IDENTIFICATION AND ANALYSIS OF DRUG-IMPREGNATED PAPER FROM PRISONS IN ENGLAND BETWEEN 2018-2020

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Asena Avci Akca BSc MSc.

Supervised by Professor Atholl Johnston

Barts and The London School of Medicine and Dentistry

William Harvey Research Institute

STATEMENT OF ORIGINALITY

I, Asena Avci Akca, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

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ABSTRACT

Introduction: Drug misuse is a global concern that has become increasingly widespread across all levels of society. Its use in prisons contributes to greater disruption and violence, as well as having a negative influence on prisoner safety, rehabilitation, and recovery. Novel psychoactive substances (NPS) in the forms of impregnated papers posted to prisoners are of a particular concern in prison settings where they are commonly used by vaping.

Aims: This study was conducted to develop a spectral database and a qualitative method to identify the variety of emerging NPS impregnated onto paper samples sent to prison inmates. The aim was to help rapid detection and identification and to demonstrate that these findings can be a good indicator of the drug prevalence and trends in prisons.

Methods: 1250 non-judicial paper samples seized from twelve English prisons between 2018 and 2020 were analysed to determine the NPSs circulating in the prisons. From each piece of paper, from different locations, believed to be impregnated with drugs, approximately 1 cm^2 of paper was cut. Samples were placed into separate 1.5 mL Eppendorf tubes with 1 mL of 50% (v/v) methanol in LCMS- grade water. Extracts were prepared from the samples by vortex-mixing (30min). A mobile phase blank was injected between the analysis of each extract to check for carryover. Extracts were screened using an Agilent Technologies 1290 Infinity II - 6545 Q-TOF LC/MS instrument with electrospray ionization in positive ion mode. It uses an Agilent Eclipse Plus C18 1.8 mm 2.1x100 mm column, maintained at 40°C. Drug separation was performed over a total of 13 min using a simple linear gradient of water (A), and methanol (B), both contained 0.01% (v/v) formic acid in 5 mmol/L ammonium formate, at a flow rate of 400 µL/min. Sample injection volume was 0.2 µL.

Results: The research findings showed that SCRA was the most prevalent drug group detected in drug-impregnated paper seizures in English prisons between 2018 and 2020 and followed by Class A drugs and Class B drugs. SCRAs had higher prevalence in male prisons whereas female prisons presented a higher prevalence of Class A drugs. Furthermore, male prisons with lower security level (category C) were appeared to have a higher prevalence of Class B, Class C and abused prescription drugs compared to category B prisons which had a higher prevalence of nicotine. Associations between drug group and sex, prison category and geographical locations were statistically analysed by Pearson chi-square test using Minitab software.

Conclusion: The outcomes of this study have provided new information regarding drug use in prison. These findings may be significant as police information and could be utilised to aid drug policymakers in prisons, particularly in the development of treatment plans addressing the most commonly misused drugs in each prison. This analysis would help in the identification of drug smuggling ways into jails, enables prison staff to pay more attention on these sites.

PRESENTATIONS & PUBLICATIONS

1. Asena Avci, Anca Frinculescu, Lewis Couchman, Atholl Johnston. Analysis of Drugs on Impregnated Prison Letter Samples Using LC/Q-TOF-MS, Poster Presentation, September 8, 2020, SOFTember Virtual Program 2020.
2. Asena Avci Akca, Anca Frinculescu, Trevor Shine, Lewis Couchman, Atholl Johnston. Qualitative Analysis of Drug Impregnated Paper Samples from England and Wales Prisons in 2019 and 2020, Poster Presentation, September 2, 2021, MSACL 2021 EU Virtual.
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4. A. Avci Akca, L. Couchman, A. Frinculescu, A. Johnston. Qualitative analysis of drug impregnated paper samples from England and Wales prisons in 2019 and 2020, Oral Presentation, November 18, 2021, VIII International Conference on Novel Psychoactive Substances (NPS) Online.
5. Asena Avci Akca, Anca Frinculescu, Trevor Shine, Lewis Couchman, Atholl Johnston. Qualitative Analysis of Drug Impregnated Paper Samples from England and Wales Prisons in 2019 and 2020, Oral Presentation, February 1, 2022, TIAFT 2022 VIRTUAL, The 58th Annual Meeting of the International Association of Forensic Toxicologists.
6. Asena Avci Akca, Anca Frinculescu, Trevor Shine, Lewis Couchman, Atholl Johnston. Identification and Analysis of Drug-Impregnated Paper from Prisons in England and Wales between 2018 And 2020, Oral Presentation, April 1, 2022, Forensic Sciences Symposium & Online IACFT Turkish Speakers' Day.
7. Asena Avci Akca, Lewis Couchman, Atholl Johnston, Anca Frinculescu, Trevor Shine. Identification and Analysis of Drug-Impregnated Paper from Prisons in England and Wales between 2018 And 2020, Poster Presentation, May 16-20, 2022, 5th Annual Current Trends in Forensic Toxicology On-Line Symposium.
8. Asena Avci Akca, Atholl Johnston, Lewis Couchman, Anca Frinculescu, Trevor Shine. P17-Analysis of drug-impregnated paper from English prisons between 2018 and 2020, Conference Abstract Publication. Toxicologie Analytique et Clinique Volume 34, Issue 3, Supplement, September 2022, Pages S93-S94. 30th meeting of SFTA- 59th meeting of TIAFT - September 2022. https://doi.org/10.1016/j.toxac.2022.06.143

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
µg	Microgram
µL	Microlitre
25I-NBOMe	4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl) phenethylamine
2A-I	2-aminoindane
2C-B	2,5-dimethoxy-4-bromophenethylamine
2C-C-NBOMe (25C-NBOMe)	*N*-(2-methoxybenzyl)-2,5-dimethoxy-4-chlorophenethylamine
2C-E	2,5-Dimethoxy-4-ethylphenethylamine
2C-I	2,5-Dimethoxy-4-iodophenethylamine
2C-T-2	2,5-Dimethoxy-4-ethylthiophenethylamine
2C-T-7	2,5-Dimethoxy-4-(n)-propylthiophenethylamine
2-FDCK	2-Fluorodeschloroketamine
3-MeO-PCE	3-Methoxyeticyclidine
4-FA	4-Fluoroamphetamine
4-FMA	4-Fluoromethamphetamine
4-MeO-PCP	4-Methoxyphencyclidine
5-APB	5-(2-Aminopropyl)benzofuran
5-IAI	5-Iodo-2-aminoindane
5-MeO-DALT	N, N-Di allyl-5-methoxy tryptamine
5-MeO-DIPT	5-Methoxy-N,N-diisopropyltryptamine
5-MeO-DMT	5-Methoxy-N,N-dimethyltryptamine
6-APB	(6-(2-Aminopropyl)benzofuran)
ACMD	Advisory Council on the Misuse of Drugs
ADF	Alcohol and Drug Foundation
AMPH	Amphetamine
AMT	Alpha-methyltryptamine
APCI	Atmospheric Pressure Chemical Ionization
BZP	Benzylpiperazine
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
CBD	Cannabidiol
CID	Collision-Induced Dissociation
CNS	Central Nervous System
CP-	Cyclohexylphenol
CP 47, 497	(2-[(1R,3S)-3-Hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol)
CP 55,940	(-)-cis-3-[2-Hydroxy-4-(1,1-dimethylheptyl)-phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol)
CSEW	Crime Survey for England and Wales
DC	Direct Current
DMAA	1, 3-Dimethylamylamine
DMT	N,N-Dimethyltryptamine
DOC	2,5-Dimethoxy-4-chloroamphetamine
DOI	2,5-Dimethoxy-4-iodoamphetamine
EDTA	Ethylenediaminetetraacetic acid
EI	Electron Impact
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
ESI	Electrospray Ionization
ESI-QTOFMS	Electrospray Ionization Quadrupole Time-of-Flight Tandem Mass Spectrometer

Abbreviation	Meaning
Etc.	Et cetera
EWA	Early Warning Advisory
FTIR	Fourier Transform Infra-Red
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
HMPPS	HM Prison and Probation Service
HPLC	High Performance Liquid Chromatography
HU-	Hebrew University
HU-210	1,1-Dimethylheptyl-11-hydroxytetrahydrocannabinol
IR	Infra-Red
JWH-	John W. Huffman
JWH-018	Naphthalen-1-yl-(1-pentylindol-3-yl)methanone
JWH-073	Naphthalen-1-yl-(1-butylindol-3-yl) methanone
JWH-250	2-(2-Methoxyphenyl)-1-(1-pentyl-1H-indol-3-yl)ethanone
JWH-398	(4-Chloronaphthalen-1-yl)(1-pentyl-1H-indol-3-yl)methanone
L	Litre
LC-MS/MS	Liquid Chromatography and Triple Quadrupole Mass Spectrometry
LC-QTOF-MS	Liquid Chromatography Quadrupole Time-of-flight Tandem Mass Spectrometry
LSA	D-Lysergic Acid Amide
LSD	Lysergic Acid Diethylamide
m/z	Mass/Charge
mCPP	Meta-Chlorophenylpiperazine
MDA	Methylenedioxyamphetamine
MDAI	5,6-Methylenedioxy-2-Aminoindane
MDBP	1-(3,4-Methylenedioxybenzyl) Piperazine
MDBZP	Methylenedioxybenzylpiperazine
MDMA	3,4-Methylenedioxymethamphetamine
MDMAI	5,6-Methylenedioxy-*N*-methyl-2-aminoindane
MDPV	Methylenedioxypyrovalerone
MeOPP	Para-methoxyphenylpiperazine
MG	Mitragynine
mg	Milligram
mL	Millilitre
MMA	3-Methoxy-4-Methylamphetamine
MMAI	5-Methoxy-6-Methyl-2-Aminoindane
mmol	Millimole
MoJ	Ministry of Justice
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
NBOMe-	N-methoxybenzyl
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
NPLC	Normal Phase Liquid Chromatography
NPS	Novel Psychoactive Substances
ODS	Octadecylsilane
ONS	Office for National Statistics
PAY	Paynantheine
PCDL	Personal Compound Database and Library
PCP	Phencyclidine
PHE	Public Health England

Abbreviation	Meaning
PIA	P-iodoamphetamine
PMMA	Para-methoxy-N-methylamphetamine
PPO	Prisons and Probation Ombudsman
PS	Psychoactive Substances
PSA	Psychoactive Substances Act
QTOF-MS	Quadrupole Time-of-Flight Tandem Mass Spectrometer
R_f	Retention factor
RF	Radiofrequency
RMDT	Random Mandatory Drug Testing
ROTL	Released on a Temporary Licence
RPLC	Reversed Phase Liquid Chromatography
s	Second
SCRAs	Synthetic Cannabinoid Receptor Agonists
SiO_2	Silica gel
TDM	Therapeutic Drug Monitoring
TFMPP	3-Trifluoromethylphenylpiperazine
TLC	Thin-Layer Chromatography
TOF	Time-of-Flight
t_R	Retention Time
UNODC	United Nations Office on Drugs and Crime
v/v	Volume per Volume
Vcap	Capillary Voltage
WEDINOS	Welsh Emerging Drugs and Identification of Novel Substances
Δ^9-THC	Delta-9-tetrahydrocannabinol

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1. INTRODUCTION

1.1 Statement of the Problem

Novel Psychoactive Substances (NPS), also known as "legal highs" or "designer drugs," are a broad category of recreational drugs that have lately arisen on the illicit drug market and whose chemical composition and/or effects may be related to well-known compounds of abuse. Many of these chemicals, however, are not new; several were created in research facilities over 30 years ago. Despite being abandoned by researchers due to psychotropic effects or other negative features, illegal drug makers have re-evaluated them for recreational use (Abbate et al., 2018).

The usage of NPS raises a number of concerns. First, technological advances allow supply sources with the capacity for continuous product innovation to be offered, resulting in rapid increases in the number of different substances available, and, despite accelerating the legal procedures in the UK for monitoring these substances, the speed of technological developments surpasses legal controls. Second, they are easily and cheaply accessible via the internet and 'headshop' outlets, as well as traditional drug traffickers (the Psychoactive Substances Act (Psychoactive Substances Act 2016) implemented during 2016 in the UK has since prohibited headshop supply). Third, by worldwide standards, the cultural acceptance of NPS usage in the UK is extremely high. Fourth, NPS are regarded to be safe or to offer minimal risk. Fifth, there are many unknowns about the identity of specific substances obtained online and on the street. Even when a novel chemical is clearly and precisely identified, there may be little knowledge available on its effects, the dangers posed by its usage, and how these risks might be mitigated (Mdege et al., 2017).

NPS constitute the most serious threat to the safety and security of the prison environment according to HM Chief Inspector of Prisons for England and Wales Annual Report 2014-15 (HM Chief Inspector of Prisons for England and Wales, 2015) . Recent data from British prisons provides cause for concern, with estimates ranging from 33 to 90% of individuals in prison commonly consuming these compounds, particularly synthetic cannabinoid receptor agonists (SCRAs). NPS are often analogues of other psychoactive chemicals manufactured in improvised laboratories with no safety protocols or human testing, posing a serious health risk to this vulnerable group and those working with them. Although prisons have extensive security measures in place to detect illicit materials, NPS can readily elude traditional detection methods because they are frequently colourless, odourless, and active in relatively low amounts. SCRAs have been sprayed onto clothing, food items, papers, and even children's paintings brought into jails, in addition to

the traditional means of supply such as hiding in human orifices. As the usage of these substances in prisons has increased, so has the number of incidents of self-harm, suicide attempts, aggressions and assaults, and ambulance calls (Corazza et al., 2020).

1.2 Rationale for Research

In many toxicology laboratories, drug screening typically involves immunoassays, which are traditionally designed for use in biological samples due to their reliance on antibody-antigen interactions. An immunoassay can detect the presence of drug(s) but it is not very specific -cross reactivity with other substances with similar structures might occur - it can produce a large number of false positive and false negative results (Harper et al., 2017). While these methods are fairly effective in screening for common drugs of abuse, they are not suited for the screening of specimens containing NPS. Immunoassays are often unable to detect NPS due to their high structural diversity or because they are too unspecific (Grafinger et al., 2020). A major goal of the present work was to develop a reference standard-based spectral library containing MS data of more than 200 NPS and related compounds for use with an LC-quadrupole time-of-flight (QTOF)-MS based screening method. The developed library will help enable the rapid detection and identification of NPS in drug-impregnated paper and other sample matrices. The library can also be used with retrospective data searching in order to detect and identify previously unreported NPS in specimens. Following the development of the LC-QTOF-MS method, suspected drug-impregnated paper samples from English prisons were screened in order to establish the applicability of the method for use with real-world specimens. It was also important to develop a comprehensive screening and confirmation method capable of detecting and identifying several hundred NPS in a single analytical run with high specificity. In order to accomplish this goal, a rapid, sensitive, and specific LC-QTOF-MS based analytical method was developed and tested in conjunction with the compound database and libraries.

1.3 Significance of Study

The study presented here has applicability to forensic science, toxicology, and law enforcement. The research provides a library for the identification of over 200 common and uncommon NPS and related compounds, created using electrospray ionization (Stolker et al.). A comprehensive LC-QTOF-MS method for screening and confirmation was also developed in conjunction with the library and tested using suspected paper samples from prisons to confirm applicability. The NPS used in the research were identified and selected on the basis of the reference standards available

from commercial suppliers, as well as citations in government documents, peer-reviewed literature, and online drug-user forums. In order to complete this research, the work was divided into three major tasks.

1.3.1 Task 1 – Development of database and spectral library

Comprehensive libraries are widely used in analytical toxicology for the identification of analytes present in specimens. However, these libraries often do not contain many NPS. In order to identify NPS, a database and library including specific spectra of these compounds must be created. This report details the creation of a database containing over 200 NPS and related compounds, as well as the generation and collection of tandem mass spectrometry (MS/MS) data for each compound using commercially available reference standards. The database and library were then used to qualitatively screen suspected paper samples from prisons to ensure applicability to real-world samples.

1.3.2 Task 2 – Comprehensive QTOF-LC/MS method

In order to detect and identify NPS and related compounds in paper samples, a comprehensive QTOF-LC/MS method for screening and confirmation of said compounds was developed according to accepted analytical method development guidelines. In the present study, we have described the identification of NPSs in 1250 suspected drug-impregnated paper samples, seized mainly in English prisons between 2018 and 2020.

1.3.2 Task 3 – Empirical Evaluation of the Results

After finishing Task 2, the results were used to determine the association between the prevalence of drug groups detected in the papers with sex, the category of the prison, and the geographical location of the prisons by running the Pearson chi-square statistical analysis test in Minitab Software.

2. BACKGROUND

2.1 New Psychoactive Substances (NPS)

NPS have a definition created by the United Nations Office on Drugs and Crime (UNODC) as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances but which may pose a public health threat" (UNODC, 2021b). UNODC states that the term "new" does not necessarily refer to new discoveries — the first synthesis of several NPS were decades ago — but to substances that have lately become present on the market. "Legal highs", "bath salts" and "research chemicals" are the terms known in the market for the NPS (UNODC, 2021b).

By altering chemical structures and spreading through the Internet, NPS have become a serious public concern. Ever since the end of the 2000s, the misuse of SCRAs, the most typical NPS, has risen significantly throughout the world (Chung et al., 2016). Currently, over 1100 distinct NPS have been reported to the UNODC EWA (Early Warning Advisory) from 133 countries and territories (UNODC, 2021a).

Some new psychoactive substances often have a lower cost and higher potency than more well-known and controlled substances. Users of these substances consist of a larger variety of people, for instance recreational drug users, people who self-medicate, those considering having a better appearance or performance, vulnerable individuals, such as people going through homelessness, and people involved in high-risk drug use. Because detection and identification of NPS is more difficult in routine screening, users also include those who are tested for drugs on a regular basis, such as people in prison, people in drug treatment, and drivers (EMCDDA, 2021a). The health risks connected with the use of NPS have grown with the emergence of highly potent drugs capable of causing unintended overdoses, including fatalities. The purity and content of NPS-containing substances are unknown, putting consumers at risk, as proven by NPS-related hospital emergency admissions and deaths (UNODC, 2020a).

2.2 Novel Psychoactive Substance Classification

Different criteria can be used to classify NPS; therefore, there are various forms of classification. The most used criteria in literature is the pharmacological effect they produce after consumption, which is related to medicine and health fields. Moreover, origin and legal condition are other common classification criteria, which are related to forensic and legal fields. Because the previous

two classifications lack chemical information, there is another criterion in the literature to classify NPS, which is based on their chemical structure (Zapata et al., 2021).

2.2.1 Pharmacological effect

The interaction between NPS and the central nervous system (CNS) is similar to that of traditional drugs for the production of the desired psychoactive effects. These substances controlled under the 1961 and 1971 Conventions, whereas dissimilar in chemistry, can be classified into six different groups with respect to the main psychoactive effect they generate (UNODC, 2018b).

2.2.1.1 Stimulants

Stimulants are used to produce a feeling of euphoria and wellbeing by increasing the synaptic levels of serotonin, dopamine, and/or noradrenaline. It is one of the largest groups of NPS, usually obtained as in powder or pill form. Stimulants have a structural relation to 3,4-Methylenedioxy methamphetamine (MDMA) (ecstasy), cocaine, and amphetamines and can be used by swallowing, inhaling ("snorting"), and, rarely, injection or rectal administration (Tracy et al., 2017). They include drugs that have been around for decades in the UK (e.g. amphetamine, cocaine, MDMA/Ecstasy), drugs that have been around for decades in other parts of the world but have only recently appeared in the UK (e.g. methamphetamine), and a variety of recently detected stimulant NPSs. Between 2009 and 2017, 148 synthetic cathinones and 136 novel phenethylamines were discovered in NPS stimulants around the world (Abdulrahim and Bowden-Jones, 2021). Cocaine is the most commonly used illicit stimulant in Europe, and its use has been on the rise in recent years (EMCDDA, 2021b).

2.2.1.2 Cannabinoids

Cannabinoids provide a pleasant state of relaxation and the sensation of being "stoned." (Tracy et al., 2017). The term "cannabinoid" involves all chemical substances, without considering structure or origin, that bind to cannabinoid receptors in the body and brain and produce effects similar to those of the *Cannabis sativa* plant. The cannabis plant produces 80 to 100 cannabinoids and approximately 300 non-cannabinoid chemicals. Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the two most important cannabinoids. The more well-known of the two is THC, which is the chemical responsible for cannabis's psychoactive effects (ADF, 2021a). As cannabis is commonly misused, the SCRAs are prevalent options that are not identified by routine urine drug analysis. SCRAs are a large and chemically different class of substances, and

functionally similar to THC. Commonly used street names for the SCRAs are “Spice” and “K2”. In their pure form, SCRAs are either solids or oils, and are used as a mixture with or sprayed onto dried plants such as cooking herbs. They are generally sold in the foiled packages and sold as incense (Dignam, 2017). They are generally used by smoking in a 'joint', mixed with tobacco. Some SCRAs are available in forms for oral consumption, inhalation in e-cigarettes, and injection. (Abdulrahim and Bowden-Jones, 2015).

2.2.1.3 Classic hallucinogens (psychedelics)

Hallucinogens are a type of substance that causes hallucinations, which are severe distortions in a person's view of reality. Some plants and mushrooms (or their extracts) contain hallucinogens, which can also be man-made. People who are using this drug frequently describe having rapid, severe mood swings as well as seeing sights, hearing noises, and feeling sensations that appear to be real but are not. Lysergic acid diethylamide (LSD) is one of the most powerful hallucinogenic substances for altering mood and perception. It is a transparent or white, odourless, water-soluble substance made from lysergic acid, a fungus-derived chemical (National Institute on Drug Abuse, 2015). LSD and psilocybin are traditional hallucinogenic drugs; most NPS psychedelics, such as 5-MeO-DALT and the NBOMe- or 2C-series, provide stimulant effects as well (Tracy et al., 2017). These drugs are commonly taken orally or sublingually with small pieces of blotter paper or ‘tabs’ on which the drug is being dried in order to provide absorption through the oral mucosa. Insufflation, smoking, rectal, and intravenous consumption are some of the less usual modes of administration (Abdulrahim and Bowden-Jones, 2020).

2.2.1.4 Dissociatives

Dissociatives are a group of psychedelic drugs. These drugs distort sensory perceptions and create feelings of disconnection or detachment from the environment and self. The term “dissociative” means disconnected from reality (ADF, 2021b). The first compounds in this group, ketamine and phencyclidine (PCP), were initially used for general anaesthesia. However, they have generally been ended due to postsurgical dissociative side effects. The range of NPS dissociatives varies between some milder than ketamine to others as strong as PCP. The prevalent variant, methoxetamine, is commonly reported for the production of stronger and longer-lasting dissociative effects than ketamine. Routes of administration for dissociatives are inhalation, swallowing, or injection (Tracy et al., 2017).

2.2.1.5 Opioids

Opioids are a broad term that includes a wide range of chemicals, from naturally occurring opiates (opium and morphine, etc.) to synthetic opioids (fentanyl and tramadol, etc.)), semi-synthetic opioids (heroin, etc.), and novel psychoactive substances having opioid properties (acetylfentanyl, butyryl fentanyl, carfentanil, furanyl fentanyl, etc.) (UNODC, 2019). Synthetic opioids involve fentanyl, fentanyl derivatives, and newly emerging analogues that share neither the fentanyl-like nor the morphine-like chemical structure but still function on opioid receptors in the brain. U-47700 and U-50488 are non-fentanyl synthetic opioids and are referred as "U-compounds" or "U-series" compounds, or informally as "Utopioids", with the "U" referable to their company of origin (Abbate et al., 2022, Baumann et al., 2020). The number of new compounds in the most recent emerging synthetic opioid class—the 2-benzylbenzimidazole analogues, commonly known as nitazenes—has begun to dominate the current novel synthetic opioid subclass of NPS (Walton et al., 2021). Brorphine is a potent synthetic opioid that has appeared as a potential substitute for the recently scheduled synthetic opioid isotonitazene (Krotulski et al., 2021). Synthetic opioids are a diverse class of drugs that operate on opioid receptors, including prescription pain medications and anaesthetics. They cause respiratory depression, sedation, euphoria, hypothermia, drowsiness, and miosis, among other consequences (EMCDDA, 2018b).

Opioids can be used in different ways: Opioid-based medications are generally in tablet form and are often taken by swallowing. For opioid-substitution treatment, which is in liquid, tablet, and film forms, oral consumption is used. Film forms are dissolved under the tongue. Heroin is usually taken by injection but is also by snorted or smoked (ADF, 2021c).

2.2.1.6 Sedatives / Hypnotics

Sedatives are CNS depressants that are used to slow down the human brain's activity. These compounds have a considerable inhibitory and relaxing impact on the brain, and they imitate several sedative and antianxiety medications such as the benzodiazepines, diazepam, and alprazolam. They are the least understood of the NPS. One reason for this is that the clinical symptoms are so similar to the known recreational drugs that identifying their exposure in a clinical context is challenging (SCIEX, 2021) (UNODC, 2021b).

