Non-targeted analysis of new psychoactive substances using mass spectrometric techniques

by

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A thesis submitted for the

Degree of Doctor of Philosophy (Science)

University of Technology Sydney

2018

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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Daniel J. Pasin, MRACI
26/02/2018

Acknowledgements

Firstly, I would like to extend my deepest thanks to my primary supervisor, Associate Professor Shanlin Fu. You have been my primary supervisor for both my honours and PhD degrees and over these last 5 years, you have been nothing but supportive, patient and encouraging. I could always rely on you to provide timely feedback and you were always happy to endorse me for post-PhD opportunities. It has been a great pleasure to complete my PhD research under your supervision.

I would also like to thank my co-supervisors, Dr. Adam Cawley and Dr. Sergei Bidny. Without your external collaboration, the success of this project could not be possible. Furthermore, I wish to acknowledge the staff at the Australian Racing Forensic Laboratory (ARFL) and Forensic Toxicology Laboratory at NSW FASS for your assistance in this project. Furthermore, I would like to thank them for the financial support throughout this project including the purchase of reference materials and providing an external scholarship top-up.

Thank you to all the UTS academic and professional staff for your assistance throughout my project even though I was not in the laboratory much - I know Ron Shimmon will miss me dearly. I would also like to thank the Centre of Forensic Science and Professor Claude Roux for allowing me so many opportunities to present my research at domestic and international conferences. This has subsequently provided me invaluable contacts that will be no doubt be beneficial in the future.

To the UTS friends that I have made over the last couple of years, thank you for making my time during my PhD and living in Sydney enjoyable - I always know that I have somewhere to stay if I am in Europe. I would also like to thank Morgan “Mo” Philp for being a great colleague putting up with me at conferences and my general nonsense.

Lastly, I want to express my deepest gratitude to my family for supporting me throughout my PhD journey both emotionally and financially. You have always encouraged me to do my best and it has allowed me to achieve something that I never thought possible and love you all dearly.

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List of abbreviations

.csv	Comma-separated value file
$[M+H]^+$	Protonated precursor ion
$[M-H]^-$	Deprotonated precursor ion
ADBICA	*N*-(1-amino-3,3-dimethyl-1-oxo-2-butanyl)-1-pentyl-1*H*-indole-3-carboxamide
25B-NBOMe/25B	2-(4-bromo-2,5-dimethoxyphenyl)-*N*-[(2 methoxyphenyl)methyl]ethanamine
25C-NBOMe/25C	2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
25D-NBOMe/25D	2-(4-methyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
25E-NBOMe/25E	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-NBOMe/25H	2-(2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
25I-NBF	***N***-(2-fluorobenzyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethanamine
25I-NBMD	*N*-(2,3-methylenedioxybenzyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethanamine
25I-NBOMe/25I	2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
25N-NBOMe/25N	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/25T2	2-(4-ethylthio-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
25T4-NBOMe	2-(4-isoproprylthio-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
25X-NBOMe	2-(2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl) derivatives
2C-B	4-bromo-2,5-dimethoxyphenethylamine
2C-B-Fly	2-(4-bromo-2,3,6,7-tetrahydrofuro[2,3-f][1]benzofuran-8-yl)ethanamine
2C-C	4-chloro-2,5-dimethoxyphenethylamine
2C-D	2,5-dimethoxy-4-methylphenethylamine
2C-E	4-ethyl-2,5-dimethoxyphenethylamine

