Detection and Profiling of Synthetic Opioids

by

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University of Technology Sydney

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

%RSD	Relative Standard Deviation
2-BEB	(2-bromoethyl)benzene
2-CEB	(2-chloroethyl)benzene
4-ANPP	4-anilino-N-phenethylpiperidine
4-FBF	4-fluorobutyryl fentanyl
ABS	Australian Bureau of Statistics
ACIC	Australian Criminal Intelligence Commission
ACMD	Advisory Council on the Misuse of Drugs
ACN	Acetonitrile
AFP	Australian Federal Police
AIDIP	Australian Illicit Drug Intelligence Program
AIHW	Australian Institute of Health and Welfare
ANN	Artificial Neural Network
AORC	Association of Official Racing Chemists
ARFL	Australian Racing Forensic Laboratory
ATS	Amphetamine-type Stimulants
CAS	Chemical Attribution Signature
CE	Collision Energy
CE-DAD	Capillary Electrophoresis – Diode Array Detector
CID	Collision-induced Dissociation
CRM	Certified Reference Material
CSV	Comma-separated Value
DALY	Disability-adjusted Life Years
DBE	Double Bond Equivalents
DCM	Dichloromethane
DDA	Data-dependent Acquisition
DEA	Drug Enforcement Administration
DIA	Data-independent Acquisition

DIPEA	Diisopropylethylamine
EIC	Extracted Ion Chromatogram
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
ESI	Electrospray Ionisation
ESI+	Positive Electrospray Ionisation Mode
EWA	Early Warning Advisory
FbF	Find by Formula
FbI	Find by Ion
FTIR	Fourier Transform Infra-red Spectroscopy
FWHM	Full Width at Half Maximum
GC-MS	Gas Chromatography – Mass Spectrometry
GPR	Gaussian Process Regression
HR	High Resolution
HRMS	High-resolution Mass Spectrometry
Hy	Hydrophilic Factor
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
IS	Internal Standard
KMD	Kendrick Mass Defect
LC-ELSD	Liquid Chromatography – Evaporative Light Scattering Detector
LC-MS	Liquid Chromatography – Mass Spectrometry
LC-QTOF-MS	Liquid Chromatography – Quadrupole Time of Flight – Mass Spectrometry
LD_{50}	Median Lethal Dose
LR	Low Resolution
m/z	Mass-to-charge Ratio
MALDI	Matrix-assisted Laser Desorption/Ionisation
MCC	Matthew's Correlation Coefficient
MCR	Multicomponent reaction
MDA	3,4-methylenedioxyamphetamine
MDF	Mass Defect Filtering
MDMA	3,4-methylenedioxymethamphetamine

MFE	Molecular Feature Extraction
MPP	Mass Profiler Professional
MRM	Multiple Reaction Monitoring
MS/MS	Tandem Mass Spectrometry
MSC	Molecular Structure Correlator
MS^E	Elevated Mass Spectrometry
MSE	Mean Square Error
NDSHS	National Drug Safety Household Survey
NIST	National Institute of Standards and Technology
NLF	Neutral Loss Filtering
NMI	National Measurement Institute
NMR	Nuclear Magnetic Resonance Spectroscopy
NPF	Non-pharmaceutical Fentanyl
NPP	N-phenethyl-4-piperidone
NPS	New Psychoactive Substances
NSO	Novel Synthetic Opioid
ONDCP	Office of National Drug Control Policy
PCA	Principal Component Analysis
PCDL	Personal Compound Database and Library
PIS	Product Ion Searching
PLS-DA	Partial Least Squares – Discriminant Analysis
PMMA	Para-methoxymethylamphetamine
QqQ	Triple Quadrupole
RFE	Recursive Feature Extraction
RMSE	Root Mean Square Error
RRT	Relative Retention Time
RT	Retention Time
S/N	Signal-to-noise Ratio
SD	Standard Deviation
SPE	Solid Phase Extraction

STRL	Special Testing and Research Laboratory
SVM	Support Vector Machine
SWATH	Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
TBAB	Tetra-N-butylammonium bromide
TIC	Total Ion Chromatogram
UHPLC-MS	Ultra High-performance Liquid Chromatography – Mass Spectrometry
UNODC	United Nations Office on Drugs and Crime
VBA	Visual Basic for Applications

Publications and Conference Proceedings

Refereed Journal Publications

1. **Klingberg, J.**, et al., *Collision-Induced Dissociation Studies of Synthetic Opioids for Non-targeted Analysis.* Front. Chem., 2019. **7**(331).