2.2.2 Chemical Structure

Pharmacological classification can be used in medical or legal disciplines, but it is inadequate in chemistry. Understanding the chemical structures of psychoactive drugs is critical in analytical toxicology and forensics. As a result of this requirement for chemical knowledge, several NPS classifications reported in the literature have begun to group NPS not only by their origin/pharmacology but also by their chemical structure (Zapata et al., 2021). The classification adopted by UNODC establishes nine categories, including (i) synthetic cannabinoids, (ii) synthetic cathinones, (iii) phenethylamines, (iv) aminoindanes, (v) tryptamines, (vi) piperazines, (vii) phencyclidine-type substances, (viii) plant-based substances, and (ix) other substances (UNODC, 2021b).

2.2.2.1 Synthetic Cannabinoids

SCRAs initially appeared in the 1970s, when researchers were investigating the endocannabinoid system and trying to create new cancer pain therapies. The first synthesis of SCRAs was done by academic laboratories or the pharmaceutical industry. The production of selective cannabinoid receptor agonists with a focus on antinociceptive function began in 1974 at Pfizer with cyclohexylphenol (CP 55,940), and was followed in 1988 by Mechoulam's lab at Hebrew University with the HU-210 molecule. The synthesis of novel cannabinoids with some of the features of Δ^9-THC was led by John W. Huffman (Wiley et al., 2011). His research focused on synthesizing small molecules that could be applied as new pharmaceutical analgesics, particularly chemicals that bind to cannabinoid receptors in the brain (CB1) and periphery (CB2). JWH-018 is one of the analgesic drug candidates synthesized by him (Alves et al., 2020).

SCRAs are compounds that have structural properties that allow them to attach to one of the known cannabinoid receptors, such as CB1 or CB2. The CB1 receptor is involved in the physiological and, in particular, psychological effects of cannabis, whereas the CB2 receptor may play a role in immunomodulation. The chemical ingredients of cannabis, such as delta-9-tetrahydrocannabinol and cannabidiol, are the only naturally occurring cannabinoids. Synthetic cannabinoids, on the other hand, include a wide range of structurally distinct molecules with the potential for future structural alterations, such as analogues and derivatives that may demonstrate affinity for either of the cannabinoid receptors (UNODC, 2015).

Many of the chemicals are not structurally linked to the so-called “classical” cannabinoids, i.e., compounds based on dibenzopyrans, such as THC. The cannabinoid receptor agonists are a distinct class of substances, most of which have lipid solubility and non-polarity features and have 22 to 26 carbon atoms. Thus, they should easily volatilize when smoked. A side-chain is a structural characteristic that requires more than four and up to nine saturated carbon atoms for maximum action. The first molecular structure shows THC, whereas the others depict examples of SCRAs found in “Spice” or other smoking combinations. There are seven major structural groups of synthetic cannabinoids: naphthoylindoles (e.g. JWH-018, JWH-073 and JWH-398), naphthylmethylindoles, naphthoylpyrroles, naphthylmethylindenes, phenylacetylindoles (i.e. benzoylindoles, e.g. JWH-250), cyclohexylphenols (e.g. CP 47,497 and homologues of CP 47,497), and classical cannabinoids (e.g. HU-210) (EMCDDA, 2015).

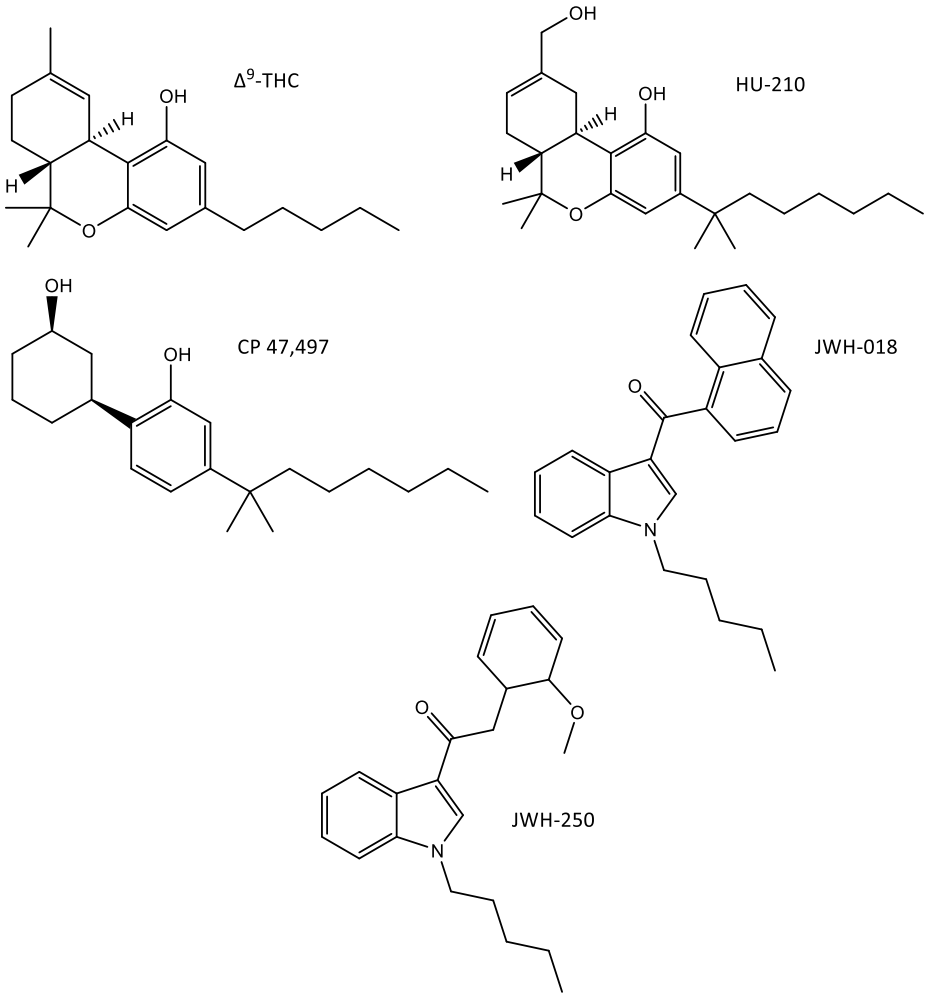

Figure 1: Molecular structures of Δ⁹-THC, HU-210, CP 47,497, JWH-018 and JWH-250

2.2.2.2 Synthetic Cathinones

Synthetic cathinones refer to a wide range of chemicals that are chemically related to cathinone, which is present in the leaves of khat (*Catha edulis*), an East African and Arabian Peninsula shrub. Khat, like other psychoactive plants, has a long history of use. Historical references to chewing khat leaves for their euphoric and stimulant effects stretch back many centuries, and this practise is still done in Somalia, Yemen, Kenya, and Ethiopia today. The dried and powdered leaves are occasionally infused to make tea, known as Abyssinian, African, or Arabian tea, or eaten as a honey-sweetened paste (Gonçalves et al., 2019). Alkaloids, glycosides, tannins, amino acids, flavonoids, vitamins, and minerals are among the many components found in khat. Chewing khat causes these chemicals to be released into the saliva, where they are quickly absorbed through the buccal mucosa and gastro-intestinal tract. Although cathine was previously thought to be responsible for the stimulating action of khat, extracts of fresh leaves of khat were found to contain cathinone, an alkaloid that is 7- to 10-fold more potent than cathine. However, cathinone is not very stable and breaks down to generate cathine and norephedrine, which is why fresh khat leaves need to be chewed soon after harvesting (Gonçalves et al., 2019).

Synthetic cathinones are cathinone derivatives. Chemically, they are members of the methylphenethylamine family, which are structurally similar to traditional amphetamines with the exception of an extra β-keto group at the amino alkyl side chain (Figure 2). The aromatic ring (R1), the alkyl side chain (R2), and the amino group (R3 and R4) are all possible modifications to the cathinone backbone structure, allowing the synthesis of an unlimited number of molecules (Figure 2) (Soares et al., 2021).

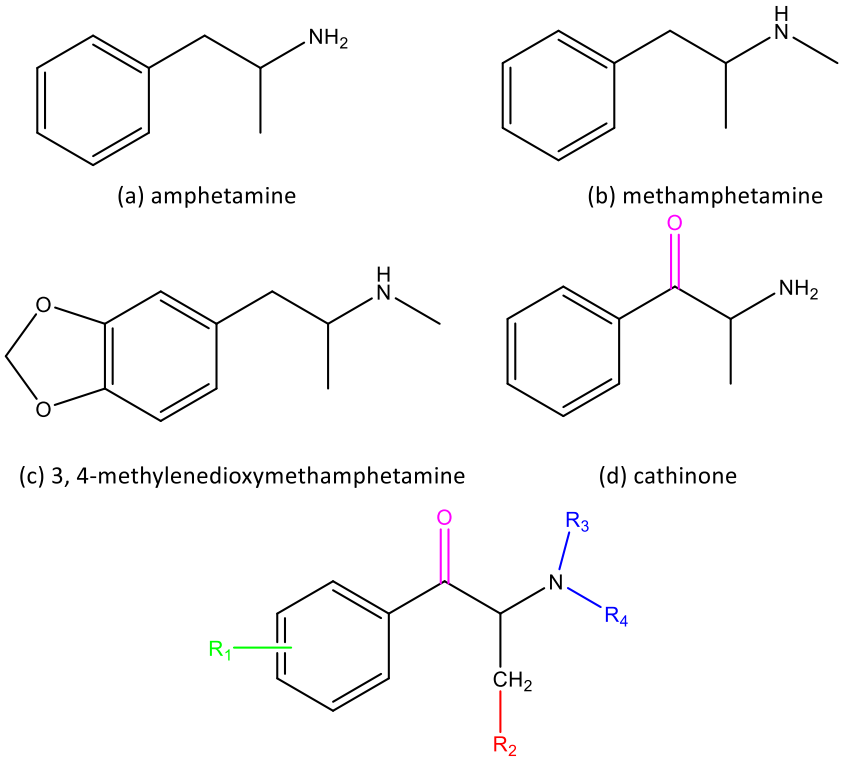

Figure 2: Chemical structures of (a) amphetamine, (b) methamphetamine, (c) 3, 4-methylenedioxymethamphetamine, (d) cathinone, and (e) general structure of synthetic cathinone derivatives

Some synthetic cathinones' molecular structures and production have long been known, but they have only recently been used recreationally. Methcathinone, a methylated derivative of cathinone, was the first synthetic cathinone designer drug, with incidents of misuse dating back to the early 1990s. Mephedrone and methylenedioxypyrovalerone (MDPV) synthesis were initially described in 1929 and 1967, respectively, although abuse was not recorded until the early 2000s. Methylone is a more recent derivative, having been patented in 1996. Following their synthesis, synthetic cathinones were disregarded until their usage as a legal substitute for MDMA was first reported on internet drug websites in 2003 and became widespread in the United Kingdom in 2009. Mephedrone is the most commonly abused synthetic cathinone in Europe, whereas MDPV and methylone are the most commonly abused synthetic cathinones in the United States (German et al., 2014).

Figure 3 depicts the chemical structures of mephedrone, methylone, methcathinone, MDPV, and methedrone.

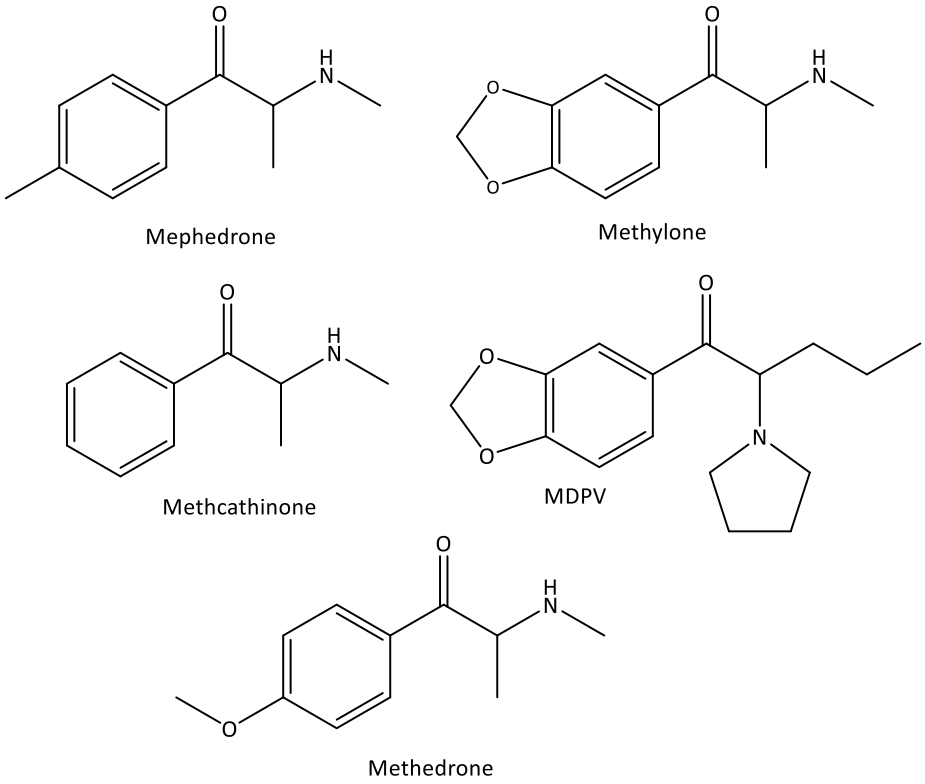

Figure 3: Molecular structures of Mephedrone (4-methylmethcathinone, 4-MMC), Methylone (βk-MDMA, 3, 4-methylenedioxy-N-methylcathinone), Methcathinone (ephedrone), MDPV (3,4-methylenedioxypyrovalerone), Methedrone (βk-PMMA, 4-methoxymethcathinone)

2.2.2.3 Phenethylamines

The word phenylethylamine refers to any structure produced from an aromatic group connected to a terminal amine by an ethyl group (Lapoint and Welker, 2022). The most basic molecule, phenethylamine, is a natural chemical that is easily converted to phenylacetic acid by monoamine oxidases. It has a noticeable structural resemblance to the neurotransmitter dopamine. Methoxy groups in positions 2 and 5 of the aromatic ring, as well as different lipophilic substituents (alkyl, halogen, alkylthio, etc.) in the 4 position, are seen in the most commonly misused phenethylamines. (Brock, 2012). Seizures of phenethylamines were first reported in the US and Europe in 2009, and since then, compounds like 2C-E, 2C-I, 4-FA, and PMMA have been reported in a number of countries around the world. Since 2011, more phenethylamines have been progressively reported to UNODC, including 4-FMA, 5-APB, 6-APB, and 2C-C-NBOMe (UNODC, 2021b). In Figure 4, examples of compounds from the phenethylamine group are displayed.

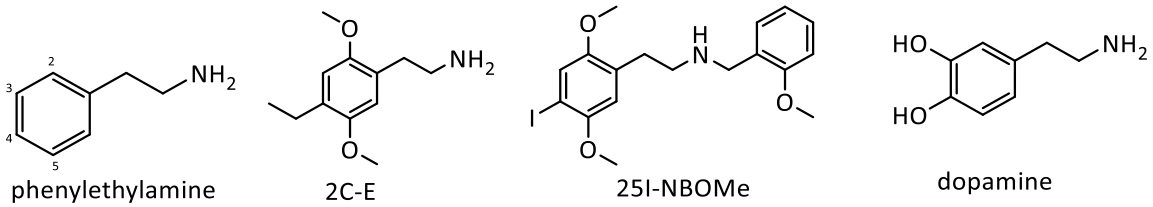

Figure 4: Chemical structures of phenethylamine, 2C-E, 25I-NBOMe and dopamine

The production of phenethylamines and amphetamine analogues has been reported in a number of studies. Phenethylamines became popular in the 1990s by Dr. Alexander Shulgin and Ann Shulgin in their book PiHKAL: A Chemical Love Story, where PiHKAL stands for "Phenethylamines I Have Known and Loved" (Shulgin and Shulgin, 1991). Alexander Shulgin, a biochemist and pharmacologist, reported the synthesis of various novel psychoactive chemicals in the 1980s and 1990s. This includes phenethylamines from the "D series" (e.g. DOC, DOI) and the "2C series" (e.g. 2C-T-7, 2C-T-2) (Rosaria Varì et al., 2018). Simple modifications to the mescaline (a natural phenylethylamine) molecule led to the production of potent hallucinogenic compounds, such as 4-bromo-2, 5-dimethoxyphenethylamine (2C-B), which Shulgin synthesised in 1974. The "2C" compounds are defined by the separation of the main amine from the phenyl ring by two carbons. A single letter in the name denotes an addition at the para position (e.g., Cl, 2C-C; ethyl, 2C-E) (Huang et al., 2012). Professor David Nichols and his research group at Purdue University in Indiana studied a new type of phenethylamine more than two decades later. Synthetic analogues of mescaline, such as 2C-B and DOB, were found to be more potent than several naturally occurring hallucinogens (Monte et al., 1997). Several chemicals were developed, including a wide variety of benzodifuranyl compounds that became known as the "FLY" (Collins, 2011). Benzodifurans, such as "FLY" (tetrahydrobenzodifuranyl) and "Dragonfly" (benzodifuranyl aminoalkanes), are hallucinogens with strong effects. The most prevalent and potent compound in this sub-group is Bromo-Dragonfly (UNODC, 2021b). Other phenethylamines, such as PMMA, which was originally synthesised in 1938 (Glennon et al., 1988), are also available as an 'ecstasy' alternative on the drug market (EMCDDA, 2003).

In terms of chemistry, phenethylamines were the most common type of designer drug available in 2014, as well as a potentially massive reservoir of novel and untested compounds (Nichols and Fantegrossi, 2014). Phenethylamines were probably the most common group since they are the easiest to synthesise and the number of ring changes that can be done on them is practically unlimited. Furthermore, depending on the substituents linked to the aromatic ring of the

phenethylamine template, phenethylamines can have psychopharmacological effects ranging from traditional hallucinogenic activity to psychostimulant action (Nichols and Fantegrossi, 2014).

2.2.2.4 Aminoindanes

Aminoindanes were reported to have considerable bronchodilating and analgesic qualities in the 1970s, but current research has revealed that they also have potent effects on serotonin release and re-uptake. These chemicals have been marketed as NPS due to their capacity to mimic the empathogenic and entactogenic effects of serotonin-releasing drugs such as MDMA (UNODC, 2013a). Aminoindanes are central nervous system stimulants that cause hallucinations. They are available in powder-filled capsules, tablets, or powder form and are administered orally or by snorting (Scott-Ham and Stark, 2016).

Due to a bridge between the α-carbon and the aromatic ring, 2-aminoindane has a stiff structure and is an amphetamine (AMPH) analogue. The synthesis of cyclic analogues of 3, 4-methylenedioxyamphetamine (MDA), MDMA, 3-Methoxy-4-methylamphetamine (MMA), and p-iodoamphetamine (PIA) that contained the 2-AI molecule was done in the 1990s. MDAI, MDMAI, MMAI, and 5-IAI are NPSs created from the chemicals listed above; all are psychoactive, and their presence on the market has been confirmed in confiscated samples of "legal highs" (Figure 5) (Pinterova et al., 2017).

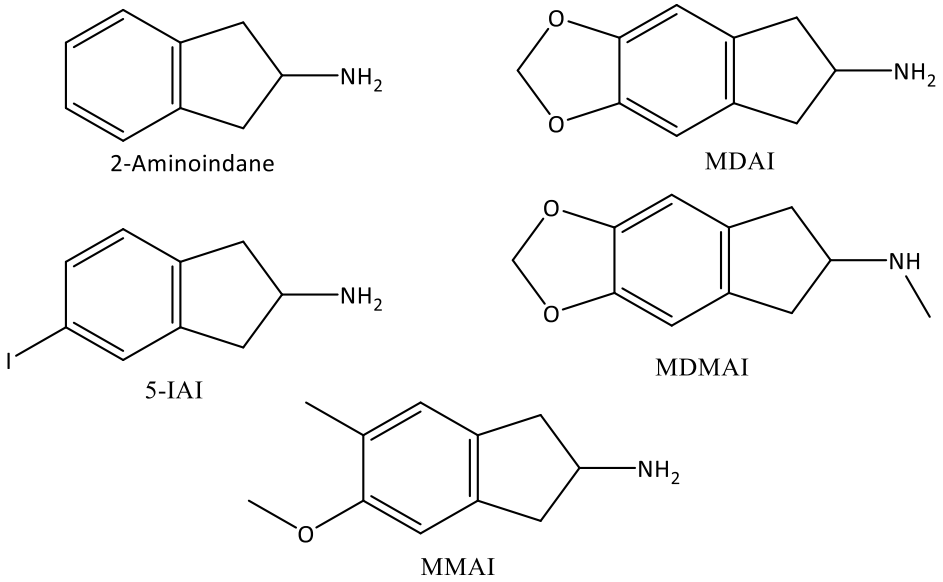

Figure 5: 2-Aminoindane and its derivatives, found in 'legal high' samples

2.2.2.5 Tryptamines

Tryptamines have a bicyclic indole ring structure with an aminoethyl molecule connected at the 3-position as their basic structure. All tryptamines get their core structure from tryptophan, which is an important amino acid in various organisms. Tryptophan is then decarboxylated by enzymes, forming tryptamine (Figure 6) (Nichols and Fantegrossi, 2014). Psilocybin, a natural hallucinogen found in certain kinds of mushrooms that have the tryptamine structure, became popular in the US in the late 1950s, but synthetic tryptamines did not appear on illicit drug markets until the 1990s (UNODC, 2013a).

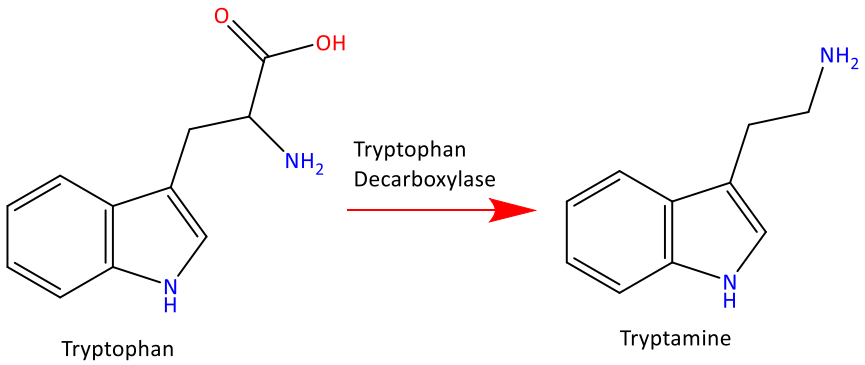

Figure 6: Conversion of tryptophan to tryptamine

After PiHKAL, the Shulgins published TiHKAL: The Continuation, where TiHKAL stands for “Tryptamines I Have Known and Loved" (Shulgin and Shulgin, 1997).

Tryptamines include endogenous serotonin and melatonin, naturally occurring chemicals such as psilocybin (magic mushrooms) and bufotenine, and totally synthesised substances such as DMT (N, N-Dimethyltryptamine) and 5-MeO-DALT. These chemicals primarily generate hallucinogenic effects, which can be visual, aural, tactile, olfactory, or temporal, and work by interfering with the serotonin neurotransmitter system (Scott-Ham and Stark, 2016). Handovsky discovered bufotenine in 1934, and Hoshino synthesised it in 1935. in 1938, Hofmann synthesised LSD, and alpha-methyltryptamine (AMT) was marketed in 1960 as an antidepressant medicine under the trade name Indopan (Zanda and Fattore, 2017). Natural tryptamines are commonly available in preparations of dried or brewed mushrooms, while tryptamine derivatives are sold in capsule, tablet, powder, or liquid form. Tryptamines are generally swallowed, sniffed, smoked, or injected. (UNODC, 2013a). Tryptamine derivatives are illustrated with chemical structures in Figure 7.