2C-G	2,5-dimethoxy-3,4-dimethylphenethylamine
2C-H	2,5-dimethoxyphenethylamine
2C-I	4-iodo-2,5-dimethoxyphenethylamine
2C-P	2,5-dimethoxy-4-propylphenethylamine
2C-T	2,5-dimethoxy-4-methylthiophenethylamine
2C-T-2	2,5-dimethoxy-4-ethylthiophenethylamine
2C-T-4	2,5-dimethoxy-4-isopropylthiophenethylamine
2C-T-7	2,5-dimethoxy-4-propylthiophenethylamine
2C-X	2,5-dimethoxyphenethylamines derivatives
3,4-DMMC	3,4-dimethylmethcathinone
4-EEC	4-ethylethcathinone
4-EMC	4-ethylmethcathinone
4-MEC	4-methylethcathinone
4-MMC	4-methylmethcathinone
5F-AB-PINACA	*N*-(1-amino-3-methyl-1-oxo-2-butanyl)-1-(5-fluoropentyl)-1*H*-indazole-3-carboxamide
5F-ADBICA	*N*-(1-amino-3,3-dimethyl-1-oxo-2-butanyl)-1-(5-fluoropentyl)-1*H*-indole-3-carboxamide
5F-APICA	*N*-(1-adamantanyl)-1-(5-fluoropentyl)-1*H*-indole-3-carboxamide
5F-CUMYL-PINACA	1-(5-fluoropentyl)-*N*-(2-phenyl-2-propanyl)-1*H*-indazole-3-carboxamide
5F-MMB-PICA	Methyl *N*-{[1-(5-fluoropentyl)-1*H*-3-indolyl]carbonyl}valinate
5F-MMB-PINACA	Methyl *N*-{[1-(5-fluoropentyl)-1*H*-3-indazolyl]carbonyl}valinate
5F-PB-22	8-quinolinyl 1-(5-fluoropentyl)-1*H*-indole-3-carboxylate
5-HT	5-hydroxytryptamine
AB-CHMINACA	*N*-(1-amino-3-methyl-1-oxo-2-butanyl)-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
AB-FUBINACA	*N*-(1-amino-3-methyl-1-oxo-2-butanyl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide
AB-PINACA	*N*-(1-amino-3-methyl-1-oxo-2-butanyl)-1-pentyl-1*H*-indazole-3-carboxamide
ADB-CHMINACA	*N*-(1-amino-3,3-dimethyl-1-oxo-2-butanyl)-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide
ADB-FUBINACA	*N*-(1-amino-3,3-dimethyl-1-oxo-2-butanyl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide
AJS	Agilent Jet Stream
AM-1241	(2-iodo-5-nitrophenyl){1-[(1-methyl-2-piperidinyl)methyl]-1*H*-3-indolyl}methanone
AM-1248	1-adamantanyl{1-[(1-methyl-2-piperidinyl)methyl]-1*H*-3-indolyl}methanone

AM-2201	[1-(5-fluoropentyl)-1*H*-3-indolyl](1-naphthyl)methanone
AM-2233	(2-iodophenyl){1-[(1-methyl-2-piperidinyl)methyl]-1*H*-3-indolyl}methanone
AM-694	[1-(5-fluoropentyl)-1*H*-3-indolyl](2-iodophenyl)methanone
ANU	Australian National University
AORC	Association of official racing chemists
APCI	Atmospheric pressure chemical ionisation
APICA	*N*-(1-adamantanyl)-1-pentyl-1*H*-indole-3-carboxamide
AR	Analytical reagent
ARFL	Australian Racing Forensic Laboratory
BB-22	8-quinolinyl 1-(cyclohexylmethyl)-1*H*-indole-3-carboxylate
bbCID	Broadband collision-induced dissociation
Bromo-DragonFly	1-(8-bromobenzo[1,2-*b*; 4,5-*b*']difuran-4-yl)-2-aminopropane
BZP	Benzylpiperazine
CA	California
CB	Cannabinoid receptor
CBD	Cannabidiol
cc	Cubic centimetres
CDC	Centre for Disease Control
CE	Capillary electrophoresis (separation technique)
CE	Collision energy (mass spectrometry)
CID	Collision-induced dissociation
cm	Centimetre
CMF	Charge-migration fragmentation
CO	Carbon monoxide
CRF	Charge-retention
CRM	Certified reference material
Da	Dalton
DART	Direct analysis in real time
DBE	Double bond equivalents
DDA	Data-dependent acquisition
DEA	Drug Enforcement Administration
DESI	Desorption electrospray ionisation
DIA	Data-independent acquisition
DOB	4-bromo-2,5-dimethoxyamphetamine
DOET	4-ethyl-2,5-dimethoxyamphetamine
DOH/2,5-DMA	2,5-dimethoxyamphetamine
DOI	4-iodo-2,5-dimethoxyamphetamine
DOM	2,5-dimethoxy-4-methylamphetamine

