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.

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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
Na_2HPO_4	Di-sodium hydrogen orthophosphate
NaH_2PO_4	Sodium di-hydrogen orthophosphate
NaN_3	Sodium azide

NATA	National Association of Testing Authorities, Australia
NCI	Negative chemical ionization
NDSHS	National Drug Strategy Household Survey
NSW	New South Wales
PCDL	Personal Compound Database and Library
PCI	Positive chemical ionisation
PMME	Polymer monolith microextraction
POCT	Point of care test
PP	Polypropylene
QC	Quality control
QID	Four times a day
QQQ	Triple quadrupole
Q–TOF	Quadrupole time-of-flight
TOF	Time-of-flight
ROSITA	Roadside Testing Assessment
RSD	Relative standard deviation
SAMHSA	Substance Abuse and Mental Health Services Administration
SIM	Selected ion monitoring
SIR	Selected ion recording
SPE	Solid-phase extraction
SRM	Selected reaction monitoring
THC	Delta-9-tetrahydrocannabinol
THCA-A	Delta-9-tetrahydrocannabinolic acid A
THC-COOH	11-nor-delta-9-tetrahydrocannabinol-9-carboxilic acid
TMCS	Trimethylchlorosilane
TMS	Trimethylsilyl
TN	True negative
TP	True positive
ULOD	Upper limit of detection
ULOQ	Upper limit of quantification
UNSW	University of New South Wales
WADA	World Anti-Doping Agency

Abstract

The main psychoactive constituent of cannabis, Δ^9-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.