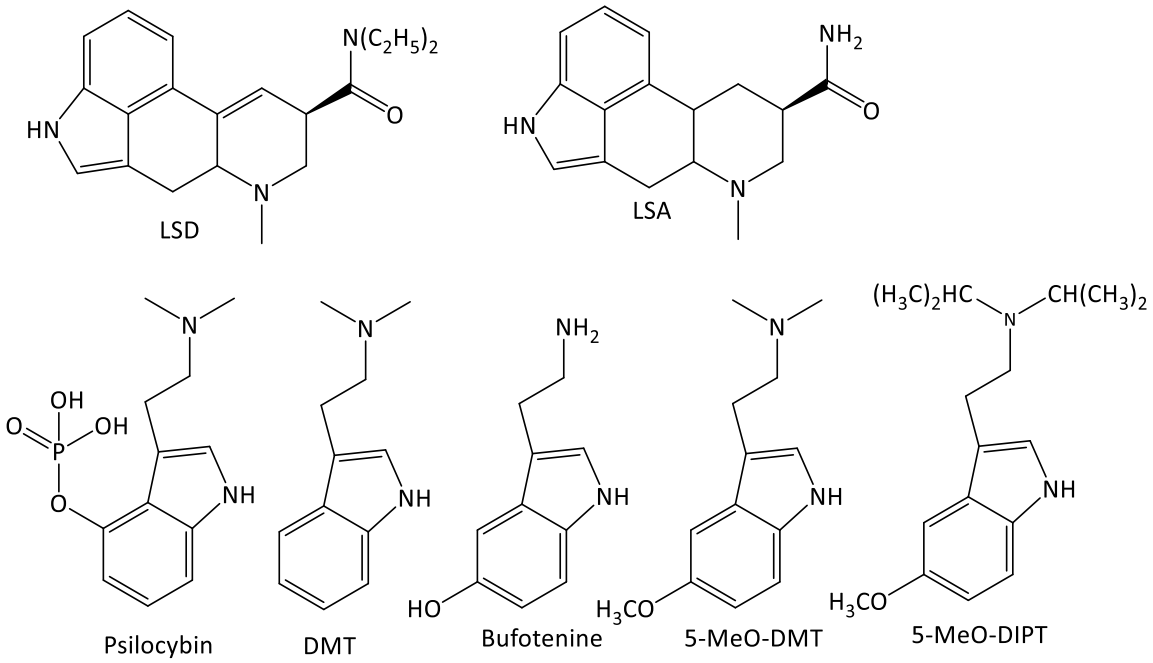

Figure 7: Chemical structures of some tryptamine derivatives

2.2.2.6 Piperazines

Piperazines are synthetic medications that were first sold as anthelminthics before being promoted as antidepressants (Zanda and Fattore, 2017). Piperazine (1,4-hexahydropyrazine) is a 6-membered heterocyclic ring cyclic chemical compound having two nitrogen atoms in opposite places (Figure 8). Piperazine is the backbone of the piperazine derivatives that are commonly used as recreational drugs; it is usually attached to an aromatic ring (Gee and Schep, 2022).

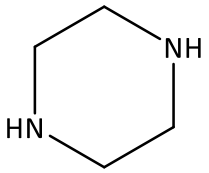

Figure 8: The chemical structure of piperazine

Piperazines, a family of synthetic drugs, have been available on the black market since the 1990s. They are split into two groups: benzylpiperazines, such as N-benzylpiperazine (BZP) and its methylenedioxy analogue, 1-(3, 4-methylenedioxybenzyl) piperazine (MDBP, MDBZP), and phenylpiperazines, such as 1-(3-chlorophenyl) piperazine (mCPP), 1-(3-trifluoromethylphenyl)

piperazine (TFMPP) (Figure 9). Except for MeOPP, all of the compounds have been described as having serotonergic and amphetamine-like characteristics (Richter et al., 2019).

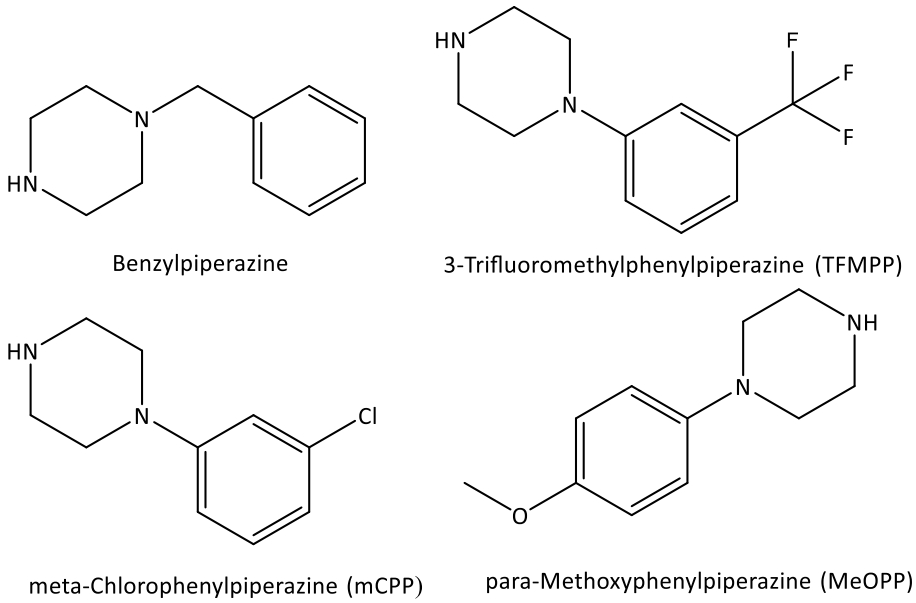

Figure 9: Chemical structures piperazine derivatives: BZP, TFMPP, mCPP and MeOPP

BZP was used to make piberaline, an antidepressant that was marketed in Hungary in the 1980s but was eventually removed. BZP first appeared in New Zealand in the late 1990s as a "legal alternative" to MDMA and methamphetamine. Its use in Europe was initially documented in Sweden in 1999, but it was only widely used as an NPS from 2004 until the European Union imposed limits on the chemical in 2008. mCPP was invented in the late 1970s and is used as an intermediate in the synthesis of various antidepressants, including trazodone and nefazodone. It is apparently more widely used than BZP in some parts of the world. To create the entactogenic effects of MDMA, TFMPP (3-Trifluoromethylphenylpiperazine) is usually invariably combined with BZP. (UNODC, 2013a).

2.2.2.7 Phencyclidine-type substances

Phencyclidine, commonly known as PCP, is a dissociative and hallucinogenic drug of abuse. Ketamine (2–(2-chlorophenyl)-2-(methylamino) cyclohexan1-one) is a similar molecule that is most widely used as an anaesthetic and has lately been researched as an antidepressant, but it has also been known to be abused (Skaugen et al., 2019). The IUPAC nomenclature for PCP is 1-(1-phenylcyclohexyl) piperidine, which is where the term PCP came from. PCP is an

arylcyclohexylamine, an achiral, lipophilic small molecule having a tertiary amine (Bertron et al., 2018).

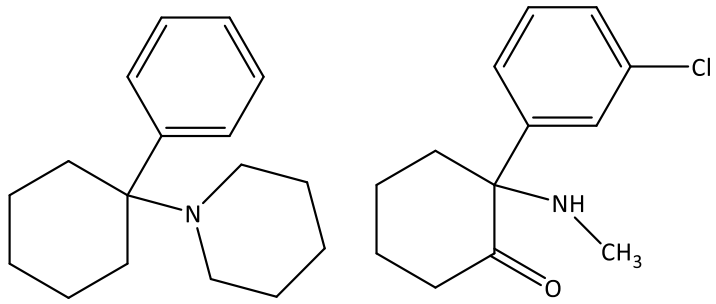

Figure 10: Chemical structures of PCP and Ketamine

In the early 1950s, phencyclidine was produced and investigated, and in 1957, it was approved for human clinical trials as an anaesthetic. PCP was withdrawn as a human anaesthetic in 1965, and the substance was offered commercially as a veterinary anaesthetic (NicDaéid and Savage, 2013). PCP has been used in veterinary medicine since 1967. PCP became a popular hallucinogenic drug in the 1960s and 1970s, owing to its low-dose dissociative and hallucinogenic effects, which might be unpleasant, severe, and long-lasting on occasion (Salter and Gunja, 2022). Following the discontinuation of phencyclidine, ketamine was produced as an anaesthetic in 1962, patented in Belgium in 1963, and approved in the United States three years later (Figure 10). Ketamine was first sold as a medical substitute for phencyclidine in the early 1970s. Ketamine was first used as a psychoactive drug in the 1980s and 1990s (UNODC, 2013a).

In 2010, the United Kingdom reported 3-methoxyeticyclidine (3-MeO-PCE) to the European Early Warning System for the first time as a "research chemical" in Europe (Figure 11). 4-Methoxyphencyclidine (4-MeO-PCP) was detected in Norway, the Russian Federation, and the United Kingdom in 2011 (Figure 11) (UNODC, 2013a).

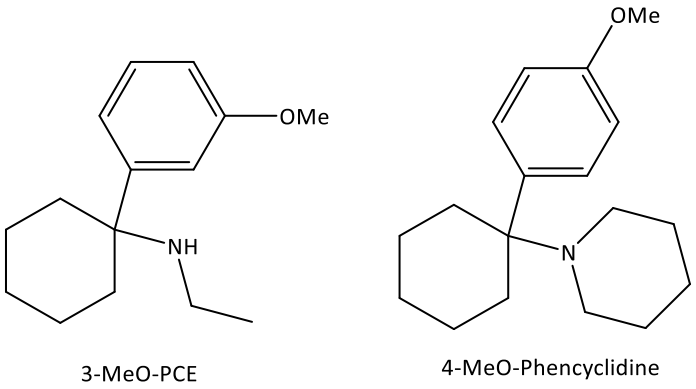

Figure 11: Chemical structures of 3-MeO-PCE and 4 MeO-PCP

PCP (also known as “angel dust”, “crystal”, or “hog”) became available on the illicit market in powder, tablet, leaf mixture, and 1 g 'rock' crystal forms in the late 1960s, referred to as 'PeaCePill,' and commonly sold as “angel dust”, “crystal”, or “hog”, usually taken orally, by smoking, snorting, or intravenous (NicDaéid and Savage, 2013). “K”, “special K”, “kit kat”, “tac”, “tic”, ”cat Valium”, “cat tranquilizer”, “vitamin K”, “ket”, “super K” are some of the street names for ketamine. Ketamine is most commonly encountered in liquid form in pharmaceutical preparations, but it is also accessible as powder and pills. The powder obtained by evaporating the original solution is commonly inhaled, smoked, or ingested (UNODC, 2013a). In recent years, 2-Fluorodeschloroketamine (2-FDCK) has emerged as a ketamine alternative among drug users. However, 2-FDCK has not been restricted or regulated in many countries, which may be due to a lack of evidence on its abuse potential (Li et al., 2022).

2.2.2.8 Plant-based substances

This group includes plants with psychoactive properties. The most frequently reported are: (UNODC, 2013c)

- Kratom (*Mitragyna speciosa*): “Kratom” is the Thai name for the plant Mitragyna speciosa Korth., which is native in Thailand and other southeast Asian countries and contains several alkaloids including mitragynine (Rust et al.) (Figure 12) and paynantheine (McGovern et al.) “Kratom” has been used as a traditional medicine to treat illnesses, including coughing, diarrhea, muscle pain, and hypertension. It is also effective in relieving opiate withdrawal symptoms for heroin or morphine addicts. “Kratom” is misused as an herbal drug of abuse mainly because of its stimulant and euphoric effects. The herbal drug has been controlled in Thailand since 1946 and in Australia since 2005 (Fu and

Stojanovska, 2013). The kratom leaves are typically chewed, brewed into a tea, or ground into a powder. Low doses have stimulating effects, but when the dose is increased, the effects become sedative (Scott-Ham and Stark, 2016).

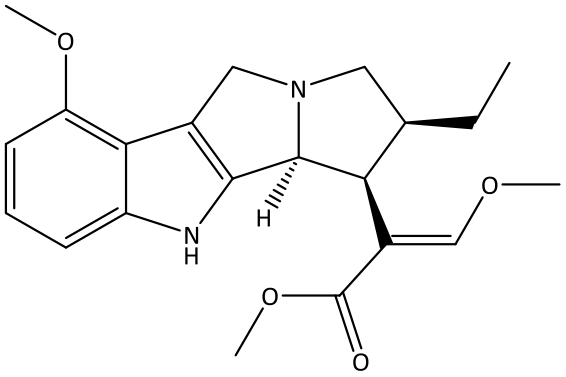

Figure 12: Chemical structure of mitragynine

- *Salvia divinorum*: *Salvia divinorum* (of the mint family Lamiaceae) is a hallucinogenic herb native to Oxaca, Mexico. Although there is no known therapeutic application for salvia divinorum or its active component, salvinorin A, it was traditionally used by the Mazatec Indians for religious ceremonies and medical purposes. *Salvia divinorum* has been used as a new psychoactive substance since the 1990s, but responders to a UNODC poll on NPS ranked it as the most prevalent plant-based substance in 2009 and the third, behind khat and kratom, in 2012 (UNODC, 2013a).

  Salvinorin A (Figure 13), a diterpene and the first non-alkaloidal hallucinogen, is the active component for *Salvia divinorum*'s psychedelic properties. The concentrations in *Salvia divinorum* leaves vary according on the growing factors and processing techniques. *Salvia divinorum* is often sold as seeds or leaves, however a combination of dried leaves and salvinorin A extracts is also available (Feng et al., 2017).

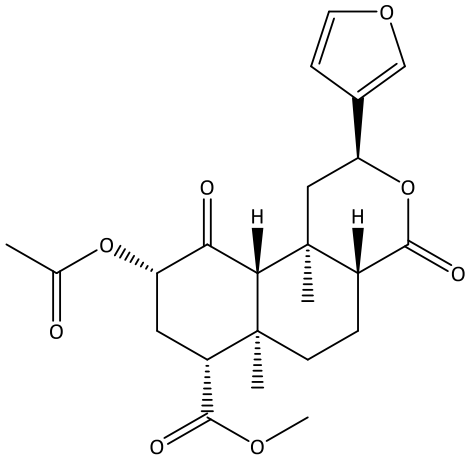

Figure 13: Chemical structure of Salvinorin A

- Khat (Catha edulis): Khat is a flowering plant found in the Arabian Peninsula and the Horn of Africa. Chewing fresh khat leaves has a long history in those tribes, stretching back hundreds of years. A number of alkaloids have been connected to the psychedelic effects of chewing khat. In 1887, the katin alkaloid was discovered, followed by cathine in 1930, and cathinone in 1975. Cathinone and, to a lesser extent, cathine are the primary stimulants found in khat leaves. Khat also includes norephedrine, which is utilised as a precursor in the production of amphetamine, among other things (UNODC, 2013c). The most frequent way to consume khat is through chewing; alternative methods include drinking as a tea, smoking, or nasal insufflation, though these are significantly less common (Gibbons and Arunotayanun, 2013).

2.2.2.9 Other substances

This category contains NPS chemicals that are structurally varied and do not fit into any of the previous categories, such as 1,3-dimethylamylamine (DMAA). This class of substances has a wide range of pharmacological properties (UNODC, 2021b).

2.2.3 Legal Condition

Drugs are classified under the Misuse of Drugs Act (MDA) 1971 based on their risk and impact on society, not necessarily the individual. It's important to note that different substances have various effects on different individuals (nidirect, 2022). The MDA 1971 (Misuse of Drugs Act 1971, 1971) covers "controlled drugs." This includes substances and products listed in Schedule 2 of the Act. That Schedule categorises controlled drugs into three classes based on their relative harms,

with Class A drugs being the most dangerous and Class C drugs being the least dangerous (Home Office, 2015). Class A drugs are the most dangerous, according to Parliament. Heroin, methadone, cocaine (including crack cocaine), ecstasy, magic mushrooms, and "crystal meth" are in this group. A Class A drug offence carries the highest punishment. Class B drugs, which include amphetamines, barbiturates, cannabis, and dihydrocodeine, are deemed less dangerous by Parliament. If some Class B drugs have been prepared for injection, they are reclassified to Class A. Amphetamines, dihydrocodeine, and codeine are examples. Parliament considers Class C drugs to be the least dangerous of the controlled substances. Benzodiazepines, steroids, and Subutex are examples (buprenorphine) (Release, 2022). For Class A drug possession, 7 years' imprisonment and/or fine and for Class A drug supply, life imprisonment and/or fine are the maximum penalties. For Class B drug possession, 5 years' imprisonment and/or a fine and for Class B drug supply, 14 years' imprisonment and/or fine are the maximum penalties. For Class C drug possession, 2 years' imprisonment and/or a fine and for Class C drug supply, 14 years' imprisonment and/or fine are the maximum penalties (House of Commons Science and Technology Committee, 2006).

Section 7 of the MDA 1971 includes the generation of regulations—currently the Misuse of Drugs Regulations 2001 (The Misuse of Drugs Regulations 2001, 2001)—that authorise activities that would otherwise be illegal under the act. The Regulations identify those who may lawfully handle specific drugs, describe the conditions under which drugs may be handled, and limit the purposes for which a specific drug may be used. They also control where a drug can be manufactured or distributed. Under the Regulations, substances are divided into five schedules based on their therapeutic value and perceived risk: Schedule 1 includes those that have no medicinal value; possession and supply are prohibited without specific Home Office approval. Schedule 2 includes those that have medicinal value but have a high abuse potential and, because of their harmfulness, are subject to special requirements relating to their safe custody, prescription, and the need to maintain registers relating to their acquisition and use, and Schedule 3 drugs include barbiturates and are subject to special prescription, though not safe custody, requirements. Schedule 4 drugs include benzodiazepines and are subject neither to particular prescription plans nor to safe custody necessities. Schedule 5 drugs include preparations that, because of their low strength, are exempt from most of the controlled drug requirements (House of Commons Science

and Technology Committee, 2006). In Table 1, examples of drugs included in Schedule 1, 2, 3, 4, and 5 according to MDR 2001 were shown:

Schedule 1:	Cannabis (including cannabis resin), ecstasy, mescaline, raw opium
Schedule 2:	Cocaine, morphine, fentanyl, amphetamine, methylamphetamine, diamorphine (heroin), ketamine
Schedule 3:	Temazepam, meprobamate, barbitone, tramadol
Schedule 4:	Diazepam, N-ethylamphetamine
Schedule 5:	Preparations containing low concentrations of heroin, cocaine, and other specified Schedule 2 drugs

Table 1: Drugs included in Schedule 1, 2, 3, 4 and 5 according to MDR 2001

2.3 Identification of Drugs of Abuse and Novel Psychoactive Substances

In this section, methods which can be used for the identification of drugs of abuse and NPS will be explained in detail.

2.3.1 Routine Forensic Toxicological Analysis

Forensic and clinical toxicology laboratories frequently use screening and confirmation techniques to detect substances in any material. In general, forensic toxicology labs use a screening technique to presumptively identify substances of abuse. When a positive result is obtained, confirmatory techniques such as gas chromatography (GC) or liquid chromatography (LC) in conjunction with mass spectrometry (MS) are required for more sensitive and specific qualitative and quantitative measurements (DeCaprio et al., 2013).

The paper sample containing drugs can be suspected by visual inspection (observable stain on the letter/envelope etc.) or tactile inspection (the texture of the paper may be different due to impregnation with drugs) in the beginning. In addition, sniffer dogs have been used to detect traditional drugs of abuse and synthetic cannabinoids in some countries, including the United Kingdom, the United States, and Canada. When samples suspected of containing drugs are detected, they are tested using in-field techniques such as ion mobility spectrometry (IMS) and/or forwarded to external forensic laboratories for confirmatory testing. External forensic laboratories use traditional methods including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) to

provide significant information even when a reference standard is not present (Vaccaro et al., 2022).

2.3.2 Presumptive Tests for Paper Samples

2.3.2.1 Colorimetric Tests

Prior to instrumental analysis, colour tests are frequently utilised in the early phases of drug identification. They enable the identification of the drug class or classes found in a sample (Kuleya and Cole, 2018). When a certain reagent reacts with a sample, a colour shift is observed. A small amount of material is typically placed on a spot plate or in a test tube, and the reagent is added. Positive controls, in which the chemical to be identified is known to exist, and negative controls (or blanks), must be performed concurrently. This eliminates the potential for false positives due to impurities or degradation products (Stuart, 2012).

Another issue with colour testing is that colour is a subjective feature that can vary over time. However, this can be addressed by using colour charts and photographically capturing the results. Although these tests are sensitive and can be relatively specific, the actual colour observed by the analyst performing the colour test is dependent on a number of factors, including the concentration of the drug, whether the drug is a salt or free base, which salt form is present, the presence of contaminants in the sample, the analyst's colour discrimination, and the conditions under which the colour test is performed (O'Neal et al., 2000).

Although presumptive tests are inexpensive, rapid, and simple to perform, they can be subjective and result in false positives when drug mixtures are present. Additionally, "false positive" results might arise when a colour shift occurs owing to the presence of certain noncontrolled compounds in the drug sample (NicDaéid and Savage, 2013). Furthermore, the test destroys the submitted material. Color tests, on the other hand, do not require a large sample size; if it can be seen, it may be tested (Harper et al., 2017).

Colorimetric tests are available for the majority of drugs of abuse, such as cocaine, various prescription opioids, amphetamines, LSD (lysergic acid diethylamide), cathinones (bath salts), heroin, and fentanyl (Harper et al., 2017). There are numerous possible indicator tests, including cobalt thiocyanate (cocaine), Dille-Koppanyi (barbiturates), Duquenois-Levine (cannabis), Mandelin (LSD, methadone or MDA), Marquis (codeine, heroin, morphine or opium), nitric acid (acetaminophen, diacetylmorphine, dimethoxymethamphetamine and mescaline), para-

dimethylaminobenzaldehyde (LSD), ferric chloride (opioid), Froehde (opioid), Mecke (opioid), Zwikker (barbiturates), and Simon's test (methamphetamine and MDMA) (O'Neal et al., 2000).

2.3.2.2 Ion Mobility Spectroscopy (IMS)

Drugs of abuse are commonly seized in prison contexts by prison officers conducting cell, inmate, or visitor searches. Sniffer dogs have been used to detect traditional drugs of abuse and synthetic cannabinoids in some countries, including the United Kingdom, the United States, and Canada. However, due to the dynamic nature of the NPS market, it is hard to maintain sniffer dogs' long-term effectiveness with these drugs. When samples suspected of containing drugs are identified, they are analysed using in-field methods such as ion mobility spectrometry (IMS) and/or sent to external forensic laboratories for confirmation (Vaccaro et al., 2022). In particular, sniffer dogs and IMS in-field monitoring were able to detect synthetic cannabinoids on paper, indicating the potential for rapid NPS detection on this matrix. IMS, on the other hand, has low selectivity and cannot clearly separate drugs (Vaccaro et al., 2022).

IMS and other rapid detection techniques have long been used to screen and preliminarily identify unknown compounds in a security context, especially for detecting trace amounts of explosives and drugs within airports (Norman et al., 2021b). IMS is used as a preliminary test for detecting NPS in suspicious objects discovered in or near the prison (Metternich et al., 2019).

IMS is a powerful analytical technique used for organic trace analysis. Analytes can be detected in a variety of matrices owing to its high sensitivity (ng range). Analytes are obtained by wiping the surface of the sample with a Teflon membrane (swab). The fast analysis times of less than 10 seconds, as well as the ease of sample collection and processing, allow for the use of mobile IMS at security points such as airports (Metternich et al., 2019). These instruments separate and identify ions by measuring their velocity through a carrier gas. Ion mobility is determined by three molecular properties: ion charge, reduced mass, and collision cross section (Harper et al., 2017).

One of the disadvantages of IMS is that drugs with identical masses and structures cannot always be separated from one another, but this is less essential practically since such instruments are utilized in a presumptive/screening mode rather than in an evidential setting (Norman et al., 2021b). It has been reported that analytes with nearly identical chemical structures, such as synthetic cannabinoids, cannot be distinguished due to the mobile IMS's limited selectivity.

Because of IMS's high sensitivity, disturbing matrix components (such as impurities or contaminants (not the target compound)) may be detected and cause an alarm as a result of a false positive result (Metternich et al., 2019).

2.3.3 Instrumental Methods in Drug Analysis

Modern forensic toxicology analyses are based on instrumental approaches. The majority of analytical instruments turn an analyte's property into an electrical or photometric signal (Smith et al., 2007). A number of laboratory instruments are available for detecting and analysing substances contained in biological specimens (ImObersteg, 2018). These analytical instruments include spectroscopic and chromatographic methods.

2.3.3.1 Spectroscopic Methods

For the detection of counterfeit substances, spectroscopic techniques are typically preferred to chromatography since they are faster, require less (or no) sample preparation, and some are non-destructive (Deconinck et al., 2013). Infrared spectroscopy (IR), Raman spectroscopy, X-ray diffraction (XRD), and NMR are spectroscopic methods that can be used in drug analysis.

2.3.3.1.1 Infra-red (IR) spectroscopy

Infrared (IR) spectroscopy is the research of the scattering, reflection, absorption, or transmission of infrared radiation in the spectral range of 800 nm to 1,000,000 nm (0.8 to 1,000 μm). The IR spectrum has three sub-regions: 12,500 to 4,000 cm^{-1} (0.8 to 2.5 μm; near IR), 4,000 to 400 cm^{-1} (2.5 to 25 μm; mid IR), and 400 to 10 cm^{-1} (25 to 1,000 μm; far IR). Just the mid IR region, which is often stated as infrared, is counted here, as it is the section commonly used in the investigation of drugs and pesticides. However, some instruments have a scan range from 5,000 to about 200 cm^{-1}; the extension to the far IR is functional for halogenated compounds and for inorganic materials (Drake, 2004).

For example, The Loop - a non-profit Community Interest Company founded in 2013 and doing drug safety testing, welfare and harm reduction services at nightclubs, festivals and other leisure events - is using Infrared spectroscopy as a main analysis technique because of its being fast, having a low cost-per-test and great detection power. It uses a Bruker Alpha FTIR spectrometer. “Fourier-transform” technology, which uses the wave-like characteristics of light to produce many different wavelengths of infra-red light, can thus develop a really accurate absorption spectrum

for a sample. A computer system then analyses the spectrum and fully compares it to the database of confirmed analyses to find a match (The Loop, 2018).

