

# **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.

Nathan Charlton

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

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

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