2. **Klingberg, J.**, et al., *Finding the Proverbial Needle: Non-targeted Screening of Synthetic Opioids in Equine Plasma.* Drug Test. Anal. 2020. **DOI:** 10.1002/dta.2893

Refereed Conference Proceedings (presenting author underlined)

1. **Klingberg J**, Shimmon R, Cawley A, Fu S. *Organic Impurity Profiling of Fentanyl Derivatives*. Oral presentation at the 8th European Academy of Forensic Science Conference (August 2018), Lyon, France.

2. **Klingberg J**, Shimmon R, Cawley A, Fu S. *Collision-induced Dissociation Studies of Synthetic Opioids for Non-targeted Screening*. Poster presentation at the 24th Australian and New Zealand Forensic Science Society International Symposium (September 2018), Perth, Australia.

3. **Klingberg J,** Cawley A, Pasin D, Fouracre C, Fu S. *Development of Non-targeted LC-HRMS Screening for Synthetic Opioids in Equine Plasma*. Oral presentation at the 72nd Meeting of the Association of Official Racing Chemists (May 2019), Paris, France.

4. **Klingberg J**, Cawley A, Shimmon R, Pasin D, Fourace C, Fu S. *Development of Non-targeted Screening Strategies for Synthetic Opioids*. Oral presentation at the Forensic and Clinical Toxicology Association Conference (June 2019), Adelaide, Australia.

5. **Klingberg J**, Cawley A, Shimmon R, Pasin D, Fouracre C, Fu S. *Development of a Non-targeted Screening Workflow for the Detection of Synthetic Opioids in Equine Plasma*. Poster presentation at the 57th Annual Meeting of the International Association of Forensic Toxicologists (September 2019), Birmingham, United Kingdom.

Abstract

Synthetic opioids are a drug class of particular concern due to their incredibly high potency and the large public health threat that they pose. These compounds have also seen significant modification, highlighting the importance of developing techniques that can detect them without relying on databases or certified reference materials. This work provides a comprehensive investigation into the detection and profiling of synthetic opioids from the perspective of both drug screening in biological matrices and analysis of seized drug samples.

Collision-induced dissociation studies were conducted on a range of different synthetic opioid standards and common product ions belonging to each opioid subclass were identified for use in non-targeted screening strategies. Product ion searching, Kendrick Mass Defect analysis and recursive feature extraction approaches were evaluated for data analysis. Product ion searching and Kendrick mass defect analysis proved effective, with estimated screening cut-offs proposed of 0.05 ng/mL and 0.1 ng/mL, respectively. Recursive feature extraction was found to have a high sensitivity for the detection of spiked compounds, however unbiased extraction of all compounds within a sample presented issues with relevance for screening.

Machine learning approaches were investigated for the identification of unknown compounds. A Naïve Bayes classification model was trained, exploiting the common fragmentation pathways identified, to predict the opioid subclass of a sample with 89.5% accuracy. Additionally, a Gaussian Process Regression model was optimised to predict the experimental relative retention time of a compound based on its molecular features. Relative retention times were predicted for 79.7% of the samples within ±0.1 of their experimental value. By using these models as complementary approaches putative identities of unknown compounds can be proposed with greater confidence before confirmation using certified reference materials.

A preliminary study was also conducted into the synthetic route profiling of acetyl fentanyl. Several common impurities were identified, as well as a number of impurities that were unique to a specific method. These impurities can provide an analyst with an indication of the method used in the synthesis of a seized sample. Furthermore, a statistical approach was taken, with the creation of principal component analysis plots and classification models. The PCA plots showed distinct separation between samples made with different methods and the trained classification models

displayed high accuracy. These results should be reviewed in context, however, as small sample sizes were used in this preliminary study.