The capacity to assess relatively heterogeneous materials and samples with poor characterisation, especially in condensed phases (such as creams, powders, and crystalline materials), is a major advantage of IR spectroscopy. IR spectroscopy can identify or confirm the presence of main ingredients in these samples, which are typically not chemically pure by nature. The use of infrared spectroscopy to establish that a sample is consistent with expectations is common (Jee, 2011). However, because most seized substances are mixtures of compounds, street items containing drugs of abuse may not be practically analysed with FTIR (Nicdaéid, 2018).

2.3.3.1.2 Raman Spectroscopy

Raman spectroscopy is an optical method that uses the inelastic scattering of light as it interacts with materials. Spectral vibrational information is obtained from the interaction of incoming radiation with the molecules of the substance (Harper et al., 2017). Raman spectroscopy is a non-destructive and quick approach to characterization of samples that doesn't require any chemical reagents and isn't affected by water or moisture (De Oliveira Penido et al., 2016).

Infrared spectroscopy and, more recently, RAMAN spectroscopy are widely employed in drug and illicit substance detection. They are both based on molecular vibrations. They can be used directly on samples in solution or powder form. They are quick and simple to use, and the resulting spectra can be thought of as a "sort of fingerprint" of each unique material. Some patterns in infrared and RAMAN spectra are characteristic of certain functional chemical groups and so provide information on chemical structure (Reniero et al., 2017).

Raman spectroscopy can identify almost any medication. It can be used to identify active pharmaceutical ingredients (APIs) and also molecules having the same chemical formula but various molecular arrangements and polymorphs. This is significant since many of the novel psychoactive chemicals that have emerged are isomers, derivatives, and analogues of many of the traditional drugs of abuse. Being able to distinguish between minor changes in physical or chemical structure substantially aids in unambiguous identification (Harper et al., 2017).

2.3.3.1.3 X-ray diffractometry (XRD)

Because of their high energy and ability to penetrate any material, X-rays have long been used to investigate the chemical and structural morphology of any element. This high energy section of

the electromagnetic spectrum has been used in the examination of crystal structure, particle size, chemical composition, nanoscale imaging, and even the observation of interior structures over the years (Hussain et al., 2021b).

Any crystalline or partially crystalline substance (i.e., chemicals that are solid and usually either clearly crystalline or powder or pill, such as methamphetamine, ketamine, and cocaine) can be detected, including those in mixtures and compounds with currently unknown structures (Trzybiński et al., 2013, Rendle, 2003). This technique is generally only applicable to solids. XRD can identify exact chemical forms but not measure them. It can detect diluents and adulterants. This method detects polymorphs as well as contaminants (common in illicit drugs) (Rendle, 2003).

One advantage of using XRD is that it does not require sample preparation and does not degrade the compound being analysed. Furthermore, only a very small amount is required (milligrams to micrograms) (Harper et al., 2017).

X-rays are highly radioactive and extremely harmful to organic cells and DNA. As a result, this method is limited to laboratory surroundings and needs extensive training and safety precautions (Harper et al., 2017). XRD is not used in forensic science to analyse common evidence such as fingerprints and bodily fluids. However, it is extremely valuable in the examination of fibres, fabrics, explosives, and archaeological evidence such as cremains (Hussain et al., 2021b).

2.3.3.1.4 Ultraviolet-visible (UV-vis) Spectroscopy

UV-Vis spectroscopy investigates the electronic changes that occur when incident electromagnetic radiation on a sample is in the 200–750 nm range, also known as the UV-Vis range (Wolstenholme, 2021). UV-vis spectrophotometers compare the intensity of light passing through a sample to the intensity of light before it goes through the sample and use this information to build a distinctive spectrum (Harper et al., 2017).

The first spectroscopic method for the forensic detection of body fluids was the use of UV-Vis light illumination. It is based on one of two processes that compounds exposed to UV-Vis radiation can go through; absorption or fluorescence emission. Most bodily fluids (such as sperm, saliva, and urine) fluoresce when exposed to a UV-Vis light source (Zapata et al., 2015).

Analytical absorption spectroscopy in the UV and visible parts of the electromagnetic spectrum has been widely utilised in pharmaceutical and biomedical analysis for quantitative purposes as well as, with some restrictions, for the characterization of medicines, contaminants, and

metabolites as well as related chemicals (Cordonnier and Schaep, 2008). These methods are highly important in forensic science since they provide results in a small amount of time. These techniques are also employed in the preliminary and confirmatory screening of forensic evidence such as drugs and poisons (Hussain et al., 2021a).

Comparable UV spectra may be provided by drugs with similar structures. UV-vis was utilised to identify MDMA, ketamine hydrochloride, cocaine hydrochloride, diazepam, phenobarbital, and barbital concentrations in the microgram range, as well as explicitly identify six separate chemicals and accurately distinguish some mixes for the first time (Li et al., 2012).

For increased selectivity and specificity, UV can be coupled with chromatographic methods. It cannot identify many substances in a mixture. Otherwise, the method will produce saturated spectra if the samples are not diluted. Compounds without a suitable chromophore provide no signal (e.g., due to the low wavelength chromophore of gamma-hydroxybutyrate (GHB), analysis by UV-vis is much more difficult without additional sample preparation), despite the fact that most drugs of abuse have a suitable chromophore owing to aromatic ring structures in their chemical composition (Harper et al., 2017).

2.3.3.1.5 Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance spectroscopy is based on the nuclear magnetic resonance phenomenon, which happens when the nuclei of particular atoms are immersed in a static magnetic field and subjected to a second oscillating magnetic field. Nuclei that are near each other have an effect on each other's effective magnetic field. This impact can be seen in the NMR spectra (Reniero et al., 2017). It is important to note that sample preparation for NMR analysis is fairly straightforward. A tiny amount of the material, around 15 mg, is typically extracted with 600 µl of a suitable deuterated solvent. The extract is transferred to the NMR tube and analysed (Souza and Lião, 2019).

The availability of a large number of positional isomers of structurally related drugs requires effective tools that provide the necessary structural information for their differentiation. NMR enables the analyst to unequivocally distinguish between different ring-substituted drug derivatives, even in the presence of diluents and other adulterants. Although certain substitution patterns resemble one another in the area corresponding to the protons of the alkyl side chain, the integrated spectrum and the pattern of the aromatic proton signals allow their distinction

from one another. While being a powerful tool for the identification of analogues, the cost of NMR spectroscopy and the technical expertise required prevent its widespread application in routine analysis (UNODC, 2006). The types of samples submitted by law enforcement officials that are amenable to forensic NMR spectroscopic examination are quite diverse. Abused substances, explosives, fire accelerants, hydrocarbon fuels, body fluids and tissues, poisons, and fingerprint reagents are examples of these (Dawson, 2006).

2.3.3.2 Chromatographic Methods

Chromatography is simply a collection of techniques for separating mixed substances by their continuous distribution between two phases. The fixed (stationary) phase is one of the two phases. A solid or a liquid that is supported by a solid. The other phase is a moving one (mobile) phase that is either a gas or a liquid and circulates constantly around the stationary phase (W. Shantier, 2020). Based on the sample type, the analytes to be separated, the column technology utilised to separate the analytes, and the type of detection equipment, each chromatographic technique has its own field of use. However, the sample must typically be in solution (either aqueous or organic) before being introduced into the chromatograph (Carlin and Dean, 2013) Depending on whether the mobile phase is a gas or a liquid, chromatography can be separated into two primary branches. Thin-layer chromatography is a type of liquid chromatography that is also known as planar chromatography (American Chemical Society, 2017).

2.3.3.2.1 Gas Chromatography (GC)

Gas chromatography (GC) can be applied to a wide range of compounds in the fields of toxicology, pharmacy, industrial chemistry, the environment, and clinics (Dawling, 2003). GC can simply be used for qualitative analysis when determining the presence of a specific drug. It is also suitable for drug profiling. Impurities and adulterants in illicit drugs can be discovered and measured in order to link a sample to a source or method of production (Stuart, 2012).

From the simplest (e.g., purity tests of individual chemicals) to the most complex (e.g., petrochemical assays of samples containing hundreds of distinct components), GC allows separation and quantitative analysis of volatile, thermally stable molecules in a wide range of mixtures. GC method produces both a broad analysis of the complete sample and detailed information on individual components of the sample for the study of complicated samples. This is due to the resolving power of long, narrow-bore (capillary) columns covered with a thin film of

stationary phase, which maximizes the capacity to separate chemical components that are closely related (Marriott, 2004).

GC separates sample components based on the partitioning of a gaseous mobile phase and a stationary phase kept in a long, tubular column. Generally, sample injection involves vaporization of the sample and its transfer to the beginning of the column. The gaseous mobile phase helps the sample components travel through the column. Unlike in other chromatographic procedures, the mobile phase just serves as a carrier, with little interaction between the sample and the mobile phase (Forbes, 2021). Molecules having more affinity for the stationary phase stay more in that phase and it therefore takes more time to get to the detector (Figure 14). The detector signal depends on the quantity of material that moves through it. Elution from the column of each compound gives a specific retention time that is the time from introduction to peak detector response (Dawling, 2003).

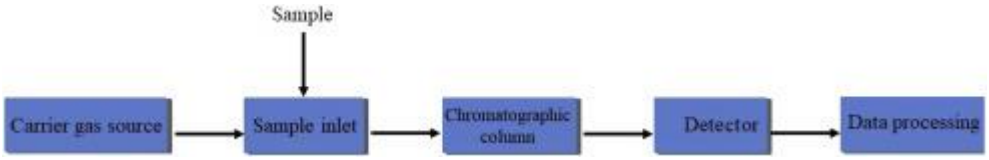

Figure 14: Basic flow pattern of GC (Wen et al., 2021) (For copyright see Appendix 5).

The analyst has two main aims: initially, each dissimilar molecule should appear in a separate band or peak with no overlapping with other constituents in the mixture; and next, these bands should have a uniform shape and be narrow. This is done partly by choosing the column stationary phase and its packing carefully and partly by adjusting the column settings (e.g., temperature ramp). Furthermore, the technique of injecting the substance into the chromatograph, the detector selection, and chemical alteration to enhance the volatility of the substances are also significant for better analysis. Improved detector performance and column performance depend on each other (Dawling, 2003).

2.3.3.2.2 Liquid Chromatography (LC)

Being able to separate and investigate complex compounds is essential for the biological and medical sciences. Chromatographic methods have provided major improvements in speed, resolving power, detection, quantification, convenience, and applicability to new sample types. The most significant one of these changes was high performance liquid chromatography (HPLC). Modern HPLC systems were presented in 1969, but they did not have wide acceptance in the

pharmaceutical area until several years later. When HPLC techniques that were able to do quantitative analysis were on the market, their convenience in pharmaceutical investigation was fully understood. In the 1990s, HPLC began to make an explosive progress, so it became the most popular analytical technique evaluated with respect to sales of instruments and scientific significance. Its current popularity arises from its useful separation of numerous sample forms, excellent resolving power, speed, and nanomolar detection levels (Kupiec et al., 2003).

HPLC is suitable for the analysis of hydrophilic, thermally labile, and high molecular mass substances. In the investigation of drugs and other poisons, HPLC has extra useful advantages such as flexibility, usually low operation costs, a range of selective detectors, and ease of automation. These characteristics can be used to assist the investigation of many compounds, such as drugs and metabolites, simultaneously. HPLC is mainly used in pharmacokinetic and metabolic analysis, the analysis of plasma concentrations of drugs given in therapy (TDM), and in examining exposure to toxic chemicals (Flanagan et al., 2007). HPLC enables the identification of unknown substances in a variety of matrices, such as physical and biological materials. HPLC is widely used in pharmaceutical science to facilitate drug discovery and pharmacokinetic investigations; in anti-doping; in the food and packaging sector; in environmental sciences; and in forensic science. It is used as an alternative to, and in addition to, gas chromatography (GC), and is especially effective when compounds are not suitable for GC (Turfus and Rodda, 2021).

The solvent system can consist of a single buffered solvent (isocratic) or of numerous solvent systems (gradient). The versatility of a gradient system in assessing a wide range of chemicals, as well as the capacity to produce a higher concentration of the substance, are both advantages. When the specimen contains unknown substances, the gradient is most commonly utilized. The isocratic method is more rapid and, as a result, more appealing to laboratories that investigate large numbers of specimens (ImObersteg, 2018).

Whereas being thermally stable for the analyte is not as critical as in GC, many substances (particularly metabolites) are not stable in biological matrices or if exposed to extremes of pH, for instance during the sample preparation step. Therefore, the column/eluent combination, the collection and storage of the sample, the selection of sample preparation method, and the detection conditions must be considered properly. Choosing an appropriate internal standard is also a significant step (Flanagan et al., 2007).

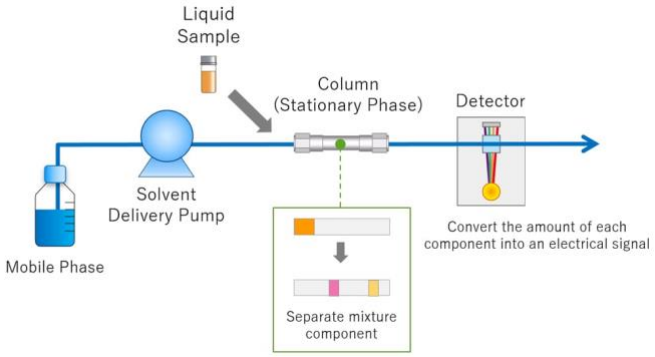

Figure 15: Schematic HPLC system (SHIMADZU, 2022) (For copyright see Appendix 5)

Functionally, the big distinction between GC and HPLC is the type of the mobile phase. Liquids cannot be compressed like gases. In other words, in HPLC, the use of smaller particle-size packings produces high effectiveness with relatively short columns at ambient temperature. Furthermore, the composition of the mobile phase can be changed to regulate retention and selectivity, providing a very high level of control over a given elution (Flanagan et al., 2007).

The interaction mechanism between the analyte and the stationary phase defines the chromatographic mode (Wellings, 2005). There are two separation modes of HPLC with respect to the polarity of the phases:

Normal Phase Liquid Chromatography (NPLC) (Figure 16): It is a method for separating the components of a mixture that involves columns packed with polar stationary phases and nonpolar or moderately polar mobile phases. Individual solute migration rates via NPLC columns are mostly determined by their polarity. Hence, less polar solutes move the fastest and hence exit the column first, followed by increasing polarity solutes that move more slowly (Cooper, 2006).

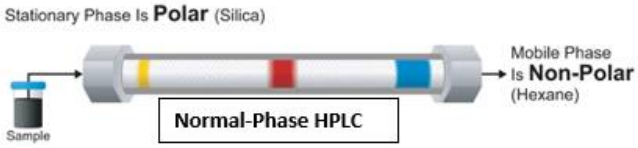

Figure 16: Normal Phase Chromatography (WATERS, 2022) (For copyright see Appendix 5)

This is represented by adsorption chromatography with the use of silica gel as the stationary phase, and compounds are held by adsorption force onto the polar silanols at the silica surface. Common mobile phases in normal phase chromatography are alkanes such as heptane or alkanes

modified by alcohol. Alcohols produce a double layer at the silica surface and miscible ions can alter these layers (Flanagan et al., 2007). Because of the polar characteristics of the stationary phase and non-polar or the less polar characteristics of the mobile phase, polar analytes have a tendency to adsorb to the surface, while nonpolar analytes have a tendency to stay in the mobile phase (Vitha, 2017).

Reversed Phase Liquid Chromatography (RPLC) (Figure 17): By the mid-1970s, the ability to bind nearly any functional group, polar or nonpolar, to silica particles and create bonded stationary phases had resulted in the rapid development of RPLC. Unlike NPLC, RPLC employs nonpolar bonded stationary phases (e.g., octadecylsilane (ODS), often known as C18), and aqueous-based polar mobile phases. Because retention is mostly determined by a solute's hydrophobicity, elution order is often reversed from that of NPLC: more polar solutes come first, followed by solutes with decreasing polarity. RPLC has proven to be suitable for a wide range of separation challenges, and it now accounts for roughly 80% of all HPLC applications (Cooper, 2006).

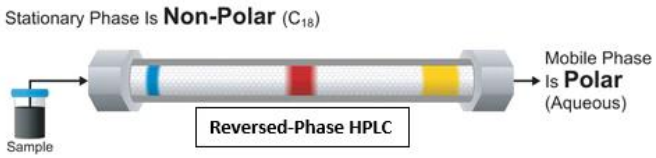

Figure 17: Reversed-Phase Chromatography (WATERS, 2022) (For copyright see Appendix 5)

In NPLC, the stationary phase is generally silica undergoes a modification by adding octadecyl (ODS, C18), octyl (C8), ethyl (C2), methyl (C1), or phenyl propyl (phenyl) silyl moieties. There are various kinds of phases based on the experimental conditions during preparation and synthesis (Flanagan et al., 2007). The most prevalent application of reversed phase chromatography is the analysis of drugs in seized samples, and the most common and efficient column is a bonded octadecyl silica column (C18). Column length, diameter, particle size, pore size, and carbon load should all be taken into account when selecting a column (UNODC, 2020b).

2.3.3.2.3 Thin-layer Chromatography (TLC)

TLC is still a useful method in a forensic laboratory despite its relative lack of specificity and resolution since it can be used as a quick and affordable preliminary test (Stuart, 2012). It can be equally applied to drugs in their pure form, to those obtained from pharmaceutical formulations, to illegally produced compounds and to biological samples. The main principle of TLC is the travel by capillary movement of a liquid phase, which is an organic solvent, through a thin, uniform layer

of stationary phase, which is mostly hydrated silica gel (SiO_2), kept on a solid or semisolid support, usually a glass, aluminum, or plastic sheet or “plate” (Poole, 2003).

By comparing spot colour and retention factor (Turfus and Rodda) values with a positive control, i.e., a drug standard, TLC can be used to presumptively detect drugs. There are numerous solvent systems that can be utilised to separate components for each drug class. The spots are then examined under UV light and/or sprayed with a development reagent that has been chosen to react with specific functional groups (Wolstenholme and Forbes, 2021). The following are some of the most common development reagents for the drug classes:

- for marijuana, Fast Blue BB (red, orange, yellow, brown);
- for opiates, Dragendorff's reagent (orange/orange-red);
- for cocaine, acidified potassium iodoplatinate reagent (blue);
- for amphetamines, Fast Black K (purple or orange-red);
- for barbiturates, mercuric chloride-diphenyl carbazone (blue-violet on pink and purple).
- for benzodiazepines (fluorescent then purple), sulfuric acid followed by acidified potassium iodoplatinate reagent;
- for LSD (purple), Ehrlich’s reagent (Wolstenholme and Forbes, 2021).

Analysts use solvent extracts of urine, stomach contents or scene residues with TLC for poison “screening” methods. Moreover, they can detect and identify many individual compounds and groups of compounds. In some situations, TLC can be thought of an extension of the color tests because the colors produced by different substances form the basis of compound detection. Nevertheless, the combination of a solvent extraction and concentration step and a chromatographic step increases both sensitivity and selectivity. If it is needed, unreacted areas can be taken and used for gas chromatography, high performance liquid chromatography, or mass spectrometry analysis (Flanagan et al., 2007).

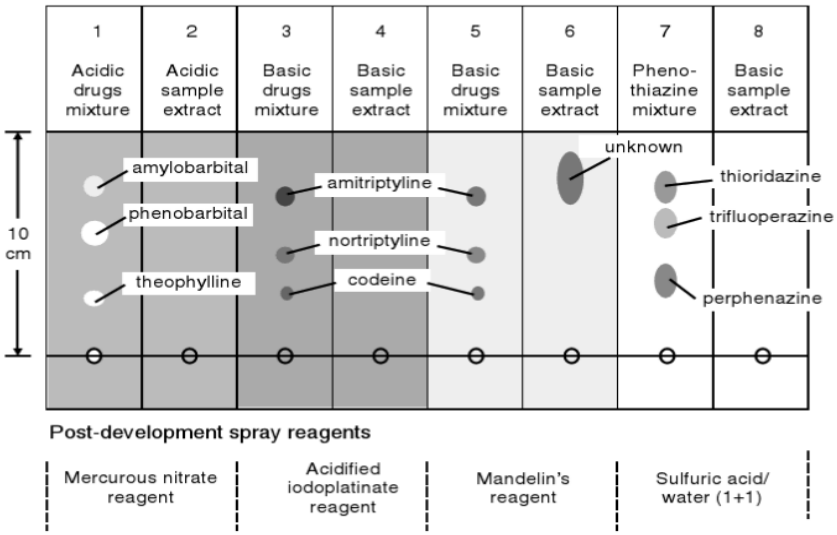

Figure 18: Example of differential visualization of a TLC plate (Flanagan et al., 2007) (For copyright see Appendix 5).

In the example shown in Figure 18, regions of the plate were covered with thin glass plates and each part was sprayed individually and successive spraying with different chemicals is an important identification method. Dragendorff's reagent, which detects alkaloids in a sample, can be used after the plate has been sprayed with ninhydrin (for ammonia) and FPN (Forrest color test) reagent (for phenothiazines), for example. Moreover, quite complex reactions may be implemented by the sequential spraying method (Flanagan et al., 2007).

2.3.3.3 Mass Spectrometric Methods

Mass spectrometry is a technique for separating substances based on molecular and atomic mass. It is the most versatile analytical tool utilised today, as it allows the analyst to identify chemical and structural information about the various types of molecules found in the material (ImObersteg, 2018). Mass spectrometry is the current gold standard in forensic drug analysis, measuring the precise molecular mass of ions as defined by their mass to charge ratio (m/z) (Tsai and Lin, 2005).

The ion source, mass analyzer, and detector are the main components of the instrument, as displayed in Figure 19. Separation, ionisation, and detection are all required in mass spectrometry. Ions generated in the ion source are transferred in the gas phase across the mass spectrometer (Harper et al., 2017). In a high vacuum, all of these ions are subjected to a changing magnetic field and separated based on their mass and charge (which is usually one). Each ion generates a current

proportional to its relative abundance at the collector. This current is then converted and displayed against the ion's mass-to-charge ratio (m/z) (NicDaéid and Savage, 2013).

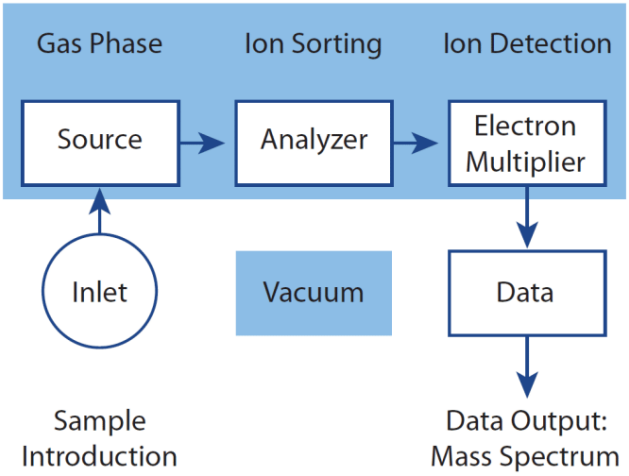

Figure 19: Components of a mass spectrometer (Vandenbroucke, 2015) (For copyright see Appendix 5)

Gas chromatography and liquid chromatography are methods for separation. Ionization can be carried out in a number of ways. Electron ionisation (EI), atmospheric pressure chemical ionisation (APCI), electrospray ionisation (Stolker et al.), matrix-assisted laser desorption ionisation (MALDI), atmospheric pressure photoionization (APPI), fast atom bombardment (De Campos et al.), and, more recently, direct analysis in real time (DART) are the most commonly used in illicit substance analysis (Harper et al., 2017).

In forensic laboratories, numerous mass analysers are employed in mass spectrometers. One of the most frequent is a quadruple mass spectrometer, which uses four rods to produce a voltage along the route of the ions. In addition, the basis of a time-of-flight (TOF) instrument is the principle that lighter ions are propelled quicker than heavier ions, resulting in short TOFs over a given distance (Stuart, 2012).

2.3.3.3.1 Gas Chromatography Mass Spectrometry (GC-MS)

In the detection of relatively low relative formula mass (M_r), volatile, thermally stable organic compounds, both EI and CI are widely utilised in GC-MS. Both employ the same main instrument and ionisation source system. Other desorption ionisation processes (such as ESI) are available. EI generates positively charged ions by removing an electron, whereas chemical ionisation can generate either positively or negatively charged species (Flanagan et al., 2020).

GC-MS is one of the most widely used techniques for the analysis of forensic drug samples. Since it is a hyphenated technique, there is a combination of the separating ability and sensitive measurement of a GC with the analyte specificity of a spectroscopic technique. Compounds can be identified by comparison of the retention time and mass spectrum of the analyte with that of a reference standard. All compounds detected by GC-MS and reported by the analyst must be compared to the current mass spectrum of the proper reference standard, preferably taken from the same instrument and worked under the same settings. Commercial mass spectral libraries or user-generated spectra should be used for reference purposes only (UNODC, 2013b).

