Detecting and quantifying cannabinoids in oral fluid

by

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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 the 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 the information sources and literature used are indicated in the thesis.

SIGNATURE: Anna Molnar

DATE: 20/10/2015
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‘Recovery of delta-9-tetrahydrocannabinol in oral fluid from polypropylene containers.’ (Oral presentation)

- The 50th Annual Meeting of the International Association of Forensic Toxicologists (TIAFT), Hamamatsu, Japan.
- 21st International Symposium on the Forensic Sciences (ANZFSS), Hobart, Australia.

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Abbreviations

11-OH-THC 11-hydroxy-delta-9-tetrahydrocannabinol
AS4760 Australian Standard 4760:2006
AS/NZS4308 Australian and New Zealand Standard 4308:2008
BSTFA N,O-bis(trimethylsilyl)trifluoroacetamide
CBD Cannabidiol
CBN Cannabinol
CI Chemical ionization
CID Collision induced dissociation
CMC Critical micelle concentration
CNS Central nervous system
DDS Drug Detection System
DRUID Driving under the influence of drugs
DW DrugWipe®
EIC Extracted ion chromatogram
ESI Electrospray ionisation
EtOAc Ethyl acetate
FDA United States Food and Drug Administration
FN False negative
FP False positive
GC Gas chromatography
HPLC High-performance liquid chromatography
LC Liquid-chromatography
LLE Liquid-liquid extraction
LLOD Lower limit of detection
LLOQ Lower limit of quantification
LOD Limit of detection
LOQ Limit of quantification
m/z Mass-to-charge ratio
MDMA 3,4-methylenedioxy-N-methylamphetamine
MRE Mean relative error
MRM Multiple reaction monitoring
MS Mass spectrometry
MS/MS Tandem mass spectrometry
MSTFA N-Methyl-N-(trimethylsilyl)trifluoroacetamide
Na₂HPO₄ Di-sodium hydrogen orthophosphate
NaH₂PO₄ Sodium di-hydrogen orthophosphate
NaN₃ Sodium azide
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>NATA</td>
<td>National Association of Testing Authorities, Australia</td>
</tr>
<tr>
<td>NCI</td>
<td>Negative chemical ionization</td>
</tr>
<tr>
<td>NDSHS</td>
<td>National Drug Strategy Household Survey</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>PCDL</td>
<td>Personal Compound Database and Library</td>
</tr>
<tr>
<td>PCI</td>
<td>Positive chemical ionisation</td>
</tr>
<tr>
<td>PMME</td>
<td>Polymer monolith microextraction</td>
</tr>
<tr>
<td>POCT</td>
<td>Point of care test</td>
</tr>
<tr>
<td>PP</td>
<td>Polypropylene</td>
</tr>
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<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>QID</td>
<td>Four times a day</td>
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<td>QQQ</td>
<td>Triple quadrupole</td>
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<td>Quadrupole time-of-flight</td>
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<tr>
<td>TOF</td>
<td>Time-of-flight</td>
</tr>
<tr>
<td>ROSITA</td>
<td>Roadside Testing Assessment</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
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<td>Substance Abuse and Mental Health Services Administration</td>
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<td>SIM</td>
<td>Selected ion monitoring</td>
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</tr>
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</tr>
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<td>Trimerthylsilyl</td>
</tr>
<tr>
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<td>True negative</td>
</tr>
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<td>UNSW</td>
<td>University of New South Wales</td>
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<td>WADA</td>
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Abstract

The main psychoactive constituent of cannabis, Δ⁹-tetrahydrocannabinol (THC), is the major target analyte for the detection of cannabis in oral fluid. While oral fluid has been used widely for drug testing purposes for a number of years, it has not been as thoroughly investigated as urine and blood testing procedures. This thesis aims to fill some of the gaps in knowledge regarding the detection of cannabinoids, particularly THC, in the oral fluid matrix.

THC is highly lipophilic and it is known that losses can occur when it comes in contact with plastic. Factors governing the interaction of THC with polypropylene in the oral fluid matrix were investigated using liquid chromatography–tandem mass spectrometry (LC–MS/MS) and gas chromatography–mass spectrometry (GC–MS) techniques. Preliminary results of the stability of THC in oral fluid stored in polypropylene containers indicated comparable THC losses under refrigerated and freezing conditions over a period of two weeks.

Delving further into the circumstances surrounding the absorptive tendencies of THC, no significant difference was found in terms of THC loss to plastic when the concentration ranged from 25–1000 ng/mL in the same volume of oral fluid. Varying the oral fluid volume (0.5–1.5 mL) while keeping THC at a constant concentration showed an upward trend with more loss associated with lower volumes. This indicated that THC adsorption is increased with greater plastic surface area to oral fluid volume. The use of Triton® X-100 significantly decreased the adherence of THC to the plastic tubes and increased the THC transfer (>96%) at all volumes tested. Addition of a surfactant to an accurately measured volume of oral fluid is a potential way to reduce the adsorption effect, while avoiding inconsistencies with oral fluid volumes generally found when using commercial collection devices. Degradation of THC during storage was also studied over a 4-week period and it was found that azide did not seem to play a significant role in preserving THC in oral fluid.
Sativex®, an oromucosal spray containing THC and cannabidiol (CBD), is indicated for the treatment of spasticity in multiple sclerosis in the United Kingdom (UK) and a number of other countries. The introduction of Sativex® to the Australian market may have implications for patients who drive since the THC may be detected by roadside drug testing procedures. Studies were carried out to determine whether or not patients taking Sativex® will test positive to THC using the DrugWipe® II Twin and Cozart® Drug Detection System (DDS) screening tests. Detectable levels of THC, CBD and cannabinol (CBN) in their oral fluid were also confirmed by LC–MS/MS. It was found that Sativex® users may test positive for THC by roadside drug testing within 2–3 h of use. Confirmatory analysis can identify Sativex® treatment through use of THC/CBD ratios, however, these ratios would unlikely be sufficient to differentiate non-medicinal cannabis use from Sativex® use if both are taken concurrently.

Analytical methods are continually evolving as more sensitive, more reliable and more user-friendly instrumentation and procedures are developed. The potential of using novel nanospray LC–chip–MS to detect and quantify cannabinoids in oral fluid was evaluated. The system was found to be unsuitable for routine analysis procedures; however it may have potential in other fields if used with a highly sensitive tandem MS.

The results presented in this thesis provide new insight into some of the difficulties faced with the detection and quantification of cannabinoids in oral fluid. The importance of determining the most appropriate collection and storage procedures for oral fluid specimens is highlighted, as is the interpretation of positive screening and confirmatory results when medicinal cannabis products are inevitably introduced.