

# **Detection and Profiling of Synthetic Opioids**

by

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Degree of Doctor of Philosophy (Science)

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.

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TBAB: tetra-N-butylammonium bromide; ACN: acetonitrile; DCM: dichloromethane; DIPEA: diisopropylethylamine.....	153
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## Abbreviations

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

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

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

<b>STRL</b>	Special Testing and Research Laboratory
<b>SVM</b>	Support Vector Machine
<b>SWATH</b>	Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra
<b>SWGDRUG</b>	Scientific Working Group for the Analysis of Seized Drugs
<b>TBAB</b>	Tetra-N-butylammonium bromide
<b>TIC</b>	Total Ion Chromatogram
<b>UHPLC-MS</b>	Ultra High-performance Liquid Chromatography – Mass Spectrometry
<b>UNODC</b>	United Nations Office on Drugs and Crime
<b>VBA</b>	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 8<sup>th</sup> 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 24<sup>th</sup> 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 72<sup>nd</sup> Meeting of the Association of Official Racing Chemists (May 2019), Paris, France.
4. **Klingberg J.**, Cawley A, Shimmon R, Pasin D, Fouracre 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 57<sup>th</sup> 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  $\pm 0.1$  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.