Because control GC-MS systems are not high-resolution instruments, the analytical data obtained does not allow for a complete identification of the chemical structure of a new unknown molecule. This method of analysis is currently widely used. It is ideal for regular control since the analysis may be completed in a relatively short period of time (in most circumstances, a few minutes to an hour), with moderate operating expenses and an investment in instruments that most laboratories can afford. This method is also capable of performing quantitative determinations under the appropriate circumstances (Reniero et al., 2017).

2.3.3.3.2 Liquid Chromatography Mass Spectrometry (LC-MS)

In analytical toxicology, liquid chromatography combined with low- or high-resolution mass spectrometry is now a significant instrument. It has the selectivity, sensitivity, and universality required for screening, identifying, and quantifying medicines, poisons, and/or their metabolites in biological samples (Maurer, 2011). The fundamental advantage of LC-MS over GC-MS is that it can analyse a relatively high number of substances. Without derivatization, LC-MS can chromatograph polar and thermally labile/high M_r analytes (Flanagan et al., 2020). ESI is the ionization technique that is often used on LC-MS systems to produce molecular ions (e.g., protonated, deprotonated, alkali metal adducts, etc.) with minimum fragmentation (Brown et al., 2020).

The use of LC/MS in toxicology is continuously rising, due in part to the convenience of sample preparation and in part to the instrument's simple extractions and absence of derivatization requirements (ImObersteg, 2018).

When LC is coupled to a mass spectrometer in forensic toxicology, it deals with the liquid phase and the mainly hydrophilic species that are dissolved in solution. Many drugs are difficult to

vaporise and are much more amenable to chromatographic separation in the liquid phase. This is particularly true of metabolites of drugs that may be present in the blood or urine (Parkin and Brailsford, 2021).

2.3.3.3.3 Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

In the LC-MS/MS method, the compounds that are separated depending on their physicochemical characteristics in the HPLC unit are proceeded to a tandem mass detector and examined in this unit. With a reference material or library data, the response of the mass detector and the retention time of the molecule may then be compared. When the separation and purification power of the chromatography method are combined with the identification capability of the MS/MS unit, analytes with different mass spectra (even with the same retention time) can be identified, confirmed and quantified. This highly specific method can be effective for the analysis of some analytes that cannot be obtained with other chromatographic methods (Anilanmert, 2018).

Tandem mass spectrometry refers to a group of techniques in which one stage of mass spectrometry, not necessarily the first, is used to isolate an ion of interest, and a second stage is used to investigate the relationship of this ion with others from which it may have been generated or which it may be generated on decomposition. In order to receive the appropriate analytical information, the two steps of mass spectrometry are linked in precise ways. There are distinct MS–MS analysis that can be conducted (Ardrey, 2003).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful confirming technique that combines the separation capabilities of standard LC with the detection capabilities of a tandem mass spectrometer, resulting in much higher selectivity. Its low detection limits enable it to be used for trace analysis as well as the testing of biological specimens such as blood and hair. LC-MS/MS is ideal for both qualitative and quantitative analysis of drugs in seized items and biological specimens due to its high sensitivity and selectivity (UNODC, 2020b).

2.3.3.3.4 Triple Quadrupole LC-MS/MS

The triple-quadrupole (QQQ) technique, which consists of three quadrupoles placed in sequence, is a common technique for doing MS/MS analysis. These are still the main mass spectrometers typically found in forensic laboratories. The mass analyzer region is made up of the first (Q1) and third (Q3) quadrupoles, which serve as standard mass filters. The RF potential is used to operate

the middle (Q2) quadrupole (Figure 20). This allows all ions to travel through it from Q1 to Q3 and the detector, as well as serving as a transitory holding zone where ions can undergo CID. Q2 is generally referred to as a collision cell. CID is caused by delivering a small amount of energy to the ions as they travel through the Q2 field and immersing them in a gas, typically argon. This tandem in space design enables the instrument to operate in modes that are unique to QQQs (Parkin and Brailsford, 2021).

The selected ion from Q1 is referred to as the precursor ion, whereas the ions generated by fragmentation of the precursor ion are referred to as product ions. The fragmentation caused in such instances is usually relatively reproducible. The third quadrupole (Q3) is used to scan the product ions or to selectively let one or more of them pass through to the detector. Change the composition of the collision gas (argon, helium, nitrogen, or xenon), the energy of the ions emitted from Q1, and the temperature and pressure in the collision cell to alter the fragmentation (Flanagan et al., 2020).

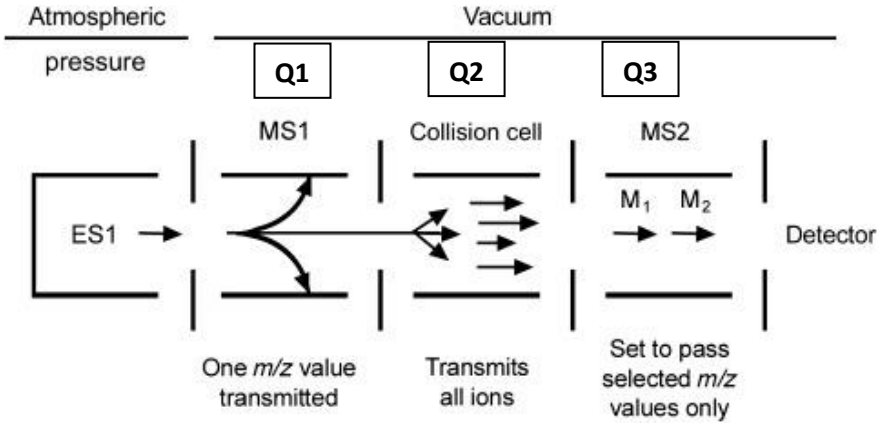

Figure 20: Schematic representation of a triple quadrupole tandem mass spectrometer (MS/MS) system (Tanna and Lawson, 2016) (For copyright see Appendix 5)

With the secondary ion detector, it is possible to complete qualitative determination by detecting the secondary ion produced by the primary ion and quantitative determination with the use of the chromatogram of the secondary ion. This process is called "Multiple Reaction Monitoring" (MRM). Although there are many molecules with the identical *m/z* value, the molecules can be distinguished from each other with a particular MRM fragmentation in LC-MS/MS, decreasing the noise with the two-stage filtering of the unnecessary ions and excluding the target ions. Thus, with the LC-MS/MS method, it is possible to quantify the substance at very low concentrations. This method is highly specific because the evaluation of the analyte is done according to molecular ion

and product ion with the retention time (t_R) (Anilanmert, 2018). The disadvantage of this targeted technique is that all other ions are lost, decreasing the information related to any peak of interest and contained throughout the chromatogram. From a forensic standpoint, the data cannot be reviewed to seek for unknowns, which may not be desired if there is only a small quantity of samples to analyze. Typically, at least two (and, if fragmentation allows, three) ions per analyte are recorded to maintain the acceptable level of certainty for the identification of substances. The quantifier (which is used for quantification if necessary) is one of these, while the others are qualifiers. The presence of these ions, as well as their relative abundances to one another, is critical for proper identification of any substance (Parkin and Brailsford, 2021).

2.3.3.3.5 Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS)

The QTOF-MS is a "hybrid" device that combines quadrupole equipment with a time-of-flight mass analyser. The QTOF-MS technique is similar to that of a triple-quadrupole mass spectrometer, except that the third quadrupole has been replaced by a time-of-flight tube (Allen and McWhinney, 2019). The features of the QTOF mass spectrometer are the result of its manufacture. The QTOF system is complicated, but the most critical elements are, apparently, the quadrupole, time-of-flight analyzer, and ionization source (Figure 21). The TOF mass analyser represents the heart of the mass spectrometer, i.e., the device able to measure the *m/z* ratios of gas-phase ions. To allow a free transition of the ions through the analyzer towards the detector, the analyzer must be operated under high vacuum conditions. The lower the pressure (typically in the range of 10^{-4} to 10^{-7} torr), the longer the mean free path of the gas-phase ions and, consequently, the better the sensitivity and mass resolution (Politi et al., 2006).

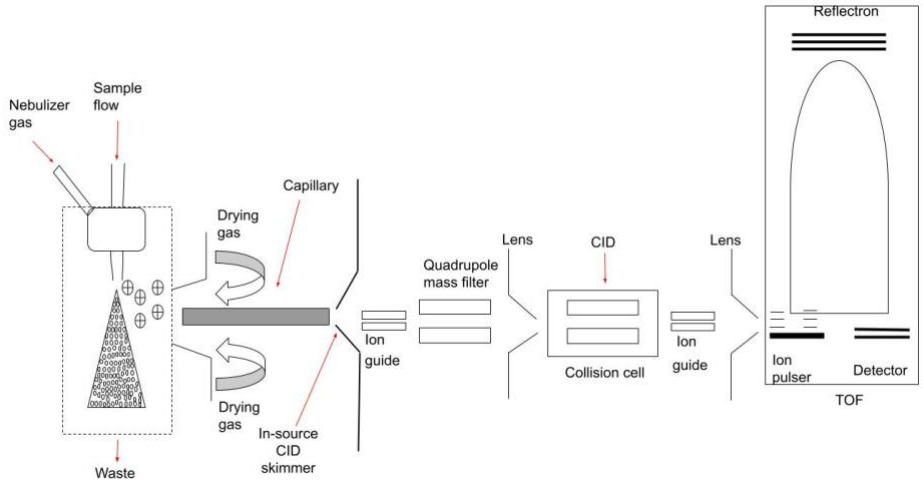

Figure 21: Schematic of the ESI-QTOFMS system

Technological developments in mass spectrometry, such as ToF and Orbitrap systems, have enabled LC-MS equipment to rapidly provide a full range of spectral analysis with the additional advantage of high mass resolving power. To facilitate identification, the latter provides selectivity as well as the ability to accurately measure mass (Sargent, 2013). The mass analyzer's resolution is defined as its capacity to differentiate between adjacent mass ions. It is a highly desirable measure of the quality of instruments since it aids in determining the mass accuracy of the instrument. There would be no advantage if equipment able to measure mass to four decimal places could not differentiate masses with differences of 0.0001. The mass resolving power of an instrument is a functional feature that refers to a mass spectrometer's capacity to provide a certain degree of mass resolution (Parkin and Brailsford, 2021). TOF devices have mass resolutions of at least 30,000–40,000, although modern benchtop Orbitrap, which is the most recent type of mass analyser to be developed, has a resolution of around 140,000 (Flanagan et al., 2020).

QTOF mass spectrometers have advantages in good mass accuracy and high mass resolution. Mass accuracy is the difference between measured (experimental) mass and theoretical mass calculated from the elemental composition of the ion. Masses can be calculated with a precision of 5–10 ppm due to the great selectivity produced by the use of narrow mass windows (Stolker et al., 2004). QTOF mass spectrometers often have mass resolution values greater than 10,000. The change in mass of the investigated compounds affects the resolution of the spectrometer. The greater the *m/z* value of the analysed compound, the easier it is to achieve higher resolution (Sekuła and Zuba, 2018).

Before the analysis of an assay sample, it must be presented into the mass spectrometer first. The basic necessity is that a substance has the ability to ionize under the applied conditions. Separation of the sample components occurs in a liquid chromatograph, and they are the first elements to go into the ionization source. The LC-MS interface has the double task of removing the solvent from the LC eluent and generating gas-phase ions from the analyte (Politi et al., 2006). There are two major types of atmospheric pressure ionization methods: electrospray ionization (Stolker et al.) and atmospheric pressure chemical ionization (APCI). These methods are commonly employed to ionise thermally labile and moderately polar organic analytes. These techniques often produce ions with no unpaired electrons, and the resulting [M + H] + molecules are known as protonated molecules (Steckel and Schlosser, 2019). All atmospheric-pressure ionisation procedures produce protonated or deprotonated molecules and various

adducts. The nature of the ions produced by ESI (protonated/deprotonated, Na+-, K+-adducts, etc.) is determined by the analyte and the experimental circumstances (De Vijlder et al., 2018).

Electrospray Ionization: In mass spectrometry, the electrospray ionisation (Stolker et al.) mode is utilised to generate ions using a spray and a high voltage. Because the source has relatively little fragmentation, ESI is referred to as a "soft ionisation" approach. The positive ionisation mode (ESI+) is utilised by default; the negative mode is employed only when necessary (for example, with labile molecules) (Reniero et al., 2017). The ions detected by mass spectrometry could be formed by the addition of a hydrogen cation, [M + H] +, or another cation, such as sodium ion [M + Na]+ (potential production of adducts in the source) (ESI+ mode), or by the removal of a hydrogen nucleus, [M H]- (ESI-mode). Water, methanol, and acetonitrile are the most often used solvents, often with organic acids added to facilitate the production of protonated species (Henderson and McIndoe, 2006). Only analytes with at least one positive elementary charge may be detected in ES+, and only analytes with at least one negative elementary charge can be detected in ES-. Molecules with basic properties can be easily ionised in ES+ by forming adducts with proton(s), whereas molecules with acidic functional groups (but no basic ones) often provide better ES- spectra (Steckel and Schlosser, 2019).

Ionic isolation is performed by selecting "precursor" ions in the first mass analyzer while ignoring any nonisobaric ions in a sample spectrum. The precursor ions are then fragmented by collision with an inert gas in a low-pressure collision chamber, and the chemically relevant fragment ions are isolated in time (ToF) using a secondary mass analyzer. Because of the low pressure in the collision chamber, only fragment ions with m/z [M±H]± precursor ions are formed in this procedure. The exact suite of ions generated in the collision cell is defined by the pressure and collision energy (Parcher et al., 2018).

The important fragmentation process of ions occurs in the collision cell. The principle of tandem mass spectrometry is that the parent (precursor) ion m_p^+ / m_p^- separated in a first analyzer is then fragmented, yielding daughter (product) ions m_d^+ / m_d^- and neutral fragments m_n.

$$m_p^+ \rightarrow m_d^+ + m_n \qquad \text{positive ion mode}$$

$$m_p^- \rightarrow m_d^- + m_n \qquad \text{negative ion mode}$$

During this process, the precursor ion is accelerated and disintegrated into product ions by collision with an inert gas. As a collision gas, nitrogen or argon is most commonly used. As a consequence of collisions with gas, ions are dissociated. The fragmentation rate of ions depends on the amount of collision energy. For a molecule with a greater mass, more energy is needed to dissociate the ions. However, the degree of ion fragmentation is also affected by the strength of each bond in a molecule (Sekuła and Zuba, 2018).

In Figure 22, solvent is running through a charged capillary (C) and forms an aerosol of charged droplets which undergoes further fragmentation because of electrostatic repulsion in order to produce a free and solvated ion (+). There is an attraction between ions and the mass spectrometer inlet orifice (I) by relative charges on the external and internal plates (Dooley, 2003).

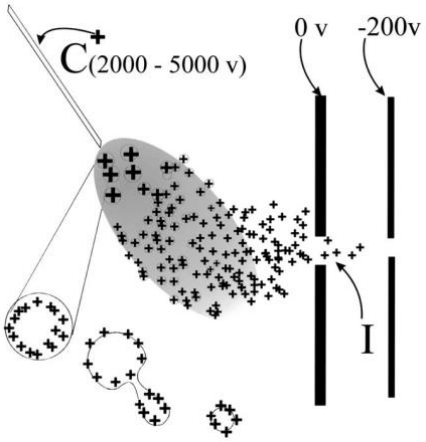

Figure 22: Schematic demonstration of the electrospray ionization process (Dooley, 2003) (For copyright see Appendix 5)

Skimmer: Another part of the mass spectrometer is the skimmer. This is the separation component of regions at different pressures in a mass spectrometer, as, in the subsequent components of the instrument, there is a growing vacuum. The other task of the skimmer is to isolate the analyte ions from the carrier gas, impurities, and residual solvent. Moreover, the ions are moved through a quadrupole ion guide followed by the lenses and go to the quadrupole mass filter. This additional quadrupole ion guide is utilized for collisional cooling and focusing of the ions going into the instrument (Sekuła and Zuba, 2018).

Quadrupole: Quadrupole analyzers are made up of four circular or, ideally, hyperbolic-sectioned rods (Figure 23). The rods must be completely parallel to one another. The quadrupole analyzer is a device that separates ions based on their *m/z* ratios by utilizing the stability of trajectories in

oscillating electric fields (Hussain et al., 2021b). A dynamic electrical field is formed by simultaneously applying a radio frequency (Turfus and Rodda) that is 180˚ out of phase to opposing pairs of rods. Each pair of opposing rods is additionally supplied with direct current (DC) of the same magnitude but opposite polarity with respect to ground. The electrical field formed in the mass analyzer functions as a filter, allowing only ions of specific masses ('resonant ions') to pass through the space between the four rods, i.e. the quadrupole is a mass-to-charge filter. Ions having differing *m/z* values (non-resonant ions) will collide with the rods and get neutralised. When a positive ion enters the area between the rods, it is pulled towards a negative rod. Increasing the DC and RF voltages allows stable paths for ions with varying *m/z* values to pass through to the detector (Hoffmann and Stroobant, 2007).

The quadrupole can work in two different modes. For single MS (or TOF-MS) measurements, the quadrupole is operated in the total transmission ion mode so that it serves merely as a transmission element, while the TOF analyzer is used to record spectra. For MS/MS (or Q-TOF-MS) measurements, the quadrupole is operated in the ion isolation mode to transmit only the specific parent ion, typically selected in a mass window from 1 to 3 *m/z* wide depending on the desire to transmit the full isotopic cluster. Mass peaks appear as resolved isotope clusters in the spectrum because of the appearance of elements with distinct isotopes in a spectrum by patterns of isotope clusters (Chernushevich et al., 2001, Barwick et al., 2006).

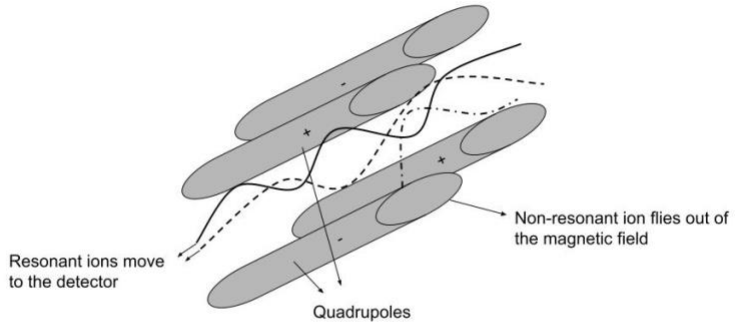

Figure 23: Quadrupole filter

Fragmented ions pass on to the TOF analyzer. However, before ions enter this part of the spectrometer, the ion beam has to be smoothed so ions going into the analyzer have the identical path distance to pass. In accordance with this aim, the quadrupole ion guide and ion focus lenses are usually used (Sekuła and Zuba, 2018).

TOF Analyzer: Ions are separated in the TOF analyzer according to their velocities as they drift in a field-free region called a flight tube after being initially accelerated in an electric field. Ions with the same kinetic energy are propelled with a constant energy towards the detector. Ions with a higher mass will have a lower velocity due to the link between kinetic energy and mass ($E_k = ½ mv^2$). As a result, the time it takes an ion to enter the detector at a certain distance from the mass analyzer's entrance can be used to determine its m/z (Parkin and Brailsford, 2021).

Normal flight times are 5–100 μs. To get an accurate measure of the flight time, the beginning time of ions, which is the time of entrance of ions going into the flight tube, needs to be precisely defined. Consequently, combination with LC needs fast electric field switching in order to provide the entrance of ions generated in the ion source to the flight tube in a short and defined time. The TOF analyzers can work in two modes: in a linear mode (the ions travel in a straight line) and in a reflectron mode (the use of mirrors to change the direction of an ion stream). However, the reflectron-type TOF is currently the most common TOF analyzer in analytical toxicology because it has a much better mass resolution of up to 20,000–30,000. A reflectron-TOF is used in modern time-of-flight analyzers to adjust for kinetic energy dispersion, which causes broad mass peaks and a spatial spread of ions with the same *m/z* but different velocities. This reflectron adjustment enables ions with the same *m/z* to arrive at the detector simultaneously. The reflectron device also extends the flight path, which improves mass resolution (Politi et al., 2006, Allen and McWhinney, 2019).

Detector: The Q-TOF detection system usually uses a microchannel-plate detector. The ion beam hits the microchannel plate and produces a stream of electrons that are altered into photons. The photons hit a photomultiplier tube and produce an amplified signal relative to ion flux (Agilent, 2015).The active elements of a TOF detector are not designed to endure the instrument's lifetime. The microchannel plates are consumables and need to be replaced on a regular basis (Laprade, 2005).

2.3.3.3.4 Mass Spectral Libraries

Mass spectral libraries are a useful tool for rapidly identifying unknown substances in a sample. Mass spectra give information on both chemical molecular weight and fragmentation (Waters, 2007). As they give a spectral reference for known standards, mass spectral libraries help in the identification of chemicals in forensic toxicological samples. Using both GC-MS and LC-MS instruments, some comprehensive libraries including thousands of chemicals have been generated. Many libraries are commercially available, such as the National Institute of Standards and Technology library (NIST). Standardized MS settings have been used in an attempt to achieve reproducible spectra across various equipment and manufacturers. For example, electron ionization-generated spectral libraries use 70 eV-standardized conditions. Initially, the creation of universal libraries was restricted by differences in LC-MS instrumentation. Different LC-MS manufacturers and methods can produce a wide range of in-source fragmentation. However, when CID is used, the spectral data is more reproducible among manufacturers than in-source fragmentation. When compared to in-source fragmentation techniques, the consistency of MS/MS spectral data is attributed to fewer experimental parameters. Collision energy and collision gas pressure are two elements that must be standardised in CID (Seither, 2018).

While substantial GC-MS and LC-MS libraries have been developed and used, many of them lack a comprehensive representation of NPS. As a result, libraries must be updated or new libraries must be created to ensure the presence of NPS spectral data. Furthermore, because new NPS arrive on the illicit drug market on a regular basis, these libraries must be able to be immediately and easily changed (Seither, 2018).

2.4 Challenges for Identification of NPS

While improvements in modern chemistry and pharmacology have resulted in the development of life-saving medications, they have also resulted in the synthesis of new or novel psychoactive compounds. NPS are frequently manufactured in improvised laboratories with no quality control or government oversight, so their structural properties, content, and unfavourable side effects are unknown. Lack of timely detection might be ascribed in part to their rapid turnover rate—by the time biochemical and toxicity research on an NPS is completed, it either disappears from circulation or is transformed into a new version to avoid regulations. This makes monitoring and regulating counterfeit substances challenging (Upadhyay, 2021).

Over 1,000 NPS have appeared in illicit drug markets in recent years. The large number of drugs and the dynamic nature of the NPS market make early detection and development of countermeasures to the emerging public health hazards connected with this trend extremely difficult (UNODC, 2020a).

The traditional drug monitoring model only takes into account a small number of well-known and banned compounds. Forensic and toxicological analytical techniques can detect, identify, and quantify compounds in seized or biological samples, depending on the method. It is important to consider that the typical approach to dealing with illegal drugs may not be appropriate for NPS. In forensic terminology, appropriate identification of drugs is critical when reviewing suspect collected samples and evaluating cases of probable intoxication, and it needs the use of reliable and accurate identification techniques (Bruni et al., 2022).

From an analytical standpoint, correct identification of NPS is a key challenge. The variety of alterations to molecules and the speed with which they appear on the market are two of the challenges that come with NPS evaluation. New compounds appear at a faster rate than analytical procedures are developed. The lack of verified standards for reference is one of the most serious issues (Peacock et al., 2019).

Similarly, the difficulties in identifying SCRAs and other NPS when they are taken into prison may be a driving factor in their availability in prison. Sniffer dogs are not trained to detect the many distinct forms of NPS, and the drug's impregnation into paper and textiles further makes it difficult for prison officials to detect (EMCDDA, 2018b).

2.5 Psychoactive substances in prisons

Drug abuse in prison is one of the problems facing the UK criminal justice system today (Ministry of Justice, 2019). According to HM Prison and Probation Service, the prevalence of drug misuse is high and it causes violence, crime and vulnerability within prisons, which is an issue for safety and the ability of prison staff to provide efficient administration. The extent of the problem is considerable and has become more challenging in recent years. Between 2012/13 and 2017/18, the degree of positive random tests for “traditional” drugs in prisons rose by 50%, from 7% to 10.6%, and drug use in prisons is now common. This is especially true in male, local, and category C prisons, which are training and resettlement prisons; most prisoners are located in these prisons. Rates of positive drug tests are divided into two groups; “traditional” drugs which are

monitored substances defined in the Misuse of Drugs Act 1971, such as opiates and cannabis; and "psychoactive substances" which have the first definition in the Psychoactive Substances Act 2016, such as synthetic cannabinoids, but many of them have now been monitored by the Misuse of Drugs Act 1971. The appearance of NPS such as SCRAs has worsened the problem, and NPS are commonly used in combination with other drugs (Ministry of Justice, 2019).

In the UK, there has been a rapid increase in accessibility and use of these drugs in the last 10 years. However, getting exact statistics about drugs in prisons is difficult, partly because of the continuous change in the use of new substances and alterations to compounds with the purpose of escaping from the law and control (Ministry of Justice, 2019).