DOT	2,5-dimethoxy-4-methylthioamphetamine
DOX	2,5-dimethoxyamphetamines derivatives
ECC	Extracted compound chromatogram
EE	Even electron
EI	Electron ionisation
EIC	Extracted ion chromatogram
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
ESI+	Positive electrospray ionisation
EU	European Union
eV	Electron volt
EWS	Early Warning System
FbF	Find by Formula
FIA	Flow injection analysis
FUB-144	[1-(4-fluorobenzyl)-1*H*-3-indolyl](2,2,3,3-tetramethylcyclopropyl)methanone
FUB-NPB-22	8-quinolinyl 1-(4-fluorobenzyl)-1*H*-indazole-3-carboxylate
FWHM	Full width at half maximum
GC-MS	Gas chromatography – mass spectrometry
GHz	Gigahertz
GUI	Graphical user interface
H_2O	Water
HCD	Higher energy collision dissociation
HESI	Heated electrospray ionisation
HPLC	High-performance liquid chromatography
HRAM	High-resolution accurate mass
HRMS	High-resolution mass spectrometry
Hz	Hertz
IDA	Information-dependent acquisition
IL	Illinois
IS	Internal standard
IUPAC	International Union of Pure and Applied Chemists
JWH	John William Huffman
JWH-007	(2-methyl-1-pentyl-1*H*-3-indolyl)(1-naphthyl)methanone
JWH-015	(2-methyl-1-propyl-1*H*-3-indolyl)(1-naphthyl)methanone
JWH-016	(1-butyl-2-methyl-1*H*-3-indolyl)(1-naphthyl)methanone
JWH-018	1-naphthyl(1-pentyl-1*H*-3-indolyl)methanone
JWH-019	(1-hexyl-1*H*-3-indolyl)(1-naphthyl)methanone
JWH-020	(1-heptyl-1*H*-3-indolyl)(1-naphthyl)methanone
JWH-030	1-naphthyl(1-pentyl-1*H*-3-pyrrolyl)methanone

JWH-073	(1-butyl-1*H*-3-indolyl)(1-naphthyl)methanone
JWH-081	(4-methoxy-1-naphthyl)(1-pentyl-1*H*-3-indolyl)methanone
JWH-098	(4-methoxy-1-naphthyl)(2-methyl-1-pentyl-1*H*-3-indolyl)methanone
JWH-122	(4-methyl-1-naphthyl)(1-pentyl-1*H*-3-indolyl)methanone
JWH-200	{1-[2-(4-morpholinyl)ethyl]-1*H*-3-indolyl}(1-naphthyl)methanone
JWH-203	2-(2-chlorophenyl)-1-(1-pentyl-1*H*-3-indolyl)ethanone
JWH-210	(4-ethyl-1-naphthyl)(1-pentyl-1*H*-3-indolyl)methanone
JWH-250	2-(2-methoxyphenyl)-1-(1-pentyl-1*H*-3-indolyl)ethanone
JWH-307	[5-(2-fluorophenyl)-1-pentyl-1*H*-3-pyrrolyl](1-naphtyl)methanone
KMD	Kendrick mass defect
kV	Kilovolt
L	Litre
LC-MS	Liquid chromatography – mass spectrometry
LLE	Liquid-liquid extraction
LLOQ	Lower limit of quantification
LOD	Limit of detection
LRMS	Low-resolution mass spectrometry
M	Moles per litre; mol/L
m/z	Mass-to-charge ratio
$M^{+\bullet}$	Radical cation
$M^{+\bullet\bullet}$	Diradical cation
MA	Massachusetts
MAE	Microwave-assisted extraction
MALDI	Matrix-assisted laser desorption ionisation
mDa	Millidalton
MDF	Mass defect filtering
MDMA	3,4-methylenedioxymethamphetamine
MDMB-CHMICA	Methyl *N*-{[1-(cyclohexylmethyl)-1*H*-3-indolyl]carbonyl}-3-methylvalinate
MDMB-FUBINACA	Methyl *N*-{[1-(4-fluorobenzyl)-1*H*-3-indazolyl]carbonyl}-3-methylvalinate
MDMB-PINACA	Methyl *N*-[(1-pentyl-1*H*-3-indazolyl)carbonyl]-3-methylvalinate
MDMC	2,3-methylenedioxymethcathinone
MDPBP	3,4-methylenedioxy-α-pyrrolidinobutiophenone
MDPPP	3,4-methylenedioxy-α-pyrrolidinopropiophenone
MDPV	3,4-methylenedioxypyrovalerone

