4. Optimized hydrolysis by comparing recoveries from three different β-glucuronidases

Sample Preparation

1. Add the desired concentration of the metabolites to 1 mL urine
2. Add 50 μL of internal standard (UR-144 PAM-D5; 1 μg/mL)
3. Add 1 mL of 100 mM sodium acetate buffer (pH 4.5)
4. Add 50 μL of β-glucuronidase from red abalone and incubate for 30 minutes at 55 ^oC
5. Add 200 μL of 10% HCl to each sample
6. Add 3 mL of extraction solvent (hexane:ethyl acetate:isopropanol; 40:50:10)
7. Rotate for 15 minutes to mix
8. Centrifuge for 5 minutes at 3000 RPM
9. Freeze bottom (aqueous) layer using acetone-dry ice bath
10. Pour organic layer into new test tube and dry down at 50 ^oC for 15 minutes
11. Reconstitute samples in 100 μL of 70:30 mobile phase A:B (0.1% formic acid in water: 0.1% formic acid in methanol) and transfer to autosampler vials

Results

Summary Table of Optimization of LC-MS/MS Parameters and Extraction Results

Compound	Parent Ion (m/z)	Daughter Ion (m/z)	Cone Voltage (V)	Collision Energy (eV)
AB-PINACA PAM	361.10	217.05 245.15	20	25
ADB-PINACA PAM	375.20	245.15 330.23	20	20
ADBICA PAM	374.20	244.21 357.06	20	15
5F-PB-22 3-CIM	250.17	118.09 206.32	30	20
JWH-018 PAM	372.10	144.98 155.05	30	35
PB-22 3-CIM	232.17	118.28 188.28	25	20
UR-144 PAM-D5	347.20	125.31 249.21	35	25
UR-144 PAM	342.16	125.31 244.24	30	25
BB-22 3-CIM	258.17	118.28 176.06	30	20
AKB-48 PAM	396.20	93.13 135.35	30	35

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Results

Chromatogram of extracted metabolites from urine (100 ng/mL)

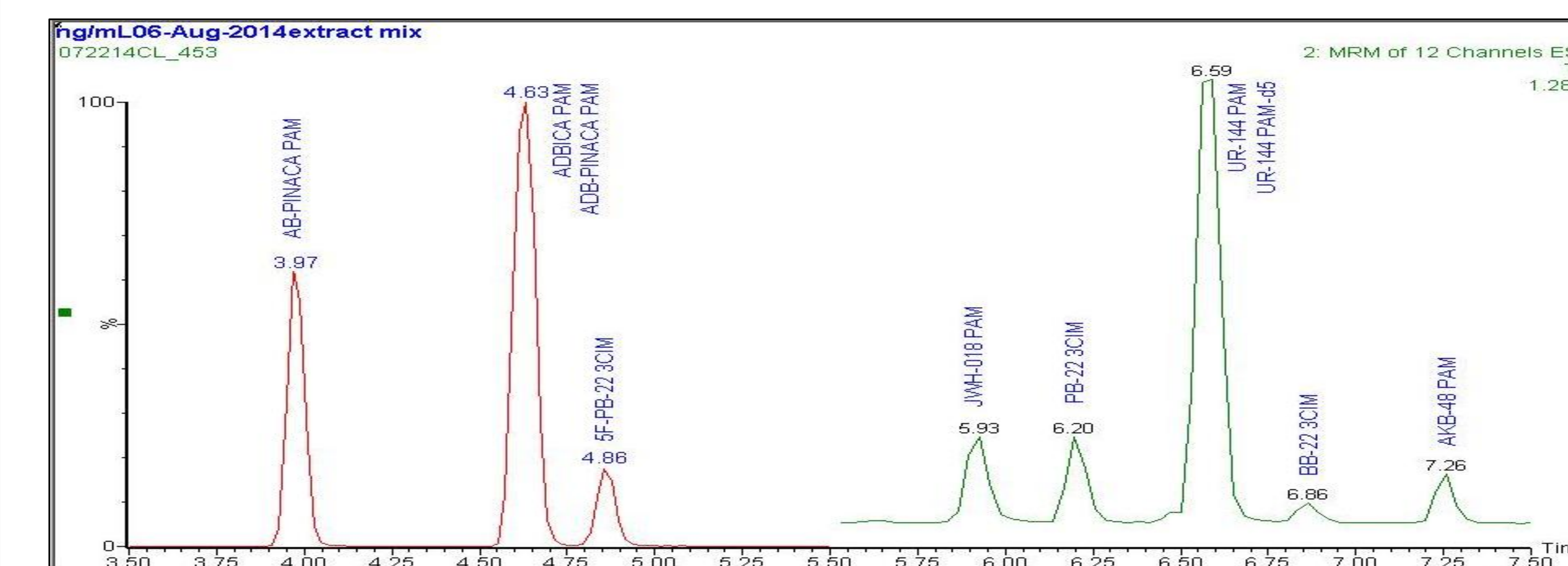

Table of extraction and LC results

Compound	Retention Time (min.)	Range (ng/mL)	R^2	Recovery
AB-PINACA PAM	4.00	1-100	0.992	87%
ADB-PINACA PAM	4.63	1-100	0.990	114%
ADBICA PAM	4.63	1-100	0.981	120%
5F-PB-22 3-CIM	4.90	1-100	0.984	43%
JWH-018 PAM	5.95	1-100	0.995	116%
PB-22 3-CIM	6.22	1-100	0.990	30%
UR-144 PAM-D5	6.59	-------	-------	-------
UR-144 PAM	6.63	1-100	0.994	111%
BB-22 3-CIM	6.86	1-100	0.992	64%
AKB-48 PAM	7.26	1-100	0.991	100%

Conclusions

- This method was able to achieve baseline separation aside from the metabolites of ADB-PINACA and ADBICA, but each analyte was identified using their specific MRM transitions.
- There was an insignificant difference in results produced by the three β-glucuronidases that were compared and there was very little difference in the efficacy of converting the glucuronide conjugate back to the carboxylic acid metabolite. β-glucuronidase from red abalone was then selected to be used based on cost and availability.
- Each analyte in the assay was linear in the range of 1-100 ng/mL ($R^2 > 0.98$) and the limits of quantitation and detection for all nine metabolites were both below 1 ng/mL.
- The recoveries obtained were satisfactory for the pentanoic acid metabolites, and even though the recoveries for the 3-carboxyindole metabolites were lower than desired, the extraction solvent used yielded the highest recovery of solvents tested.

References

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