Drug use in prisons might be different and might not certainly be used by the people who are currently using illegal drugs. Psychoactive substance use has become an important issue in prisons in England and Wales in recent years. SCRAs are the most commonly used drugs in prisons. They mimic the effects of cannabis (Wheatley et al., 2015). The popularity of NPS has been increased because they are undetectable by conventional screening methods, they are relatively affordable, and their legal status is perceived, as well as they help to relieve someone of boredom, they are being used as a defence mechanism or as a method of self-medication, yet there is rising evidence that there may be a change in this situation. SCRAs cause unpredictable consequences on people maybe because of that, these drugs attract some prisoners who intend to try or be more daring with their drug use. Developments in screening, together with stricter implementation of prison policies, the launch of smoke-free prisons and the Psychoactive Substances Act provisions, might have been put together to have an influence on NPS use in prison (PHE, 2015).

2.5.1 Prevalence

According to data from the Random Mandatory Drug Testing (RMDT) programme, which provides the measurement of the level of drug misuse in prisons, dissuade from drug misuse between prisoners, and detects prisoners who might be in need of help from substance misuse services, there is a rise for drug finding in prisons, which increased to 18,435 incidents from 13,118 incidents, a rise of 41% between the year ending March 2018 and the year ending March 2019 (Ministry of Justice, 2019).

In the Psychoactive Substances Act 2016, "psychoactive substance" is defined as any substance that is capable of producing of a psychoactive consequence in a person who uses it and is not an

exempted substance. These substances are controlled drugs, medicinal products, alcohol or alcoholic products, nicotine, tobacco products, caffeine or caffeine products, or any substance which is normally consumed as food and does not include an illegal component (UK Government, 2016). Psychoactive Substances are the most common drug type in prison, and they were found in 51% of all positive samples, passing cannabis, opiates, and buprenorphine by a huge difference (Figure 24) (Ministry of Justice, 2019). In September 2016, Her Majesty's Prison and Probation Service became the first prison service in the world to introduce innovative mandatory drug tests for psychoactive substances. It has been a criminal offence to possess psychoactive substances in prison and more than 300 sniffer dogs have been trained specifically to detect these drugs. Body searches and metal-detecting technology are used in every prison across the estate, and the effectiveness of body scanners and other technology are in examination. In the year to March 2019, the most common types of illicit items found in prisons were drugs, which included 18,435 incidents of finds; 11,448 mobile phones; 9,785 weapons; 6,484 alcohol; and 5,909 tobacco (Figure 25) (Ministry of Justice, 2019).

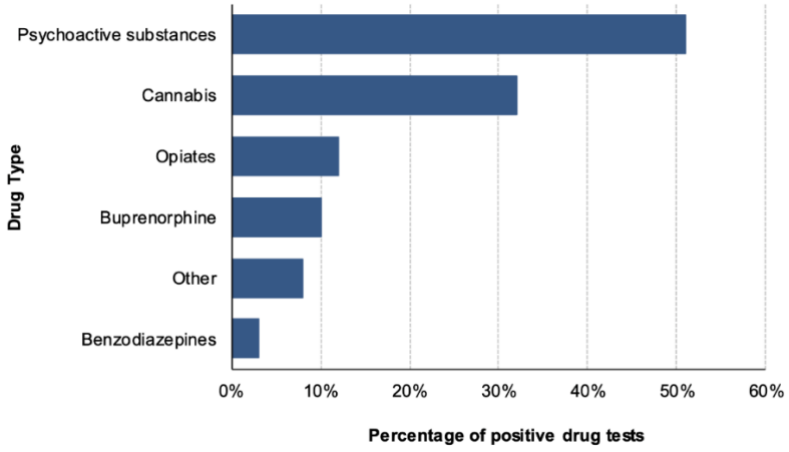

Figure 24: Positive drug tests by drug type (including PS), the 12 months ending March 2019 (Ministry of Justice, 2019) (For copyright see Appendix 5)

Psychoactive substances were the drug type accounting for the greatest number of incidents where drugs were found in the 12 months to March 2019 (Figure 25). Psychoactive substances, as stated in the Psychoactive Substances Act 2016, were detected in 6,699 cases in 2019, more cases than any other drug type in this time interval. There was an increase in the finds of all drug types in 2018 when compared with 2019. The largest increase was in Class A drugs, which rose from 408 finds in 2018 to 1,755 finds in 2019 (Ministry of Justice, 2019).

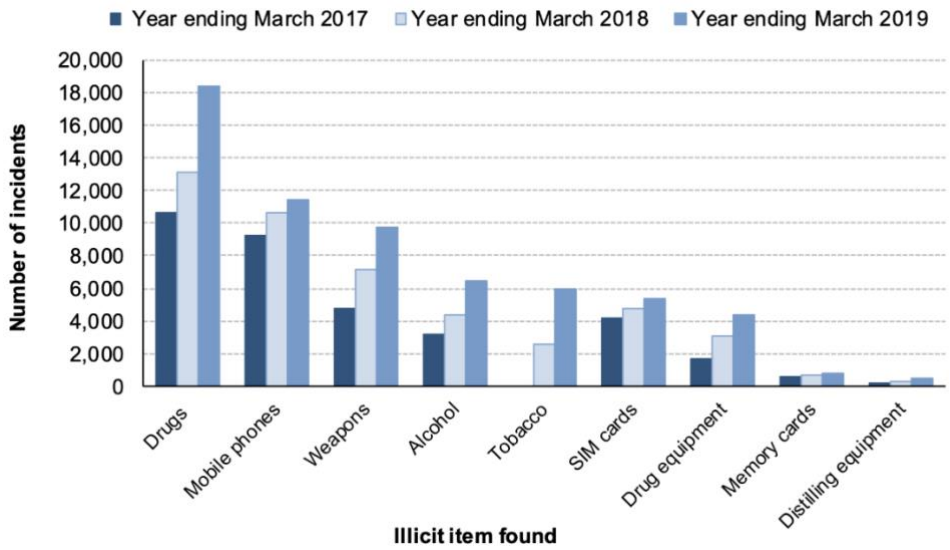

Figure 25:Number of incidents where illicit items were found in prisons, the 12 months ending March 2017 to the 12 months ending March 2019 (Ministry of Justice, 2019) (For copyright see Appendix 5)

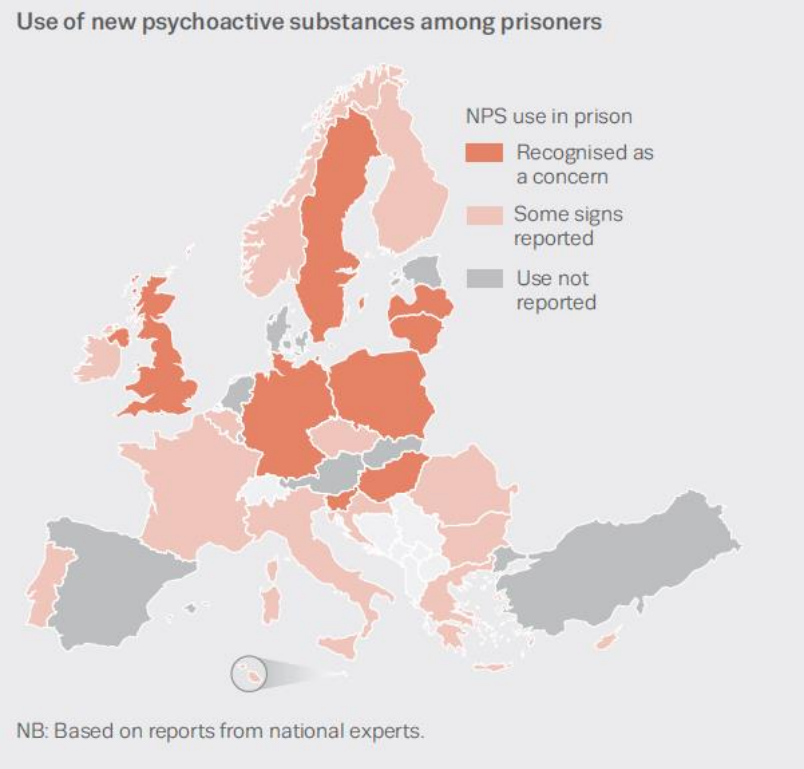

Figure 26: Use of NPS among prisoners (EMCDDA, 2019) (For copyright see Appendix 5)

A recent EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) review identified NPS use in prisons in 22 countries (Figure 26), with SCRAs identified as posing the main challenge.

NPS were linked to a wide range of physical and mental health harms, whether through acute intoxication or chronic consumption (EMCDDA, 2019).

2.5.2 Types of NPS used in prison

Recently, there has been an increase in the variety of NPS used in European prisons. However, it continues to be uncertain how much of this is associated with developed control works in prisons. NPS use in prison should be considered within the scope of broader polydrug use, which may include alcohol, traditional drugs, and prescription medication misuse in prison, with accessibility in this situation as one of the significant factors to use. Accessibility and cost, rather than the special preferences of the user, have an effect on choosing particular drugs in prison. For example, in Poland, it is reported that there is an alteration from the use of traditional drugs in prison to the use of NPS patterns that remains poorly understood and merits further formal research investigation (EMCDDA, 2018b).

NPS includes numerous different kinds of drugs, with completely dissimilar properties. The four most important types which are reported in the prison environment are synthetic cannabinoids, synthetic cathinones, new benzodiazepines, and new synthetic opioids. For 16 countries, data on the various types of NPS used in prisons are presented in Table 2 (EMCDDA, 2018b).

	Synthetic cannabinoids	Synthetic cathinones	New synthetic opioids	New benzodiazepines
Finland	•	•	•	•
Latvia	•	•	•	•
Poland	•	•	•	•
Sweden	•	•	•	–
Czech Republic	•	•	•	–
Italy	•	–	•	•
Cyprus	•	•	–	–
Germany	•	•	–	–
France	•	•	–	–
Hungary	•	•	–	–
Lithuania	•	•	–	–
Croatia	•	–	–	–
Ireland	•	–	–	–
Norway	•	–	–	–
Slovenia	•	–	–	–
United Kingdom	•	–	–	–
Total	16	10	6	4

Table 2: Groups of NPS identified by experts as being used in prison (EMCDDA, 2018b) (For copyright see Appendix 5)

2.5.2.1 Synthetic Cannabinoid Receptor Agonists (SCRAs)

There is accumulating evidence that the use of SCRAs has become a serious issue in prisons in the United Kingdom, where the problem, including violence and non-fatal and fatal intoxications, is relatively well documented. Moreover, in the United Kingdom, drug-related and other issues within prisons (such as assaults and suicides) have reached record highs and a significant part of the disruptive behaviour has been associated with the problems caused by t he use of NPS, specifically SCRAs (EMCDDA, 2017b). In the EMCDDA study, it was found that SCRAs are the most prevalent group of NPS used in prisons. Data on the type of drugs used was not provided by all countries. It is reported that SCRAs are the type of NPS identified to be used in prison by the 16 countries that provided information on this issue (Table 2) (EMCDDA, 2018b).

They were commonly sold as "lawful" alternatives to cannabis, although they have different effects than cannabis. Normally, SCRAs are traded as "herbal smoking mixtures". Sellers of SCs might also provide them as powders and tablets, substances that resemble cannabis resin and e-liquids for consumption in electronic cigarettes (EMCDDA, 2017a). SCRAs have usually been classified under the street names 'Spice', 'K2', 'Black Mamba', etc. They have been the greatest class of novel substances controlled by the EMCDDA and are becoming more and more different, with 179 identified since 2008 and 10 reported in 2017 (EMCDDA, 2018a). SCs are very different in chemical aspects, and due to difficulties in analytical detection, they may be undetected or under-reported (EMCDDA, 2018b).

2.5.2.2 Synthetic Cathinones

Synthetic cathinones are the second most prevalent type of NPS in prison, with 10 countries giving information on their use in prison settings; less countries stated new synthetic opioids (6 countries) and new benzodiazepines (4 countries) being consumed in prison (Table 2) (EMCDDA, 2018b).

Synthetic cathinones are the next prevalent class of novel substances monitored by the EMCDDA, with 130 identified overall, 14 identified for the first time in 2016, and 12 reported in 2017 (EMCDDA, 2018a).

2.5.2.3 New synthetic opioids

Synthetic opioids are a wide group of drugs that have an effect on the opioid receptors, and they involve prescription painkillers and sedatives. The use of these substances has consequences such

as respiratory depression, sedation, euphoria, hypothermia, tiredness and miosis, which is too much contraction of the pupil of the eye. A total of 38 novel synthetic opioids have been identified in Europe's drug market since 2009, with 13 reported for the first time in 2017. This involves 28 fentanyl derivatives, 8 of which were reported for the first time in 2016 and 10 in 2017. The new fentanyl products are very effective substances that cause a significant risk to individual and public health (EMCDDA, 2018a).

The consumption of new synthetic opioids in prison has resulted in some negative health problems. It is reported in Latvia that the rising consumption of new synthetic opioids in prison has caused more overdoses and a rise in injecting, with needle-sharing, in prison. Using the same injecting apparatus raised risks which involved the contraction of blood-borne infections, for example, human immunodeficiency virus and the hepatitis C virus (EMCDDA, 2018b).

2.5.2.4 New benzodiazepines

They have a chemical relationship with prescription benzodiazepines. Some 23 new benzodiazepines are being controlled by the EMCDDA and 3 of them were identified for the first time in Europe in 2017. Some of them are provided as tablets, capsules, or powders under their specific names. In other circumstances, forgers use these substances to generate fake varieties of frequently prescribed anti-anxiety medications, like diazepam and alprazolam, which have direct sales on the illegal drug market. More than 0.5 million tablets were seized between 2015 and 2016, including new benzodiazepines such as diclazepam, etizolam, flubromazolam, flunitrazolam, and fonazepam, representing a two-thirds increase over the previous year (EMCDDA, 2018a).

2.5.3 Consequences of taking psychoactive substances in prison

2.5.3.1 Physical and Mental Health Problems

Users in prison have a desire for some positive effects such as sedation, relaxation, euphoria, and altered perception. They are using it with the purpose of 'taking a holiday' or feeling of 'escape' from prison life. The most commonly reported negative effects on health are cardiac, psychiatric, and neurological. Frequently reported instantaneous effects are anxiety, cardiovascular problems such as chest pain, racing heart and collapse, psychosis, hypertension and seizures (HMPPS, 2019a).

In the EMCDDA study, it is reported that there are a large number of physical and mental health problems related to acute intoxication and chronic use of synthetic cannabinoids in prison (EMCDDA, 2018b). Paranoia, psychosis, aggression and violence against others, self-harm, and suicide are examples of serious mental health harms of drug use. A trend of synthetic cannabinoid-related toxicity has also been observed, with first generation compounds primarily showing cannabis-like undesirable effects, second generation compounds showing cardiovascular/stimulant toxicity, and third generation compounds showing neurological toxicity associated with CNS depression (Shafi et al., 2020).

Regular consumption of NPS can cause both psychological and physical addiction (HMPPS, 2019b). Classic signs are severe craving, rapid tolerance due to enhancing the dose every day to acquire the desired effects, and withdrawal indicators when consumption suddenly discontinues. In addition, in the chronic users of SCRAs, there have been dependence and withdrawal symptoms (Users Voice, 2016). In 7 countries (Germany, Finland, Italy, Latvia, Lithuania, Poland, and the United Kingdom), non-fatal overdoses associated with NPS, mainly SCRAs, have been reported. Moreover, it is stated that the consumption of synthetic cannabinoids can have long-lasting adverse effects, and the custody and healthcare teams may have to deal with the effects for months after consumption (PHE, 2017).

In recent years, there has been an increase in concerns from personnel working in UK prisons about secondary exposure to psychoactive drug fumes, which are commonly considered as synthetic cannabinoids. There have been reports (both in the media and from prison officials) of staff related illnesses ranging from minor symptoms such as headaches and disorientation to more severe symptoms caused by secondary exposure to drug fumes while working in prisons. The compounds of concern are frequently referred to as NPS (Paul et al., 2021).

2.5.3.2 Impact on prison management and the prison environment

There is an increase in the obtainability and consumption of NPS in prison, and this situation disturbingly affects the prison staff, with security matters and a breakdown of the prison establishment (EMCDDA, 2018b). NPS use in prisons results in a rise in the extent of violence in many countries (Germany, Finland, Poland, Sweden, and the United Kingdom) and a rise in bullying and aggression. The average price of NPS in prisons can be up to 10 times the street cost. Many of them have an intense effect in the beginning. A person who uses NPS regularly can develop a tolerance and it can cause their habits to increase and they can go into debt with drug

suppliers (HMPPS, 2019b). Drug abuse contributes to a cycle of disruption and violence, resulting in a decreased or unstable regime, which might encourage inmates to turn to drugs and alcohol due to unpredictability and a lack of purpose. The debt incurred as a result of drug supply, distribution, and usage is also a substantial source of violence, intimidation, and self-harm throughout the estate, harming both staff and other inmates (HMPPS, 2019a).

In Poland and the UK, it is reported that the rise in the number of emergency calls associated with the use of synthetic cannabinoids in prisons can directly influence prison routines (EMCDDA, 2018b). For each prisoner who is taken to a hospital, one or more people from prison staff has to leave the prison building, which has a direct influence on the arrangement of other activities in the prison, like education, sports, or work activities. When they have less opportunity to participate in important activities, it may cause boredom among prisoners; it has been regarded as one of the major factors contributing to substance use in prison. Moreover, due to an escalated amount of emergency calls, health experts who are employed in the prison setting might have less time to manage the routine prison healthcare. These problems may directly affect whole prisons and the welfare of their prison population (EMCDDA, 2018b).

A disturbing side of SCRA use has been revealed, with inmates being spiked with high-dose SCRA to entertain other prisoners. Prisoners were given SCRA free of charge but were asked to use large amounts for the fun of other prisoners rather than observing the drug's effects for resale within the prison (ACMD, 2020).

2.5.4 Drug supply into prisons

External visits; postage; prison employees; over prison walls; individuals coming to or leaving prison; and new methods are the main ways for smuggling illicit drugs into prison, many of which are identical to those used to smuggle other items (Blakey, 2008).

The ‘throw-over’ method: Drugs can be thrown over prison walls, however this method is heavily dependent on the architecture and location of the prison. Drugs may be concealed in a variety of materials when thrown over the walls, including within tennis balls, dead animals such as birds or rats, or other objects (The Economist, 2013).

Drones: Over the last decade, technological advancements have influenced how drugs are smuggled into prisons. Drones, for example, have been used to transport goods into prison

grounds rather than directly throwing drugs over prison walls. Different methods may be utilised concurrently and in combination to avoid detection and keep supply (EMCDDA, 2022b).

External visits: According to research, visits can be used to bring illicit drugs into prison where they can be used, sold, or swapped for other goods and services. Drugs are wrapped in small packages and hidden internally, in clothing, or in other items in some situations; the packages are delivered to the prisoner either mouth to mouth or hidden in objects (e.g., food and drink). To prevent detection during a post-visit search, the inmate will have to conceal the package internally (Tompkins, 2016, Penfold, 2005). Moreover, some external subcontractors in prison, such as cleaning companies, waste disposal trucks, and canteen distributors, have been identified as enablers for the supply of NPS in prison, and they have been reported by countries as potential sources of supply. Another common supply route reported is the distribution through the prison cafeteria: pre-packed food packages, like coffee, noodles, or crackers, can be utilised for hiding NPS (EMCDDA, 2018b).

NPS impregnated post parcels or letters: Another way of bringing NPSs into prison takes advantage of the fact that they are obtainable in a liquid form. SCRAs and synthetic opioids can be dissolved in a solvent, such as acetone, and can be soaked onto paper and tobacco by spraying or soaked into fabrics (Ford and Berg, 2018). NPS impregnated post parcels or letters by spraying the paper were sent to prisons and it has been defined as a way of getting NPS into prisons in many countries (Finland, Germany, Hungary, Lithuania, Poland, Sweden, and the United Kingdom). They can then be cut into small pieces and smoked. Different forms of paper materials have been stated to be used, such as children's drawings and fake legal correspondence (UK Focal Point, 2018). NPS in this form has some health hazards due to the potential presence of "hot-spots" - parts on the paper with a high concentration of the active compound and which may be associated with a high risk of overdose. Moreover, according to anecdotal data from the United Kingdom, there is a potential rise in the use of liquid NPS in vaping pens, which might be a possible adjustment to the recent implementation of the smoking ban in UK prisons (EMCDDA, 2018b).

People returning to prison: Many people enter prison or re-enter after court trials or periods of release, resulting in a continuous circulation of inmates. People may hide drugs internally before entering prison, making them difficult to detect. Drug-addicted prisoners who want to have a supply of drugs for their first days in prison, either to deal with withdrawal symptoms or to sell

for other products, regularly engage in this practise. Non-users may also bring drugs into prison in order to make money (Penfold, 2005, Tompkins, 2016).

2.5.5 Security categories of prisons

In England and Wales, the prison estate is divided into several categories based on the level of security provided by each facility. The purpose of categorisation is to evaluate the risks posed by a prisoner in terms of (Ministry of Justice, 2016): the likelihood of escape; the risk of harm to society if an escape happens; and control issues affecting the prison's security and stability, as well as the safety of those inside.

Following these evaluations, the prisoner is placed in the lowest security category possible to mitigate the potential consequences. England and Wales have different security categories in their prison systems, ranging from category A (highest security) down to category D (lowest security). In male prisons, there are four prison categories:

Category A	These are high-security prisons. They hold male prisoners who, if they escaped, would pose the greatest risk to the community, the police, or national security.
Category B	These are either local or training prisons. Local prisons house inmates who have been sentenced or are on remand from a local court, while training prisons accommodate long-term and high-security prisoners.
Category C	These are training and resettlement prisons, with the majority of inmates housed in category C prisons. They give prisoners the opportunity to build their own talents so that when they are released they can find work and reintegrate into the community.
Category D	These prisons provide minimal security and allow qualifying offenders to spend the majority of their day outside the prison on licence to work, study, or for other resettlement objectives. Only prisoners who have been risk-assessed and considered appropriate for open settings are housed in open prisons.

Table 3: The four security categories of UK male prisons (Ministry of Justice, 2022)

Female inmates can be housed at one of four levels of security:

Category A	Prisoners for whose escape would pose a significant risk to the public, the police, or the state's security, and the goal must be to prevent escape.
Restricted Status	Any female, young person, or young adult prisoner imprisoned or on remand whose escape would pose a major risk to the public and who needs to be housed in designated secure accommodation.
Closed Conditions	Prisoners for whom the highest levels of security are not required but who pose too big a threat for open confinement or for whom open confinement is inappropriate.
Open Conditions	Prisoners who pose a low risk can be fairly trusted in open conditions and are appropriate for open settings.

Table 4: The four security categories of UK female prisons (Ministry of Justice, 2016)

3. DEVELOPMENT OF DATABASE AND SPECTRAL LIBRARY

3.1 Introduction

The existence of NPS in the illicit drug market, and thus in toxicological cases, is not a new phenomenon. Besides this, as NPS get more complex, analytical approaches for detecting and identifying them must be improved to keep step with new ones (Favretto et al., 2013). Illicit producers modify novel psychoactive chemicals in order to avoid legislation that restricts their public use. A simple action such as removing, adding, or relocating a functional group in the chemical compound can prevent a drug from being under legal control. When these modifications are applied, the structures of the emerging substances no longer represent those of illicit substances, putting them outside the scope of controlled substance regulations (Grabenauer et al., 2012). Methods for screening by GC-MS and LC-MS have been generated, but both typically involve the use of libraries with distinctive mass spectra (Broecker et al., 2011). There are various advantages to employing ESI over EI, such as the ability to maintain an unbroken molecular ion, which results in improved confidence in identification since ESI ionisation is a "softer" or less powerful method than EI. ESI systems are also not restricted to using just volatile, thermally stable compounds as EI sources in GC. Historically, the building of ESI-based libraries for LC techniques has involved the use of triple quadrupole mass spectrometry, although these instruments are known to have low resolution, making identification of some NPS difficult, especially those with very highly similar accurate masses (Stein, 2012, Meyer and Maurer, 2016).

The current work includes the development of a compound database for 229 different chemical substances thought to be potential NPS, metabolites, and related substances.

3.2 Materials and Methods

3.2.1 Chemicals and Standards

Drug standards were obtained from Analytical Services International Ltd. and TICTAC Communications Ltd. and were provided as solids or as standards in solution (Figure 27). HPLC Grade Methanol (RATHBURN, Scotland, UK) and Optima™ LC/MS Grade Water (Fisher Chemical, Loughborough, UK) were used to prepare 1 mg/mL stock solutions from the neat solid standards. Solvents used for liquid chromatography included methanol (HPLC grade) from Rathburn and water (Optima™ LC/MS Grade) from ThermoFisher Scientific. Liquid chromatography additives

used included formic acid (Optima LC/MS) and ammonium formate (99%) from ThermoFisher Scientific.