MFE	Molecular feature extraction
MFG	Molecular formula generator
mg	Milligram
MI	Michigan
mL	Millilitre
mM	Millimoles per litre; mmol/L
mm	Millimetre
MMB-FUBINACA	Methyl *N*-{[1-(4-fluorobenzyl)-1*H*-3-indazolyl]carbonyl}valinate
MO	Missouri
MPBP	4-methyl-α-pyrrodinobutiophenone
MS	Mass spectrometry
MS/MS or MS^2	Tandem mass spectrometry
MSC	Molecular Structure Correlator
MS^n	Multistage tandem-mass spectrometry
MΩ	Megaohm
N_2	Nitrogen gas
NaCl	Sodium chloride
ng	Nanogram
NH_3	Ammonia
NJ	New Jersey
NL	Neutral loss
NLF	Neutral loss filtering
NMI	National Measurement Institute
NMR	Nuclear magnetic resonance
NPS	New psychoactive substances
NSW	New South Wales
OE	Odd electron
OH	Ohio
PA	Pennsylvania
PCA	Principal component analysis
PCDL	Personal compound database and library
PET	Positron emission tomography
PFAC	Perfluoroalkyl compounds
PiHKAL	Phenethylamines I have known and loved
PLE	Pressurised liquid extraction
pNLC	Precursor neutral loss chromatogram
ppm	Parts per million
PPP	Pyrrolidinopropiophenone
PTR	Proton transfer reaction
QLD	Queensland
QqQ	Triple quadrupole

QTOF	Quadrupole time-of-flight
R^2	Coefficient of determination
rpm	Revolutions per minute
s	Seconds
S/N	Signal-to-noise ratio
SA	South Australia
SALLE	Salting-out assisted liquid-liquid extraction
SCX	Strong cation exchange
SIEVE®	Statistical Iterative Exploratory Visualization Environment
SPE	Solid-phase extraction
SRI	Selective reagent ionisation
STA	Systematic toxicological analysis
SWATH®	Sequential window acquisition of all theoretical spectra
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
TCC	Total compound chromatogram
TCMP	Tetramethylcyclopropyl
THC	Δ^9-tetrahydrocannabinol
TIC	Total ion chromatogram
TiHKAL	Tryptamines I have known and loved
TOF	Time-of-flight
TX	Texas
UK	United Kingdom
UNODC	United Nations Office of Drugs and Crime
UR-144	(1-pentyl-1*H*-3-indolyl)(2,2,3,3-tetramethylcyclopropyl)methanone
USA	United States of America
V	Volt
VBA	Visual Basic for Applications
XLR-11	[1-(5-fluoropentyl)-1*H*-3-indolyl](2,2,3,3-tetramethylcyclopropyl)methanone
α-PVP	α-pyrrolidinovalerophenone
Δ	Mass error
µg	Microgram
µL	Microlitre

Publications and conference proceedings

Refereed journal publications directly related to this project

1. **Pasin, D.**, Cawley, A., Bidny, S., Fu, S. (2017) Current applications of high-resolution mass spectrometry for the analysis of new psychoactive substances: a critical review. Analytical and Bioanalytical Chemistry, doi: 10.1007/s00216-017-0441-4.
2. **Pasin, D.**, Cawley, A., Bidny, S., Fu, S. (2017) Characterisation of hallucinogenic phenethylamines using high-resolution mass spectrometry for non-targeted screening purposes. Drug Testing and Analysis, doi: 10.1002/dta.2171.
3. Cawley, A., **Pasin, D.**, Ganbat, N., Ennis, L., Smart, C., Greer, C., Keledjian, J., Fu, S., Chen, A. (2016) The potential for complementary targeted/non-targeted screening of novel psychoactive substances in equine urine using liquid chromatography-high resolution accurate mass spectrometry. Analytical Methods. 8(8): 1789-97, doi: 10.1039/C6ay00156d

Refereed journal publications from other related research activities

1. Bidny, S., Gago, K., Chung, P., Albertyn, D., **Pasin, D**. (2017) Simultaneous screening and quantification of basic, neutral and acidic drugs in blood using UPLC-QTOF-MS. Journal of Analytical Toxicology. 41(3): 181-95, doi: 10.1093/jat/bkw118.
2. **Pasin, D.**, Bidny, S., Fu, S. (2015). Analysis of new designer drugs in post-mortem blood using high-resolution mass spectrometry. Journal of Analytical Toxicology. 39(3): 163-71, doi: 10.1093/jat/bku144

Refereed conference proceedings (presenting author underlined)

