Further Research Concerning the Detection of Oxidation Products of THC-COOH Following Urinary Adulteration

PhD Thesis: Science

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Certificate of Authorship and Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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28/11/2014

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In closing, I offer to all those dear to me a quote from the animated television series "Metalocalypse", from the episode titled "Dethhealth" (Schnepp 2009). The main characters of this show humorously ponder how to return a false-negative drug test result:

Pickles: Dudes, we party too hard, so our bodies are in terrible shape. We gotta trick the doctor by making it seem like we're in really good shape. And there's only one way to do that. Bleach... Here, drink this Murderface...

Skwisgaar: Uhh, maybe this ams a stupid question, buts, why don'ts we just pours bleach into our cups of...urines?

Pickles: No! Drink the bleach!

Nathan: Bleach is healthy. It's mostly water. And we are mostly water. Therefore, we are bleach.

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List of Abbreviations

11-OH-THC	11-Hydroxy-THC, 11-Hydroxy-Δ^9-tetrahydrocannabinol
APCI	Atmospheric Pressure Chemical Ionisation
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
cAMP	Cyclic Adenosine Monophosphate
CAN	Ceric Ammonium Nitrate
CB_1	Cannabinoid Receptor Type 1
CB_2	Cannabinoid Receptor Type 2
CBD	Cannabidiol
CBG	Cannabigerol
CBN	Cannabinol
CEDIA	Cloned Enzyme Donor Immunoassay
CI	Chemical Ionisation
COSY	Correlation Spectroscopy
DEA	Drug Enforcement Administration
DNA	Deoxyribonucleic Acid
EIA	Enzyme Immunoassay
EIC	Extracted Ion Chromatogram
ELISA	Enzyme-Linked Immunosorbent Assay
EMIT	Enzyme Multiplied Immunoassay Technique
ESI	Electrospray Ionisation
FBI	Federal Bureau of Investigation
FPIA	Fluorescence Polarization Immunoassay
GC	Gas Chromatography
GC-MS	Gas Chromatography–Mass Spectrometry
HFBA	Heptafluorobutyric Anhydride
HMBC	Heteronuclear Multiple-Bond Correlation Spectroscopy

HPLC-MS	High-Performance Liquid Chromatography–Mass Spectrometry
HSCQ	Heteronuclear Single-Quantum Correlation Spectroscopy
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LLE	Liquid-Liquid Extraction
MRM	Multiple Reaction Monitoring
NIDA	National Institute on Drug Abuse
NMR	Nuclear Magnetic Resonance Spectroscopy
PAD	Programmable Absorbance Detector
PCC	Pyridinium Chlorochromate
PFPA	Pentafluoropropionic Anhydride
PFPOH	Pentafluoropropionic Alcohol
PVP	Polyvinylpyrrolidone
QToF-MS	Quadrupole Time-Of-Flight Mass Spectrometer
SAMHSA	Substance Abuse and Mental Health Services Administration
SRM	Selected Reaction Monitoring
THC	Tetrahydrocannabinol, Δ^9-Tetrahydrocannabinol
THC-COOH	11-nor-9-Carboxy-THC, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol
THC-COOH-d9	Deuterated 11-nor-9-Carboxy-THC
THCV	Tetrahydrocannabivarin
TIC	Total Ion Chromatogram
UV-Vis	Ultraviolet–Visible Spectroscopy

Abstract

In Australia and throughout the world, cannabis is one of the most widely used recreational substances. Whilst the recreational use of cannabis remains widely controversial, and the detection of its use in a range of biological matrices is of vital importance for drug testing laboratories and law enforcement agencies. The detection of drugs of abuse is critical in various areas, including pre-employment and post-incident drug screening, and sports drug testing.

The use of cannabis by an individual may be ascertained by identifying the main metabolites of the major psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (THC), in biological matrices such as urine. The principal metabolite of THC is 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), and may be detected in urine in both its free and glucuronide-bound form, with detection of either regarded as compelling evidence for the use of cannabis by an individual.

Detection of THC-COOH by a range of instrumental techniques in drug testing laboratories is well established. However, this metabolite is known to be susceptible to reaction with certain adulterants. Adulteration of urine samples with oxidising adulterants has been shown to effectively mask cannabis use through reaction with THC-COOH. As such, the primary goals of this research are to assess the efficacy of a range of adulterants on the detection of THC-COOH in vitro, ascertain whether novel reaction products specific to the reaction of THC-COOH with selected adulterants form, and to assess the potential of these compounds to act as markers of both cannabis use and urine adulteration.

Successful detection of a range of reaction products of THC-COOH was achieved, and three adulterants selected for further research: pyridinium chlorochromate, Betadine and bleach. Structural elucidation of these reaction products was attempted, and validated methods were developed for the quantitative detection of THC-COOH and qualitative detection of the targed reaction products following urine adulteration. Kinetics, pH and stability studies demonstrated that these reaction products formed under a range of pH and sample storage conditions, and critically, remained detectable for at least twenty days following adulteration.

Detection of these potential markers of urine adulteration was also successfully achieved through the adulteration of authentic cannabis-positive urine specimens. This detection in authentic urine specimens is considered significant, as it highlights the potential for these novel compounds to be incorporated into current drug testing regimes employed by drug testing laboratories, and a potential means by which both cannabis use and urine adulteration may be conclusively identified.