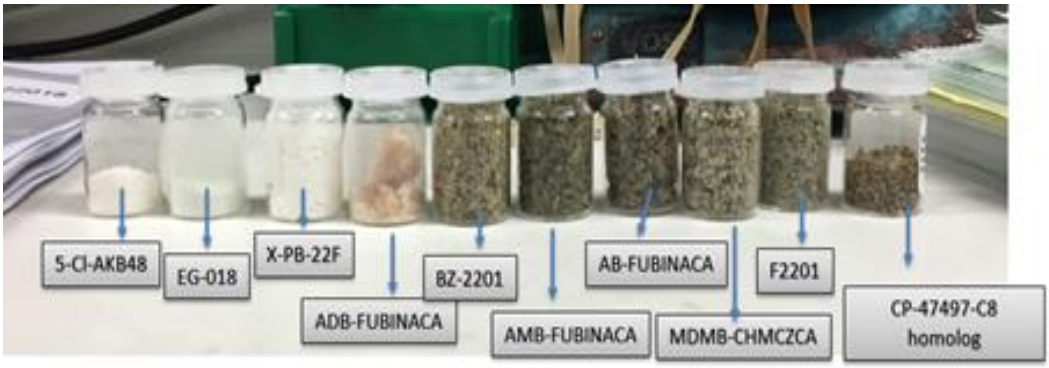

Figure 27: Examples of drug standards from TICTAC Communications Ltd.

The drugs used to build the database were listed, and the information about which drugs were provided by which company was given in Appendix 4. It was critical to include any compounds that might emerge on the street market. Chemical compounds that could be used as potential NPS entities in this study were found in a variety of publicly available sources. Peer-reviewed literature research articles and reviews, government publications, commercial standard supplier documentation, and websites including online drug forums were all very helpful in selecting compounds for inclusion in the database.

3.2.2 Sample Preparation

Reference solutions were prepared from the reference standards of internal standards at concentrations of 10 mg/L in MeOH. Working solutions were then prepared from the reference solutions at a concentration of 1 mg/L in MeOH.

3.2.3 Instrumentation and Software

Analytical instrumentation included an Agilent 1290 Infinity II HPLC system coupled to an Agilent 6545 Accurate-Mass QTOF MS (Agilent Technologies, Santa 41 Clara, USA) (Figure 28). The QTOF-MS was operated in positive-ion electrospray mode with a Jet Stream ESI Technology. Agilent MassHunter LC/MS Acquisition software for the 6200 series TOF/6500 series QTOF (Version B.08.00, Build 8058.0) and MassHunter Qualitative Analysis software (Version B.08.00) were used to acquire and process the data. MassHunter Personal Computer Database Library (PCDL) Manager software (Version B.08.00, Build 8209.0) was used to create the high-resolution MS/MS

spectral library and the compound database. ChemSketch (ACD/ChemSketch Freeware 2018.1) was used to create and import the chemical structure of each drug into the PCDL.

Figure 28: Agilent Technologies 1290 Infinity II – 6545 Q-TOF LC/MS instrument with electrospray ionization in positive ion mode

3.2.3 Method

Collection of spectral data for the MS/MS spectral library was done. Diluted standards (1 mg/L) were individually injected directly into an Agilent 6545 series Quadrupole Time-of-Flight (QTOF) mass spectrometer with Jet Stream ESI ion source coupled to an Agilent 1290 Infinity Series Binary Pump system.

Electrospray ionization analysis was performed for the acquisition of MS/MS data for all standards. The injection volume of the ESI method is 2 µL with direct injection from the auto sampler into the ion source. The mobile phases consisted of 5 mmol/L ammonium formate and 0.01% (v/v) formic acid in water (A) and in methanol (B), pumped at 0.400 mL/min. A positive mode ESI targeted MS/MS method was used to collect the data. The quadrupole used a medium isolation mass window of 4 amu. The source parameters were: drying gas temperature of 250˚C; drying gas flow of 12 L/min; nebulizer pressure of 35 psi; sheath gas temperature of 350˚C; sheath gas flow of 12 L/min. Scan source parameters used included: VCap voltage 3500 V; nozzle voltage 300 V; fragmentor 150 V. For SCRAs, three separate collision cell energy levels were used in the

collision induced dissociation (CID): 10, 20, and 40 eV. For other drugs, one collision cell energy level was used in the CID; 30eV. The MS range was 40-1,000 m/z. The MS acquisition rate was 3 spectra/s. The MS/MS range was set to 40-600 m/z with an MS/MS acquisition rate of 2 spectra per second. Two reference ions, provided by the reference mass solution, were monitored with mass correction to ensure proper instrumental calibration throughout analysis; 121.0509 m/z and 922.0098 m/z.

3.2.3 Development of the comprehensive compound database

Chemical compounds to be counted as potential NPS substances were determined from several public sources, such as peer-reviewed literature research papers and reviews, government reports, commercial standard provider documents, and websites such as online drug forums. When a potential NPS was detected, chemical and structural data were confirmed by searching many supporting sources for each drug. Once it was confirmed, the structure of each drug was logged using the ChemSketch software. Then, the chemical structure of each drug was introduced into the database as a .mol file with the help of the MassHunter PCDL Manager software. A chemical structure search was implemented on SciFinder in order to get the CAS Registry Number and IUPAC name. Another search was performed on ChemSpider to get a ChemSpider number. For each drug in the database, data including compound name, chemical formula, monoisotopic mass, chemical structure, and IUPAC name were recorded to the database, as well as CAS registry number and ChemSpider number if found.

3.2.4 Development of the spectral library

To add the spectral data to the MS and MS/MS spectral libraries, a compound needs to have a base peak of at least 10^3 counts. If the NPS standard accomplished this condition, then the drug data and fragment ion spectrum from each collision energy were transferred to a compound exchange format (.cef) file and then introduced into the PCDL using PCDL Manager Software.

The “Find by Formula” function of the Qualitative Analysis software was used to isolate a targeted compound from the chromatogram. The MS/MS spectra were then extracted and exported into the PCDL using the “Send Spectra to PCDL” function.

3.3 Results and Discussion

3.3.1 Compound database

To add all possible NPSs to the full compound database, searches were done in the literature and on the web. Firstly, government documents were checked to find substances of potential concern. With literature reviews, important data can be found and, from further reports, the existence of designer drugs in real cases can be described. Product searches from chemical manufacturers were also helpful for getting information about compounds. Online drug forums were also reviewed to generate information about new NPS compounds being discussed by drug users. Though such sources are not evaluated by experts and their accuracy may be questionable, any drugs that may be evolving on the street market should be added to the database.

A total of 229 distinctive structural drugs which were believed to be possible NPSs were found and included in the compound database. Most of them are classified under the cannabinoid, cathinone, tryptamine, piperazine, and phenethylamine structural groups. Compounds from different classes were recorded. For each compound, street or common name, chemical formula, monoisotopic mass, chemical structure, and IUPAC name were included in the database. Supplementary data (CAS numbers and ChemSpider numbers) was also recorded if obtainable. A list of all compounds to build the database was provided in Appendix 4. Figure 29 shows an illustrative screen shot of the compound database created by the software (MassHunter PCDL Manager). Other than the data mentioned above, the record for each compound also shows whether MS spectral data was obtained, that is, “Num Spectra” column. The creation of the compound database is a continuing and active work; since new drugs are produced and described for avoiding current law, they are added to the resource.

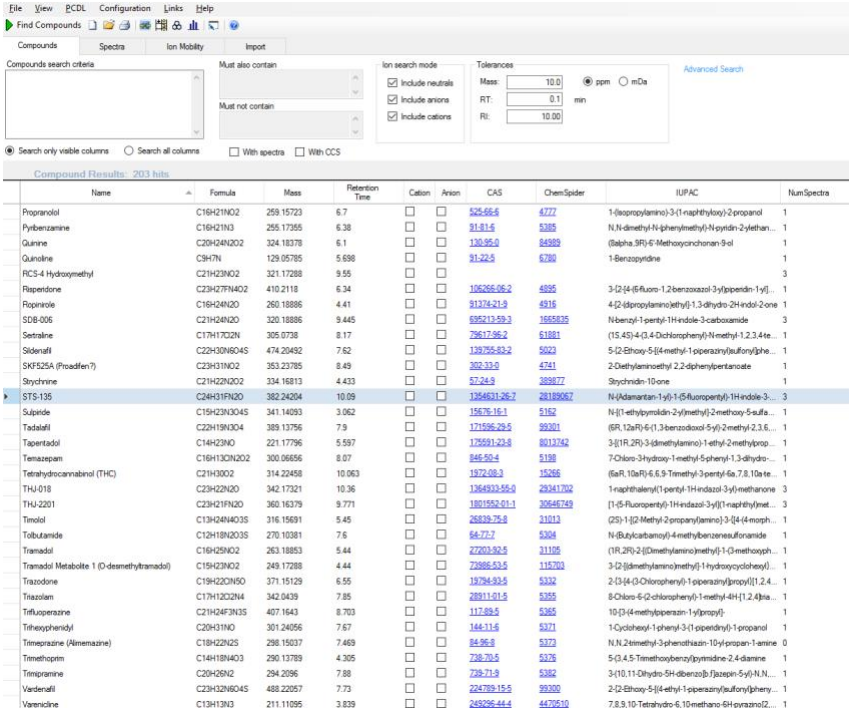

Figure 29: An image of the PCDL software that was used to create the database and the MS/MS spectral library

3.3.2 Development of the high-resolution MS/MS spectral library

229 drug standards that were chosen from the substances in the compound database were obtained for the generation of a high-resolution MS/MS spectral library. With this aim, each drug standard was firstly analysed via ESI by QTOF-LC/MS. A targeted method for each standard was generated. Source and acquisition components were selected in order that it could be compared to formerly generated libraries utilising the same instrument. Three set collision cell energies (10, 20, and 40 eV) were used to boost the production of fragments of the target ions. In order to be eligible for addition to the MS/MS library, each designer drug standard had to get a base peak of at least 1000 counts, a necessity which confirmed that background noise was at minimum in the MS/MS spectral data.

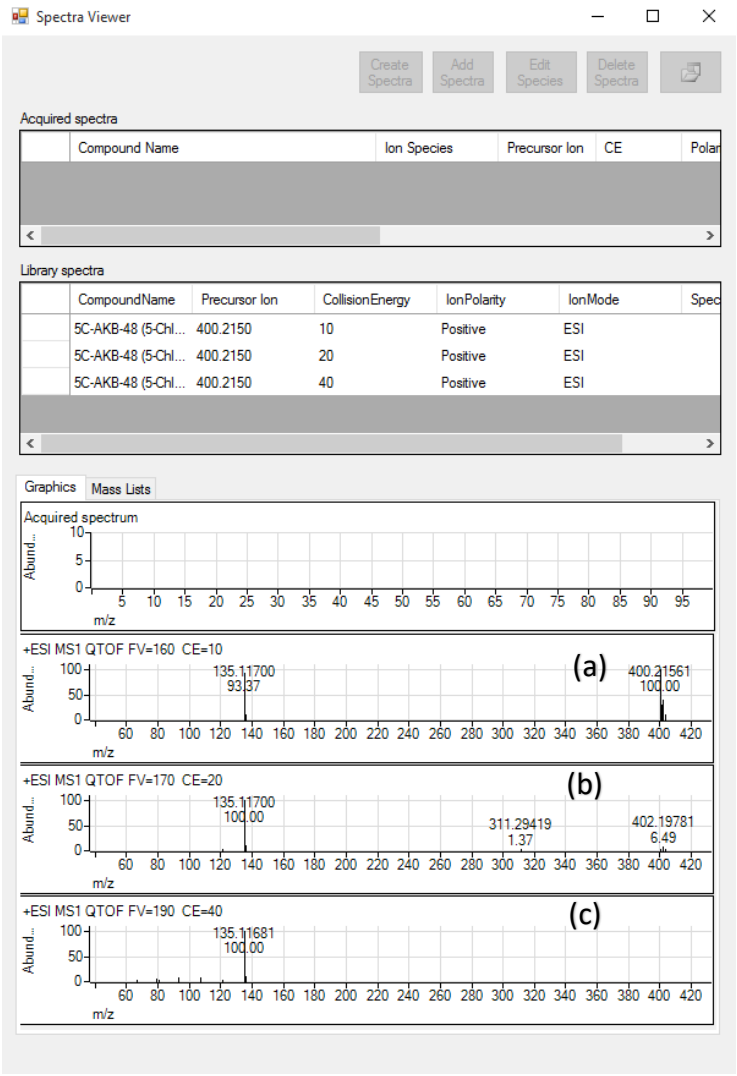

Figure 30: Pictures of the PDCL software displaying the MS/MS spectral data of 5C-AKB-48. MS/MS spectral data is presented at distinct collision energy levels: a) 10eV, b) 20 eV, and c) 40 eV.

In addition, all the information on relative ion abundances of each ion at each of the three collision energies for all standards is shown in the spectra viewer above the m/z values on the spectra for each ion. (Figure 30). Retention time information is achieved by extracting the chromatogram at the m/z value of the drug standard (Figure 31).

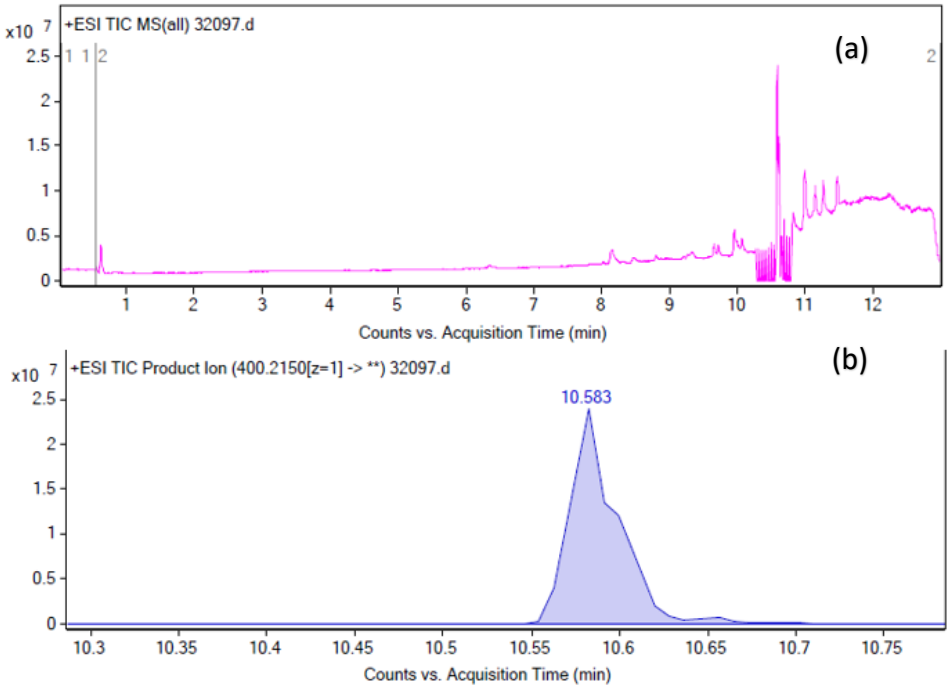

Figure 31: Total ion chromatogram of 5C-AKB-48 (a) and extracted ion chromatogram for 5C-AKB48 standard (b)

Moreover, with the molecular structure viewer on the database software, chemical structure information can be seen (Figure 32).

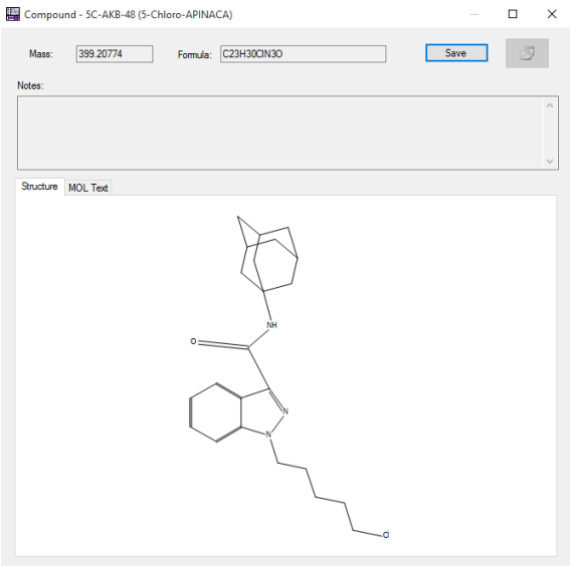

Figure 32: Image of molecular structure viewer for 5C-AKB-48 from the molecular structure viewer

4. LC-QTOF-MS METHOD

4.1 Introduction

In this research, it is reported that the development of a qualitative method in order to detect and confirm the presence of NPSs on impregnated papers coming from prisons using liquid chromatography quadrupole time of flight mass spectrometry. The method was used for the analysis of paper samples suspected to be impregnated with NPSs and related compounds seized from twelve English prisons between 2018 and 2020. The aim of this study was to show the usefulness of analysis of such samples from prisons for monitoring, to investigate the influence of legislative amendments in drug producers on English prison illicit drug markets, to improve rapid detection, to determine prevalence, and finally to lower the amount of supply and harm as a consequence of drug use in prisons.

4.2 Materials and Methods

4.2.1 Chemicals

Solvents used for liquid chromatography and for the preparation of samples included methanol (HPLC grade) from Rathburn and water (Optima™ LC/MS Grade) from ThermoFisher Scientific. Liquid chromatography additives used included formic acid (Optima LC/MS) and ammonium formate (99%) from ThermoFisher Scientific.

4.2.2 Preparation of Samples

The letters with envelopes were hand delivered, sealed in individual evidence bags, to TICTAC. From each piece of paper or envelope, approximately 1 cm square of paper was cut from different locations and placed into separate 1.5 mL Eppendorf tubes with 1 mL of 50% (v/v) methanol in LC-MS-grade water. In between the preparation of each letter sample, gloves were changed and the scissors and work surface were cleaned down with 50% (v/v) methanol in LC-MS-grade water. Extracts were prepared from the letters by vortex-mixing (30 min). A portion of the supernatant (300 µL) was then transferred to a new vial for direct analysis. The injection volume was 0.2 µL. A mobile phase blank was injected between the analysis of each extract to check for any carryover. Sample preparation is displayed in Figure 33.

Due to their ability to dissolve a wide range of NPS, methanol or ethanol are usually used as solvents. Substance can be extracted from the paper sample and dissolved in methanol solution

after 30 minutes of vortex mixing. A portion of the supernatant of the extracted solution can be used for direct analysis.

For paper samples, 30 minutes was the optimum extraction time in methanol/water. When they were vortex-mixed for 15 minutes, the peaks in the chromatogram were poor (the peak areas were very small). Longer mixing times may be required to transfer all of the compounds in the impregnated paper sample into the solvent due to low active content of the paper. The drugs were dissolved in a solvent and then impregnated into these papers. If mixing time was optimized, the dissolved amount in the solvent would be higher and it would give a better peak on the chromatogram.

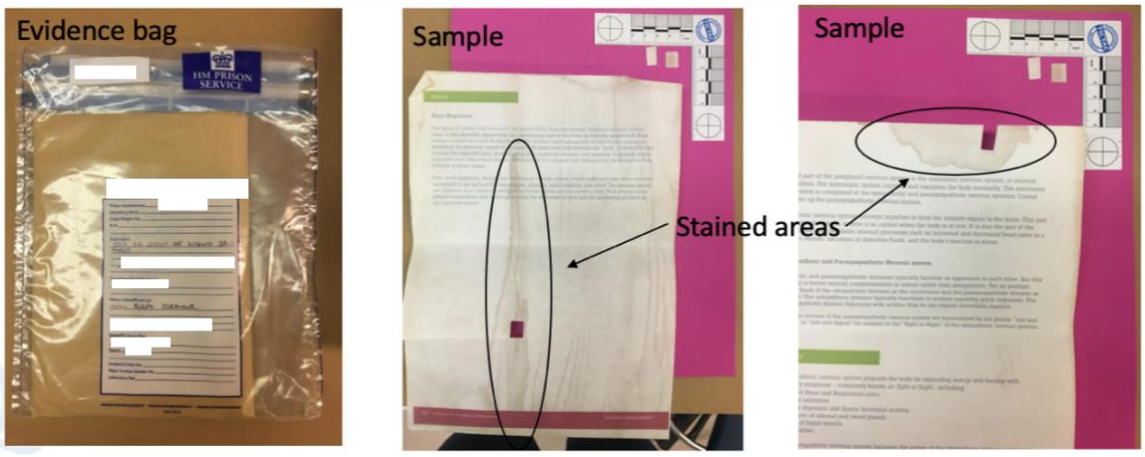

Figure 33: Example of sample preparation

4.2.4 Analysis of collected samples

For this study, 356 drug-impregnated paper seizures from 5 prisons in 2018, 332 drug-impregnated paper seizures from 7 prisons in 2019 and 562 drug-impregnated paper seizures from 5 prisons in 2020 were collected. Individual paper samples from these seizures were prepared for the analysis. The samples used in this research were non-judicial samples seized by the HM Prison Service. Some NPSs can be soaked in active doses into herbal material, paper, clothing, and other items before being smoked or vaped (EMCDDA, 2018b). In this study, there were envelopes, letters, cards, photographs, rule 39 letters*, books, newspapers, tissue paper, rolling papers, bills, stamps, cheques, puzzle books, playing cards etc. Some samples were seized from prisoners directly (intercepted during a pass between prisoners, found in the prisoner's hand or given to the prisoner by a visitor, etc.) or were found wrapped in tissue hidden at the bottom of the bin or thrown in socks from a spur. Immediately following the seizure, samples were packed

in tamperproof polythene evidence bags and carefully kept. Figure 34 displays the variety of drug-impregnated samples from prisons.

* Prison Rule 39 states that a prisoner's correspondence with the courts and their legal counsel may only be opened, stopped, or read under certain conditions. Rule 39 applies to both correspondence sent to and received by the prisoner. This type of correspondence is commonly referred to as a "Rule 39 letter"(Prisons&Probation Ombudsman, 2015).

Figure 34: Examples of seized items submitted for analysis.(a) sample M18098097: birthday card; (b) sample S0185578: pieces of papers with e-cigarette capsules; (c) brown vape capsules with a piece of paper sample N08311067; (d) sample M18707480: burnt roll of paper; (e) sample N05240030: a letter with 15 pounds cheque; (f) sample L00364648: 1 stained card; (g) sample NM0015081: tissue with green papers inside; (h) sample M19177904; a puzzle page; (i) sample N04571159_A: A4 sheets (prisoner forms);(j) sample NS0029860: 1 letter with a stained envelope; (k) sample M0200171: Royal Mail 1st class stamps; (l) sample L0001828; book with stained pages.

There was no date information from the laboratory for the submission of the seizures to the laboratory. Therefore, we cannot say anything about the stability of drugs in impregnated paper samples when interpreting the findings.

In Figure 35, an example of a sample preparation is shown. From the stained areas, 1cm^2 pieces were cut and prepared for the analysis.

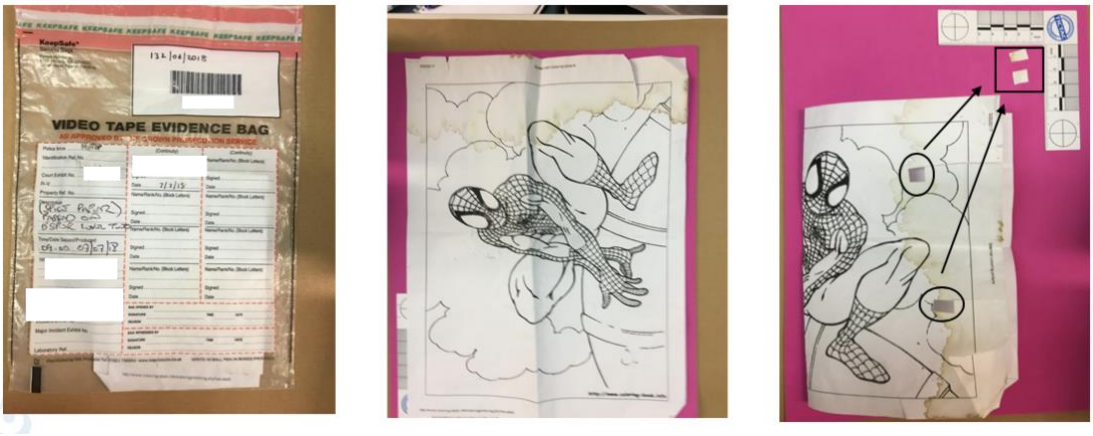

Figure 35: Example of a sample preparation

There were different types of seizures in this study. Some of them were a mix of different types, and some of them were just one type. Table 5 shows the number of seizure types received from the prisons:

seizure type	number of seizure type	seizure type	number of seizure type
letter	861	A4 sheets	7
envelope	15	picture	1
pieces of paper	142	cloth labels	1
notebook	2	address book	1
book	2	letter + photos	2
rule 39 letter	27	diary + letter + card	1
crossword puzzle	1	letter + photo + stamp	1
diary	2	tissue paper	3
photos	5	card + photos	3
drawing	8	card + stamp	1
card	66	letter + money	1
stamps	1	lined paper + tissue paper	1
letter + card	34	letter + envelope + ash	1
card + drawings	1	pieces of paper (burned)	7
letter + stamp	6	letter + puzzle page	1
letter + piece of envelope	4	rizla paper	1
letter + envelope	12	pieces of paper + magazine	1
pieces of drawings	1	Black paper in envelope	1
letter + drawings	2	letter + cheque	1
letter + piece of newspaper	1	piece of envelope	1

seizure type	number of seizure type	seizure type	number of seizure type
puzzle book	1	letter + pieces of paper	2
sudoku book	1	ash	1
pieces of purple rain paper	2	notebook + calendar + envelope	1
book pages	1	letter + bill	1
A4 notepad	1	game cards + letters	1
card + envelope	4	paper	4
Total number of seizures		1250	

Table 5: Number of different types of paper seizures collected from prisons between 2018-2020 in this study

After the sample was run with Q-TOF-LC/MS with the developed method in this study, the total ion chromatogram was processed with Agilent Mass Hunter Qualitative Analysis Navigator Software.