1. **Pasin, D.**, Cawley, A., Bidny, S., Fu, S. Evaluating the use of Kendrick Mass Defect Analysis for rapid discovery of new psychoactive substances in non-targeted screening approaches. 55th Meeting of The International Association of Forensic Toxicologists. Boca Raton, United States of America. Jan 6-11, 2018.
2. **Pasin, D.**, Cawley, A., Bidny, S., Fu, S. Characterization of Cannabinoids Using High-Resolution Mass Spectrometry for Non-Targeted Screening. 21st Triennial Meeting of the International Association of Forensic Science. Toronto, Canada. Aug 21-25, 2017.
3. **Pasin, D.**, Cawley, A., Bidny, S., Fu, S. Non-targeted screening of new psychoactive substances using liquid chromatography-high resolution mass spectrometry. *Royal Australian Chemical Institute Centenary Congress.* Melbourne, Australia. July 23-28, 2017
4. **Pasin, D.**, Cawley, A., Bidny, S., Fu, S. The use of collision-induced fragmentation pathways of hallucinogenic phenethylamines for the detection and identification of novel analogues. Australian and New Zealand Forensic Science Society 23rd International Symposium on the Forensic Sciences. Auckland, New Zealand. Sept 18-22, 2016.
5. **Pasin, D.**, Cawley, A., Bidny, S., Fu, S. The application of mass defect filtering in data mining of high-resolution mass spectrometry data for non-targeted screening strategies of new psychoactive substances. 54th Meeting of The International Association of Forensic Toxicologists. Brisbane, Australia. Aug 28-Sept 1, 2015
6. **Pasin, D.**, Fu, S., Cawley, A. An investigation into the collision induced dissociation pathways of synthetic cathinones using high-resolution mass

spectrometry for non-targeted screening purposes. 7th European Academy of Forensic Science Conference. Prague, Czech Republic. Sept 6-11, 2015.

7. **Pasin, D.**, Fu, S., Cawley, A. Preliminary investigation into the use of mass defect filtering for data reduction and non-targeted screening strategies for new psychoactive substances (NPS) using high-resolution mass spectrometry. 7th European Academy of Forensic Science Conference. Prague, Czech Republic. Sept 6-11, 2015.
8. **Pasin, D.**, Fu, S., Cawley, A. Collision-induced dissociation pathways of hallucinogenic phenethylamines (2C-X) and their N-(2-methoxybenzyl) derivatives (NBOMe) using high-resolution mass spectrometry for non-targeted screening purposes. 53rd Meeting of The International Association of Forensic Toxicologists. Florence, Italy. Aug 30-Sept 4, 2015 (poster).
9. Cawley, A., **Pasin, D.**, Ganbat, N., Ennis, L., Smart, C., Greer, C., Keledjian, J., Fu, S., Chen, A. Validation of non-targeted high-resolution accurate mass spectrometry analysis in forensic toxicology: A case study in NBOMe detection. 53rd Meeting of The International Association of Forensic Toxicologists. Florence, Italy. Aug 30-Sept 4, 2015.
10. Cawley, A., Ganbat, N., Ennis, L., **Pasin, D.**, Smart, C., Keledjian, J., Fu, S., Chen, A., Mariani, M., Jones, D. The potential of complementary targeted/untargeted high-resolution accurate mass screening strategies for advanced sports anti-doping. Royal Australian Chemical Institute National Congress. Adelaide, Australia. Dec 7-12, 2014.
11. **Pasin, D.**, Bidny, S., Fu, S. Detection and quantification of 40 new designer drugs in post-mortem blood using high-resolution mass spectrometry. Australian and New Zealand Forensic Science Society 22nd International Symposium on the

Forensic Sciences. Adelaide, Australia. Aug 31-Sept 4, 2014.

12. **Pasin, D.**, Bidny, S., Fu, S. Analysis of new designer drugs in post-mortem blood using high resolution mass spectrometry. Forensic and Clinical Toxicology Association Inc. Meeting. Sydney, Australia. Dec 2-4, 2013.
13. Bidny, S., Kelly, G., Gago, K., David, M., Duong, T., **Pasin, D**. The application of high-resolution mass spectrometry and ultra-performance liquid chromatography in forensic toxicology for the simultaneous screening and quantification of basic, neutral and acidic drugs in blood. Forensic and Clinical Toxicology Association Inc. Meeting. Sydney, Australia. Dec 2-4, 2013.

Abstract

The proliferation of new psychoactive substances (NPS) has become problematic for forensic drug chemistry and analytical toxicology laboratories that rely on the use of targeted screening methods for the detection of analytes. In order to detect novel NPS derivatives, non-targeted or general unknown screening workflows need to be implemented. Recently, high-resolution mass spectrometry (HRMS) has become the workhorse for general drug screening due to its ability to collect full scan MS and MS/MS data, which can be retrospectively interrogated and has been identified as a potential tool for non-targeted screening.

