UTS UNIVERSITY OF TECHNOLOGY SYDNEY

The development and validation of screening test methods for the presumptive detection of New Psychoactive Substances

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Thesis submitted in fulfilment of the requirements for the degree of **Doctor of Philosophy (Science)**

under the supervision of Prof. Shanlin Fu, Dr. Ronald Shimmon and Dr. Morgan Alonzo

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Certificate of authorship and originality

I, Laura Clancy, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Mathematical and Physical Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Abbreviations

µPAD paper-based microfluidic analytical device

25B-NB4OMe 4-bromo-2,5-dimethoxy-*N*-[(4-methoxyphenyl)methyl]-benzeneethanamine

25B-NBF 4-bromo-*N*-[(2-fluorophenyl)methyl]-2,5-dimethoxy-benzeneethanamine

25B-NBOMe 2-(4-bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25C-NB3OMe 4-chloro-2,5-dimethoxy-*N*-[(3-methoxyphenyl)methyl]-benzeneethanamine

25C-NBOMe 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25D-NBOMe 2-(4-methyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25E-NBOMe 2-(4-ethyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25G-NBOMe 2-(3,4-dimethyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25H-NB4OMe 2,5-dimethoxy-*N*-[(4-methoxyphenyl)methyl]-benzeneethanamine

25H-NBOMe 2-(2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25I-NB3OMe 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-(3-methoxybenzyl)ethan-1-amine

25I-NBMD *N*-(benzo[d][1,3]dioxol-4-ylmethyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethanamine

25I-NBOH 2-(((4-iodo-2,5-dimethoxyphenethyl)amino)methyl)phenol

25I-NBOMe 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25iP-NBOMe 2-(4-isopropyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25-NBOMe 2,5-dimethoxy-*N*-(2-methoxybenzyl) phenethylamine derivatives

25N-NBOMe 2-(4-nitro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25P-NBOMe 2-(4-propyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25T2-NBOMe 2-(4-ethylthio-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25T4-NBOMe 2-(4-isopropylthio-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25T7-NBOMe 2-(4-propylthio-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

25T-NBOMe 2-(4-methylthio-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine

2C-B 2,5-dimethoxy-4-bromophenethylamine

2C-D 4-methyl-2,5-dimethoxy-benzeneethanamine

2C-E 4-ethyl-2,5-dimethoxy-benzeneethanamine

2C-H 2,5-dimethoxy-phenethylamine

2C-I 4-iodo-2,5-dimethoxy-benzeneethanamine

2C-T-2 4-ethylthio-2,5-dimethoxy-benzeneethanamine

2C-X 2,5-dimethoxy-phenethylamine derivatives

30C-NBOMe 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-(3,4,5-trimethoxybenzyl)ethanamine

4-FMC 4-fluoromethcathinone

4-MEC 4-Methyl-*N*-ethylcathinone

4-MMC 4-methyl-*N*-methylcathinone

$5HT_{2A}$ 5-hydroxytryptamine 2A serotonin receptor

ACD acetaldehyde

ACN acetonitrile

AFP Australian Federal Police

ANOVA analysis of variance test

ATR attenuated total reflectance

ATS amphetamine-type substances

BZP 1-benzylpiperazine

conc. concentrated

CYMK cyan, yellow, magenta, black

DAD diode array detector

DART	direct analysis in real time
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEA	Drug Enforcement Administration
DIMS	Drug Information and Monitoring System
DPIC	Dutch Poisons Information Centre
EI	electron ionisation
ELISA	enzyme linked immunosorbent assays
EMCDDA	European Monitoring Centre on Drugs and Drug Addiction
EWA	Early Warning Advisory
FTIR	Fourier transform infrared spectroscopy
GC	gas chromatography
h	hours
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSB	hue, saturation, and brightness
HSL	hue, saturation, and lightness
HTML	hexadecimal colour
IR	infrared
JWH-018	1-naphthalenyl(1-pentyl-1*H*-indol-3-yl)-methanone
JWH-073	(1-butylindol-3-yl)-naphthalen-1-ylmethanone
LC	liquid chromatography
LOD	limit of detection
LSD	lysergic acid diethylamide
MANOVA	multivariate analysis of variance test

