Searching for Potential Markers for Monitoring the Presence of Opiates in Urine Exposed to Oxidising Adulterants

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.

Susan Luong

DATE

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Table of contents

List of figures

List of tables

Abbreviations

1D	one-dimensional
^{1}H-NMR	one-dimensional proton NMR
^{1}H-^{1}H COSY	two-dimensional correlation spectroscopy NMR
^{1}H-^{13}C HSQC	heteronuclear single quantum coherence spectroscopy
^{1}H-^{13}C HMBC	heteronuclear multiple bond correlation spectroscopy
2D	two-dimensional
2-nitro-M6G	2-nitro-morphine-6-glucuronide
2-nitro-MAM	2-nitro-6-monoacetylmorphine
2-nitro-MAM-TMS	trimethylsilyl derivative of 2-nitro-MAM
3-MAM	3-monoacetylmorphine
6-MAM	6-monoacetylmorphine
6-MAM-TMS	trimethylsilyl derivative of 6-MAM
AIDDC	Australian Illicit Drug Data Centre
APCI	atmospheric pressure chemical ionisation
AS/NZS 4308	Australian/New Zealand Standard™ 4308
BSTFA	*N,O*-bis(trimethylsilyl)trifluoroacetamide
C6G	codeine-6-glucuronide

$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
CEDIA	cloned enzyme donor immunoassay
CID	collision induced dissociation
CNS	central nervous system
DEA	Drug Enforcement Administration
DPC	diphenylcarbazide
EIC	extracted ion chromatogram
EI-MS	electron impact-mass spectrometer
ELISA	enzyme linked immunosorbent assay
EMIT	enzyme multiplied immunoassay
EPO	erythropoietin
ESI	electrospray ionisation
ESI-MS	electrospray ionisation-mass spectrometry
FPIA	fluorescence polarisation immunoassay
GC-MS	gas chromatography-mass spectrometry
h	hour(s)
HCl	hydrochloric acid

HPLC	high performance liquid chromatography
ICP-MS	inductively coupled plasma-mass spectrometry
KNO_2	potassium nitrite
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC-MS/MS	liquid chromatography tandem mass spectrometry
LLE	liquid-liquid extraction
M3G	morphine-3-glucuronide
M6G	morphine-6-glucuronide
MALDI	matrix assisted laser desorption ionisation
MeOH	methanol
min	minutes
MRE	mean relative error
MRM	multiple reaction monitoring
MS	mass spectrometry
MSTFA	*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide
m/z	mass-to-charge
NaOH	sodium hydroxide

NMI	National Measurement Institute
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
QQQ-MS	triple quadrupole-mass spectrometer/spectrometry
QTOF-MS	quadrupole time-of-flight mass spectrometer/spectrometry
R_f	retention factor
R_t	retention time
RIA	radioimmunoassay
RSD	relative standard deviation
SAMHSA	substance abuse and mental health services administration
SIM	selective ion monitoring
SPE	solid phase extraction
THC	Δ^9-tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-Δ^9-tetrahydrocannabinol
TIC	total ion chromatogram
TLC	thin layer chromatography
TMB	tetramethylbenzidine
TMCS	trimethylchlorosilane

UNODC	United Nations Office on Drug and Crime
WADA	World Anti-Doping Agency

Abstract

Urine is a long accepted biological matrix used for the detection of prescription and illicit drug use in the population. In today's society, there is still a social stigma attached to individuals that have been found to be using contraband drugs. Being labelled a "drug addict" or a "drug cheat" in sports can potentially be detrimental to a person's reputation. As such, it is not surprising to learn that they are motivated to discover and utilise new and ingenious ways of circumventing routine drug testing protocol. A very effective method for doing so is to purposefully tamper a urine specimen to invalidate the results of a drug test.

Currently, urine samples deemed to be tampered are not analysed further for drugs of abuse as the presence of the target analytes may be significantly deteriorated or even undetectable using routine testing methods. One pathway for the mechanism of action of commercially available urine adulterants is through oxidation.

The research carried out in this project has shown that following exposure of six opiates (6-MAM, morphine, codeine, codeine-6-glucuronide, morphine-3-glucuronide and morphine-6-glucuronide) to various oxidising adulterants (nitrite, PCC and hypochlorite), stable reaction products were identified in urine. The structures of 12 reaction products were elucidated using high resolution mass spectrometry and nuclear magnetic resonance spectroscopy, where possible. The reaction products were characterised to be: 2-nitro-MAM, 2-nitro-morphine, 2-nitro-M6G, codeinone, 14-hydroxycodeinone, 6-*O*-methylcodeine, 8-hydroxy-7,8-dihydrocodeinone, a lactone derivative of C6G, morphinone-3-glucuronide, 7,14-dihydroxy-6-MAM, a 7,8-di-keto analogue of 6-MAM and a 7,8-di-keto analogue of morphine.

In all cases, the original opiate abundances were found to be diminished or undetectable. However, the reaction products were found to be stable for at least seven days using LC-MS. Reaction mechanisms for the formation of the 2-nitro analogues and codeinone were also proposed. The formation of the 2-

nitro analogues was hypothesised to follow an electrophilic substitution reaction. The production of codeinone was suggested to be initiated by the chromium (VI) complex found in PCC.

It was discovered that both nitrite and PCC caused a decrease in the response of the CEDIA 6-AM and opiate assays, respectively. In addition, the morphine/codeine ratios (used during confirmation testing) were found to be affected by the presence of PCC, due to the loss of both native and internal standard species.

The exposure of the opiates to hypochlorite in water resulted in the detection of several potential reaction products. However, it is disadvantageous that they appear to be relatively unstable, only forming under narrow hypochlorite concentration ranges. Due to these reasons, further investigation was not pursued.

Finally, an in-house quantitative NMR procedure for the certification of reaction product material was demonstrated using 2-nitro-MAM and 2-nitro-morphine following their syntheses and isolation. This method can be used as a quick alternative to certifying material through commercial institutions when there are constraints with time and funding.

Overall, the research carried out in this project has laid the groundwork for future work concerning the use of the reaction products as markers for monitoring the presence of opiates in adulterated urine. Due to its relative stability, ease of formation and detection, the identified reaction products show potential for their incorporation into drug testing programs as a way of monitoring opiate positive urine specimens adulterated with nitrite.