Top-down screening approaches involving the selection of abundant precursor ions is difficult in toxicological analyses particular when analytes of interest exist at low concentrations. Mass defect-based top-down screening approaches were developed and evaluated for the detection of low concentration analogues. Application of mass defect filtering (MDF) on fortified and authentic samples revealed that the efficacy of this technique was dependent on sample complexity, chromatographic resolution and, more critically, software availability and/or capability. An in-house Microsoft Office Excel-based KMD analysis software was developed using the Visual Basic for Applications (VBA) programming language. Briefly, the software workflow involves the importation of single or multiple comma-separated value (.csv) files, followed by the calculation of KMD values for each mass-to-charge (*m/z*) entry normalized to – CH_2. The data can then be filtered by *m/z* range, intensity, mass defect and even/odd mass. KMD values which match the user-defined values (up to 8 different values can be monitored simultaneously) are highlighted and isolated for easy visualization. These *m/z* values can then be extracted using the corresponding native data processing

software to observe the presence of distinct chromatographic peaks for the selected *m/z* values. The program was capable of rapidly interrogating numerical MS data from multiple files acquired by major HRMS platform vendors. In addition, differential analysis software was also evaluated for the detection of anomalous signals not present in control samples, however, this technique requires representative control matrices in addition to supplementary data processing software that is not always provided by HRMS vendors or requires separate purchase.

Bottom-up screening strategies involve the monitoring of common product ions and neutral losses (NLs) for particular subclasses, where aligning chromatographic peaks for multiple product ions or NLs may indicate the possible presence of a novel NPS analogue. Collision-induced dissociation (CID) studies were performed on synthetic cathinone, hallucinogenic phenethylamine and synthetic cannabinoid derivatives to determine key product ions and NLs. 2C-X and DOX derivatives had common losses of NH_3, CH_6N and C_2H_9N and common product ions at *m/*z 164.0837, 149.0603 and 134.0732 for 2C-X derivatives and *m/z* 178.0994, 163.0754, 147.0804 and 135.0810 for DOX derivatives. The 25X-NBOMe derivatives had characteristic product ion spectra with abundant ions at *m/z* 121.0654 and 91.0548, together with minor NLs corresponding to 2-methylanisole and 2-methoxybenzylamine and $C_9H_{14}NO$.

Product ion pairs *m/z* 117.0573/105.0699, 131.0730/105.0699, 145.0886/119.0855, 159.1043/133.1012 149.0635/123.0605 and 161.0835/135.0804 were indicative of different substituted traditional cathinone derivatives. Methylenedioxycathinone-type cathinones did not exhibit common product ions but instead exhibited NLs of 18.0106 (H_2O), 48.0211 (CH_4O_2) and 76.0160 Da ($C_2H_4O_3$). The presence of *m/z* 98.0964, 112.1121 or 126.1277 and a NL of 71.0735 Da was indicative of synthetic cathinones that contain a pyrrolidine ring such as the α-pyrrolidinophenone-type and

methylenedioxy-α-pyrrolidinophenone-type cathinones. Product ions *m/z* 105.0699 and 119.0855 were indicative of unsubstituted and methylphenyl α-pyrrolidinophenone-type cathinones, respectively. While *m/z* 149.0233 was indicative of methylenedioxy-α-pyrrolidinophenone-type cathinones.

Naphthoylindole derived synthetic cannabinoids exhibited major product ions at *m/z* 155.0491, 169.0648, 183.0804 and *m/z* 185.0597 while 2-iodobenzoylindole and TMCP derivatives exhibited the product ion *m/z* 230.9301 and *m/z* 125.0961, respectively. Product ions corresponding to the linker-core-tail were observed at *m/z* 214.1226 (PICA), 232.1132 (5F-PICA), 215.1179 (PINACA), 233.1085 (5F-PINACA), 240.1383 (CHMICA), 241.1335 (CHMINACA), 252.0819 (FUBICA) and 253.0772 (FUBINACA). Furthermore, the presence of *m/z* 144.0444, 158.0600 and 145.0402 were indicative of the indole, 2-methylindole and indazole acylium cations.

These strategies were applied retrospectively to authentic forensic casework samples that were confirmed to contain NPS analogues at relatively low concentrations. All analytes of interest were detected using a combination of top-down and bottom-up screening strategies. Overall, these strategies offer a vendor-agnostic approach for the detection of NPS analogues that can be implemented immediately for samples of interest.