mCPP	1-(3-chlorophenyl)piperazine
MDMA	3,4-methylenedioxy-*N*-methylamphetamine
MDPBP	3,4-methylenedioxy-α-pyrrolidinobutiophenone
MDPV	methylenedioxypyrovalerone
MeOH	methanol
Mescaline-NBOMe	3,4,5-trimethoxy-*N*-[(2-methoxyphenyl)methyl]-benzeneethanamine
min	minutes
MPBP	4-methyl-α-pyrrolidinobutiophenone
MPPP	4-methyl-α-pyrrolidinopropiophenone
MS	mass spectrometry
NIJ	National Institute of Justice
NIR	near-infrared
NMR	nuclear magnetic resonance
NPF	Non-pharmaceutical fentanyls
NPS	new psychoactive substances
NQS	1,2-napthoquinone-4-sulphonate
***o*-TCBQ**	3,4,5,6-tetrachloro-1,2-benzoquinone
PIHKAL	'Phenethylamines I Have Known and Loved'
PMA	*para*-methoxyamphetamine
PMMA	*para*-methoxymethamphetamine
ppm	parts per million
QQQ-MS	triple quadrupole mass spectrometry
QTOF-MS	quadrupole time of flight mass spectrometry
RGB	red, green, blue
RT	room temperature

RYB	red, yellow, blue
SCRA	synthetic cannabinoid receptor agonists
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
TCBQ	2,3,5,6-tetrachloro-1,4-benzoquinone
TFMPP	1-(3-trifluoromethylphenyl)piperazine
TLC	thin layer chromatography
UK	United Kingdom
UN	United Nations
UNODC	United Nations Office on Drugs and Crime
UR-144	(1-pentyl-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
USA	United States of America
UV	ultraviolet
WHO	World Health Organisation
Δ^9-THC	delta-9-tetrahydrocannabinol

Abstract

The growing number of new psychoactive substances (NPS) necessitates the need to identify these drugs under any circumstance. Rapid, on-site presumptive tests are integral to identifying unknown substances in case work, healthcare settings, and harm reduction situations. There are currently a wide variety of colour tests available for the identification of traditional illicit drugs (e.g. cocaine, amphetamines), however, specific tests for the detection of NPS are limited. The inability of current presumptive test methods to selectively identify the large variety of NPS indicates the need for the development of such techniques that can provide information about the presence of these drugs in unknown materials. This research has developed a thoroughly validated colour test method utilising 2,3,5,6-tetrachloro-1,4-benzoquinone that is capable of selectively identifying NBOMe compounds, with significant potential implications for application in case work settings. This test method was then further investigated to improve the portability and the discrimination of the colour change results. Mobile phone applications were assessed for their ability to utilise the inbuilt camera to record RGB values which may be attributed to a drug, drug class, and even a concentration range. Statistical analysis of the RGB values was completed to further determine the applicability of this method. Promising results indicate that NBOMe analogues can be distinguished from other illicit compounds. The further optimisation of the predictive model would be of benefit for the extension of this study. The translation of the NBOMe test, along with three other colour test methods for the detection of synthetic cathinones (neocuproine-copper reagent), piperazines (1,2-naphthoquinone-5-sulphonate reagent), and fentanyl analogues (naphthoquinone reagents), to paper-based systems was assessed for ease of portability and the potential for development of a multiplexed device. The combination of these tests, and therefore their results combined into a single device, would assist in the identification process of a range of NPS without the need for multiple test kits. A proof-of-concept study was carried out to assess the possible visualisation of NBOMe compounds in oral fluid samples. The addition of colour test reagents to spiked oral fluid samples, or the inclusion of a paper chromatographic method, demonstrated the ability to detect NBOMe compounds. While there is still optimisation required, these methods showed great potential to be used in the presumptive identification of NPS and in particular, NBOMe analogues. This research demonstrates the promise of simple, affordable, and rapid methods to be utilised as on-site testing devices for NPS.