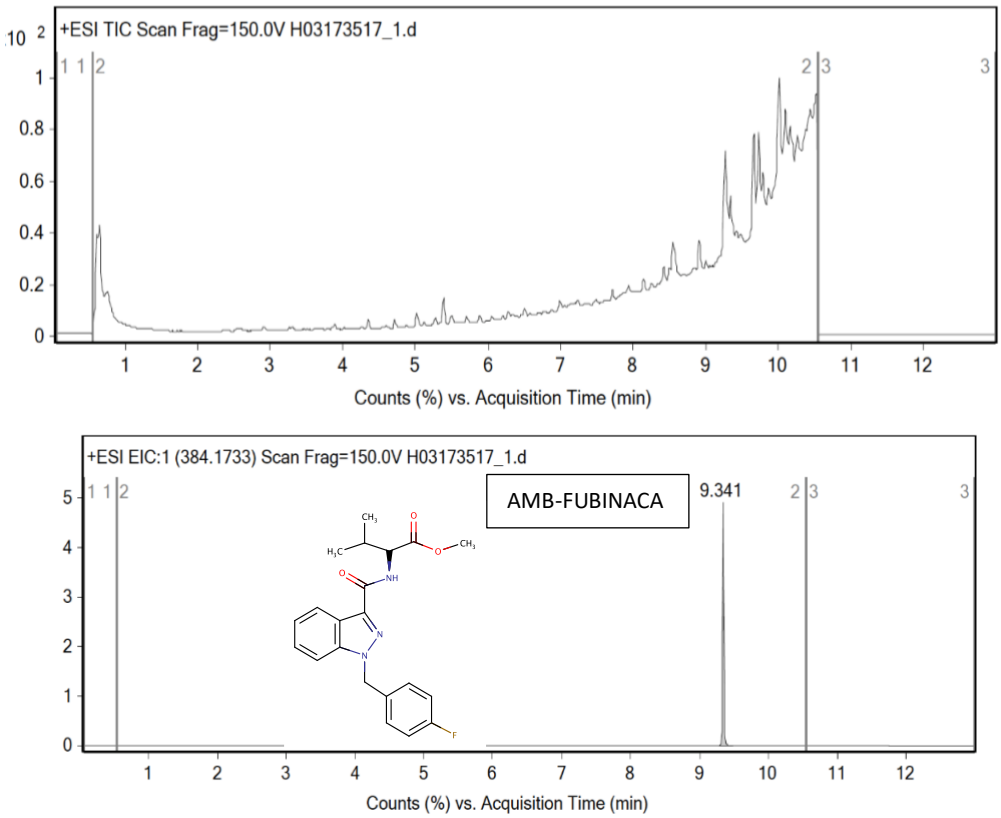

Figure 36: Total ion chromatogram for the sample (Psychoactive Substances Act 2016), extracted ion chromatogram for AMB-FUBINACA at 9.341 min

AMB-FUBINACA was detected in this sample. After extracting it from the total ion chromatogram, a single and clean peak was obtained at 9,341 min (Figure 36). When the fragment ion spectrum was checked, the fragment ion spectra were matched (Figure 37).

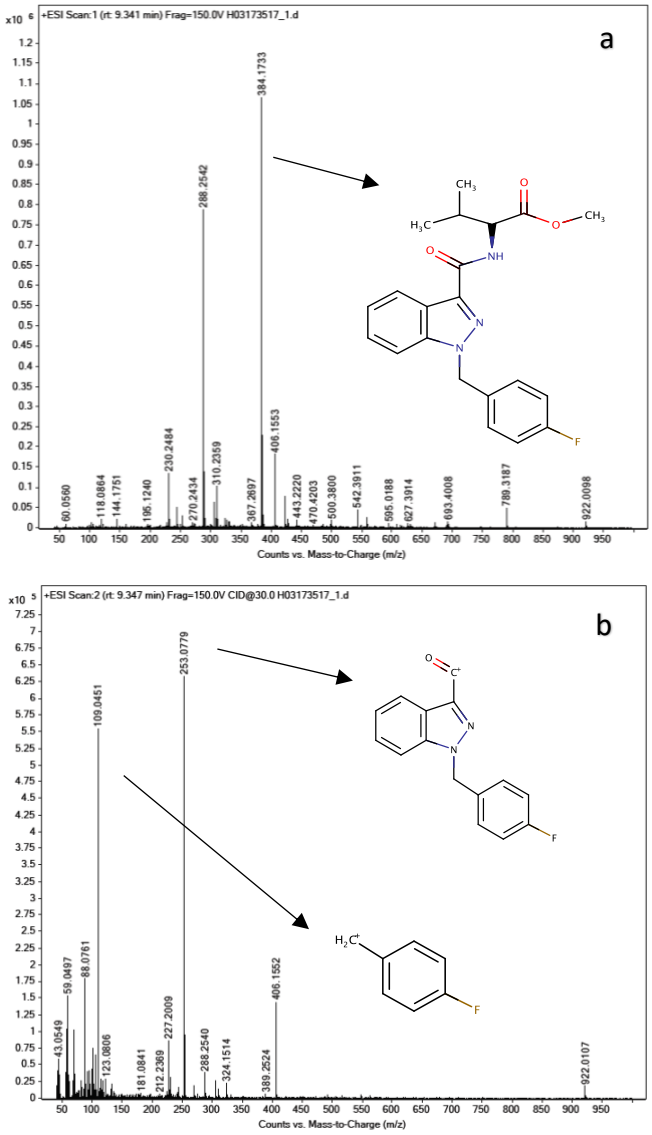

Figure 37: Mass spectrum at 9.341 min (a) and fragment ion spectrum at 30 eV collision energy (b)

4.2.3 Instrumentation and Software

Analytical instrumentation included an Agilent 1290 Infinity II HPLC system coupled to an Agilent 6545 Accurate-Mass QTOF MS (Agilent Technologies, Santa 41 Clara, USA). The QTOF-MS was operated in positive-ion electrospray mode with Jet Stream ESI Technology. Agilent MassHunter LC/MS Acquisition software for the 6200 series TOF/6500 series QTOF (Version B.08.00, Build 8058.0) and MassHunter Qualitative Analysis software (Version B.08.00) were used to acquire and process the data. MassHunter Personal Computer Database Library (PCDL) Manager software (Version B.08.00, Build 8209.0) was used to identify the drug in the paper sample.

Electrospray ionization analysis was performed for the acquisition of MS data of the paper samples. The injection volume of the ESI method is 0.2 µL with direct injection from the auto sampler into the ion source. The mobile phases consisted of 5 mmol/L ammonium formate and 0.01% (v/v) formic acid in water (A) and in methanol (B), pumped at 0.400 mL/min. A positive mode electrospray ionization (Stolker et al.) MS method was used to collect the data. The source parameters were: drying gas temperature of 250˚C; drying gas flow of 12 L/min; nebulizer pressure of 35 psi; sheath gas temperature of 350˚C; sheath gas flow of 12 L/min. Scan source parameters used included: VCap voltage 3500 V; nozzle voltage 300 V; fragmentor 150 V. One collision cell energy level was used in the CID: 30 eV. The MS range was 40-1,000 m/z. The MS acquisition rate was 3 spectra/s. The gradient profile was 5% B in 0–1 min and 12 min, and 100% B in 10–12 min. The column used was an Agilent Zorbax Eclipse Plus C18 Rapid Resolution HD (2.1×100 mm, 1.8 µm). The column was maintained at a temperature of 40˚C. Two reference ions, provided by the reference mass solution, were monitored with mass correction to ensure proper instrumental calibration throughout analysis; 121.0509 m/z and 922.0098 m/z.

The method used is the same as described earlier in section 3.2.3, except the injection volume is 0.2 µL here and there is no isolation mass window. Injection volume was reduced due to saturation on the chromatograms of some samples; that is, the concentrations of the drugs may be high enough that they can cause this saturation and can cause carryover. LC parts of the method have to be exactly the same because otherwise the retention times would not match. The important thing about the analysis of real samples is that the MS/MS done was untargeted, so a targeted method was used to build the database, then the method was run in an untargeted method. MS acquisition was untargeted and it was matched against the library generated before. When a library was being built, the Q with the Q-TOF was used to isolate the compound and MS/MS was done so a good library spectra was obtained.

4.3 Conclusion

The Q-TOF LC/MS method was used in conjunction with the compound database that was developed to qualitatively screen 1250 drug-impregnated paper seizures from English prisons between 2018 and 2020. Positive identification was achieved for the majority of the compounds present in the papers, showing potential for real-world applicability.

5. RESULTS

This study analysed 2499 individual seized paper samples originating from 1250 seizures from twelve English prisons, collected between 2018 and 2020. The prisons that provided samples ranged from category A, B, C and closed in security level. They were located across six regions in England and included 10 male and 2 female prisons. The ten male prisons were based in five regions across England, namely Yorkshire and the Humber (3), South West (2), South East (2), Eastern (2), and West Midlands (1). The two female prisons were based in two regions across England, namely Greater London (1) and South East (1). In order to analyse the data collected, the detected substances were grouped according to the type of active compounds identified. The groups used were as follows: nicotine/cotinine, abused medicines (non-prescription), prescription medicines, other medicines (consisting of drugs not abused and over-the-counter drugs), synthetic cannabinoid receptor agonists (SCRAs), miscellaneous active compounds (Misc.), Class A drugs, other Class B drugs (excluding cannabis, SCRAs, and prescription), Class C drugs, and no drugs detected. 'Miscellaneous active compounds' included any samples that did not fit into the above groups, such as caffeine. "No drugs detected" was used to indicate samples in which no active compounds were identified after the run on the Q-TOF LC/MS. Moreover, a metabolite of cocaine, benzoylecgonine, metabolite of methadone (EDDP), metabolites of tramadol (tramadol-N-oxide, N-desmethyltramadol) and metabolites of heroin (6-MAM, acetylcodeine, papaverine, noscapine, meconin) were detected and counted as the main compounds. Over the course of this study, no new active compounds were identified. Details of the drug groups detected in this study can be found in Appendix 2. Caffeine and phenacetin were the most detected cutting agents in the impregnated seized paper samples in this study. Levamisole was also detected. These cutting agents are commonly seen in cocaine and heroin detected samples.

Statistical Analysis of Results

Results that were compared against different variables such as sex, location and security level underwent statistical analysis to establish whether the variables were dependent on one another. Minitab software was used to carry out a Pearson Chi-square test on each drug type against sex, location and security level, with percentage prevalence data used to calculate the X^2 and critical values to account for differences in the quantity of samples for each variable. The null hypothesis described no significant association between the two variables tested and was accepted for critical values of $p>0.05$, whilst critical values of $p\leq0.05$ were considered to show dependence

between the two variables tested. If the p value is between 0.05 and 0.01, there is a statistically significant association between the variables. If the p value is between 0.01 and 0.001, there is highly significant association between the variables. If the p value is smaller than 0.001 , the association between the variables is considered as very highly significant. Details of statistical analysis of this study can be found in Appendix 1.

5.1 Analysis of 2018-2020 results

The results obtained from the 1250 seizures - 2499 individual papers and 5107 substances detected in total- are summarised in Figure 38. The 5017 substances detected in this study include 75 individual drugs (Appendix 2) in different drug categories. Graphs were plotted based on the total number of substances detected, the total number of individual paper samples, and the total number of seizures. There are samples that include multiple drug groups and which include multiple drugs from a drug group. Calculations based on the total number of individual paper samples and seizures were made by considering if an individual paper sample or seizure contains a substance detected from any drug group it is counted as 1 regardless of how many substances from the drug group it contains, and if not, it is counted as 0. For example, we have two evidence bags (E1 and E2) and four individual paper samples (E1_1, E1_2, E2_1 and E2_2). SCRA, Class A drug, and nicotine were detected in E1_1 and only SCRA was detected in E1_2. SCRA and Class B drugs were detected in E2_1 and SCRA and nicotine were detected in E2_2. According to our calculation, it is reported as 100% of the individual paper samples contain SCRA, 25% of the individual paper samples contain Class A drug and 50% of the individual paper samples contain nicotine. 100% of the evidence bags contains SCRA, 50% of the evidence bags contain Class B drug and 100% of the evidence bags contain nicotine. Because a single seizure or individual paper sample may contain more than one type of drug, the overall number of seizures or individual paper samples will be greater than the total number of drug types detected. Within each drug group, the totals represent the sum of the sub-groups. Because a single seizure or individual paper sample may contain various types of drugs, sum totals will include certain seizures or individual paper samples more than once. In the analysis of the results and percentage calculations, this case needs to be considered.

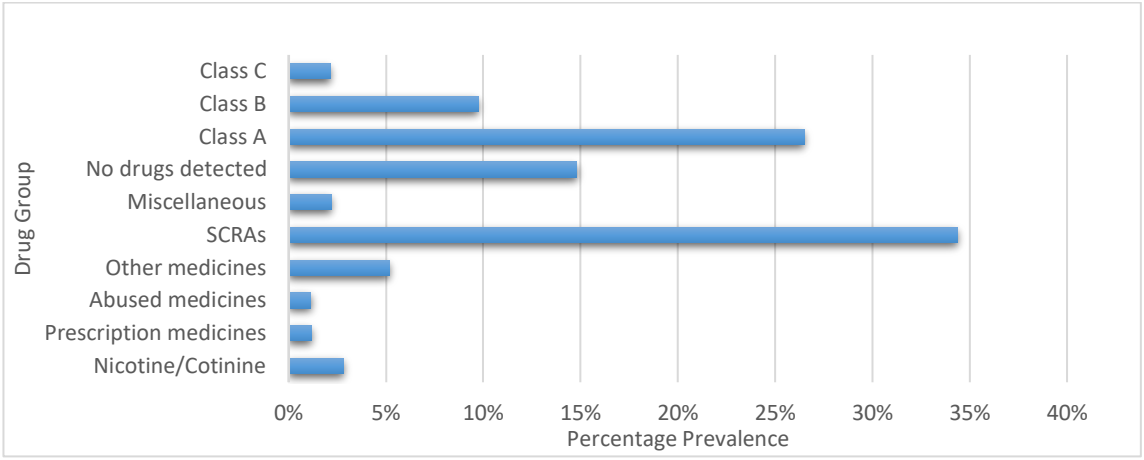

Figure 38: Drug prevalence in England prisons based on the number of substances detected in drug-impregnated papers between 2018-2020

Based on the substances detected: SCRAs were found to be the most prevalent drug in the prisons participating in this study, making up 34.4% (1755 substances) of all the substances detected (5107 substances). It was followed by Class A drugs with 26.5% (1353 substances) of all the substances detected. These were followed by Class B drugs, which were present at 9.7% (497 substances). Other medicines and nicotine/cotinine compounds followed, consisting of 5.2% (265 substances) and 2.8% (143 substances) of the total substances detected. Overall, 14.8% (756 substances) of the totall substances detected were not found to contain any active compounds (Figure 38). For all three calculations based on different data sizes, the ratio is nearly the same for the prevalence of the drug types detected in the impregnated paper samples.

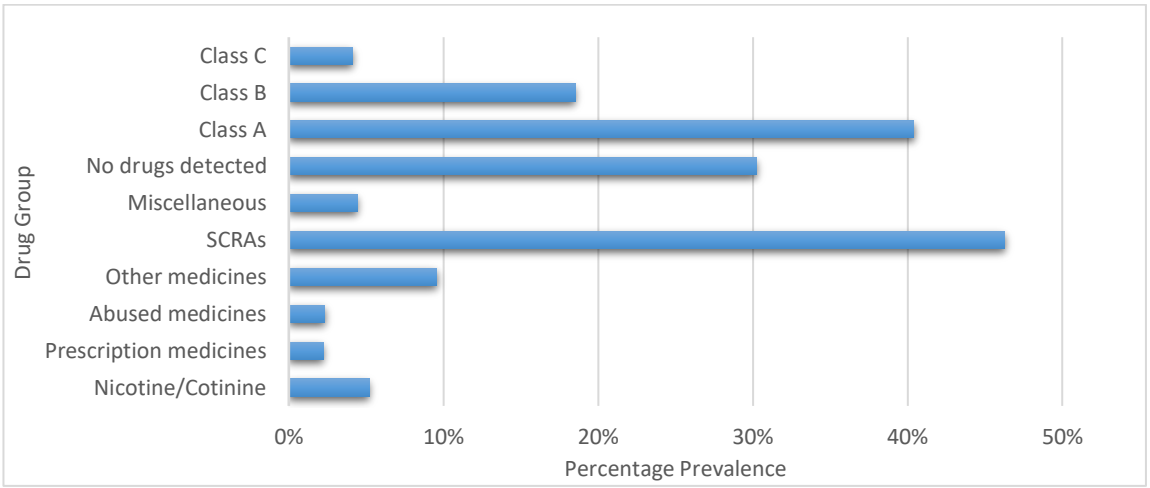

Figure 39: Drug prevalence in England prisons based on the number of drug-impregnated individual paper samples analysed between 2018-2020

Based on individual paper samples analysed: 1157 individual paper samples originating from 651 seizures were found to contain at least one SCRA. SCRAs were found to be the most prevalent drug in the prisons participating in this study, making up 46.3% (1157 individual papers) of the total number of individual papers analysed (2499 individual papers). It was followed by Class A drugs with 40.4% (1010 individual papers) of the individual papers analysed. These were followed by Class B drugs, which were present at 18.5% (463 individual papers). Nicotine/cotinine compounds followed, consisting of 5.2% (130 individual papers) and followed by miscellaneous with 4.4% (111 individual papers) and Class C drugs with 4.1% (103 individual papers) of the total individual papers analysed. Overall, 30.3% (756 individual papers) of the all individual papers analysed were not found to contain any active compounds (Figure 39).

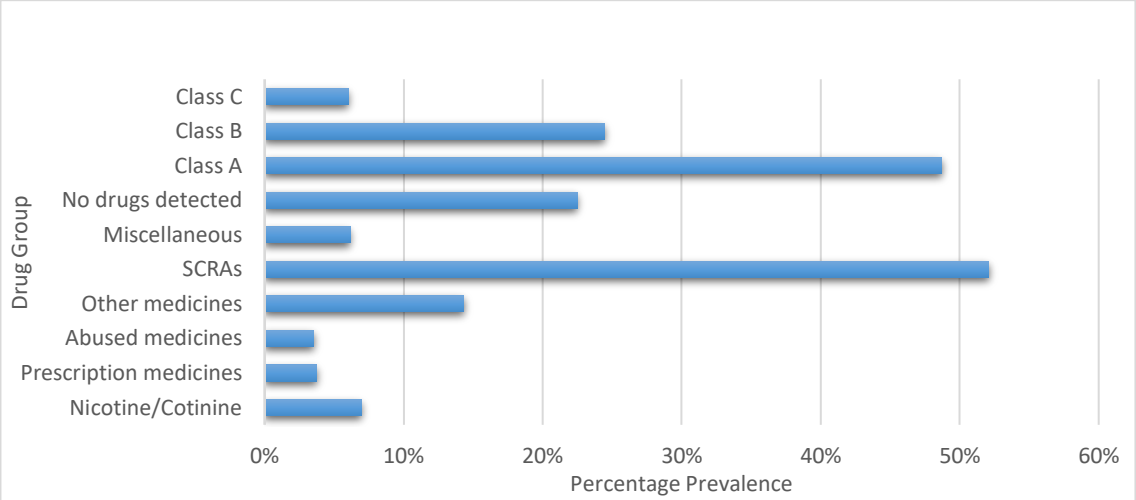

Figure 40: Drug prevalence in England prisons based on the number of drug-impregnated seizures between 2018-2020

Based on seizures collected: Of the 1250 seizures, 52.1% (651 seizures) were found to contain at least one SCRA. It was followed by Class A drugs with 48.7% (609 seizures) of all the seizures. Class B and other medicines followed, consisting of 24.5% (306 seizures) and 14.3% (179 seizures) of all the seizures. Overall, 22.5% (281 seizures) of all the seizures were not found to contain any active compounds (Figure 40).

In response to the implementation of prison smoking bans in England and Wales (Psychoactive Substances Act 2016) and to facilitate smuggling, a shift from SCRA impregnated herbal materials (64 % of submitted samples) to papers and cards sprayed with, or soaked in, SCRA-containing solutions (14 % of submitted samples) was observed (ACMD, 2020). SCRAs are commonly used in

prisons. In England and Wales, from 2018 to 2019, they were identified in more random drug tests of prisoners than cannabis. The high prevalence of SCRAs in prisons between 2018 and 2019 was supported by findings from the UK Focal Point (UK Focal Point, 2021). This trend was seen outside of prisons too. From 2018 to 2019, the use of NPS was reported in the last year by 0.5% of Crime Survey for England and Wales (CSEW) participants. 24% of these participants stated that their most recent NPS use was a herbal smoking mixture, most likely a SCRA (Home Office, 2019). SCRA was first introduced to the UK as a "legal high," with marketing mostly directed at young people in city centres (Peacock et al., 2019), but its use has now shifted significantly to rough sleepers and prison populations around the country (Gray et al., 2021, Norman et al., 2020).

The most common type of SCRA found was 5F-ADB, making up 67.7% (784 individual paper samples) of the 1157 SCRA detected individual paper samples, followed by AMB-FUBINACA (30.3%, 351 individual paper samples), 5F-MDMB-PICA (23.5%, 272 individual paper samples), and 4F-MDMB-BINACA (10.0%, 116 individual paper samples). Other SCRAs detected included NM-2201, MDMB-4en-PINACA, ADB-FUBINACA, ADB-CHMINACA, ADB-BUTINACA, MDMB-CHMICA, MMB-2201, and EMB-FUBINACA, AB-FUBINACA, 5F-AKB-48, 5C-AKB-48, 5F-MPP-PICA, and MDMB-CHMINACA. The difficulty of traditional drug testing to detect synthetic cannabinoids, combined with the drugs' odourless nature in comparison to cannabis, has been the main driving force for synthetic cannabinoid use by prisoners (EMCDDA, 2018b, Reuter and Pardo, 2017). The presence of these compounds in prisons, on the other hand, has been a rising source of concern, owing to the increasing number of uncontrolled synthetic cannabinoids being found on the UK market each year, as well as the consequences they have on mental and physical health. Prisoners have reported depression, nausea, convulsions, and temporary paralysis as a result of SCRA use (EMCDDA, 2018b). Nigel Newcomen, the Prisons and Probation Ombudsman (PPO), said in November 2016 that he had reported 64 deaths in prisons between June 2013 and April 2016, where the prisoner was known or strongly suspected of having taken NPS prior to their death (PHE, 2015). For the health and safety of those in the prison system, it is critical to track the prevalence and use of SCRAs in prison.

Class A drugs made up a smaller proportion of the individual paper samples than SCRAs, which was 40.4% (1010 individual paper samples out of 2499 individual paper samples). In this proportion, the most common Class A drug found was cocaine, making up 87.7% (913 individual paper samples) of the 1010 individual paper samples analysed in this group. This was followed by MDMA (24.9%, 252 individual paper samples), heroin (12.3%, 124 individual paper samples), and

morphine (2.6%, 26 individual paper samples). Other Class A drugs detected in the drug-impregnated papers are methadone, 2C-B, oxycodone, oxymorphone, mescaline, PMMA, and meperidine. These findings vary slightly from those of the EMCDDA, which stated that heroin was amongst one of the most commonly reported drugs used in UK prisons (EMCDDA, 2018c). Furthermore, depressant substances like heroin or cannabis have typically been chosen by prisoners over stimulants like cocaine and MDMA because they are easier to manage and help pass the time (HM Chief Inspector of Prisons for England and Wales, 2015). These differences could be due to the availability or cost of different types of Class A drugs, or they could be affected by gender variation in drug use.

Other Class B drugs, except cannabis, SCRAs and prescription drugs, made up 18.5% (463 individual paper samples) of all 2499 individual paper samples analysed. The most common Class B drug detected is ketamine, which accounts for 88.8% (411 individual paper samples) of the 463 individual paper samples analysed in this group. This was followed by amphetamine (14.0%, 65 individual paper samples) and codeine (3.7%, 17 individual paper samples). Other Class B drugs detected in the drug-impregnated paper samples are methylone, ethylone, methaqualone, adnd dihydrocodeine. Although there is insufficient data on ketamine use in UK prisons, the prevalence of ketamine use among adults in England and Wales is 0.8 percent, the highest on record (UK Focal Point, 2021).

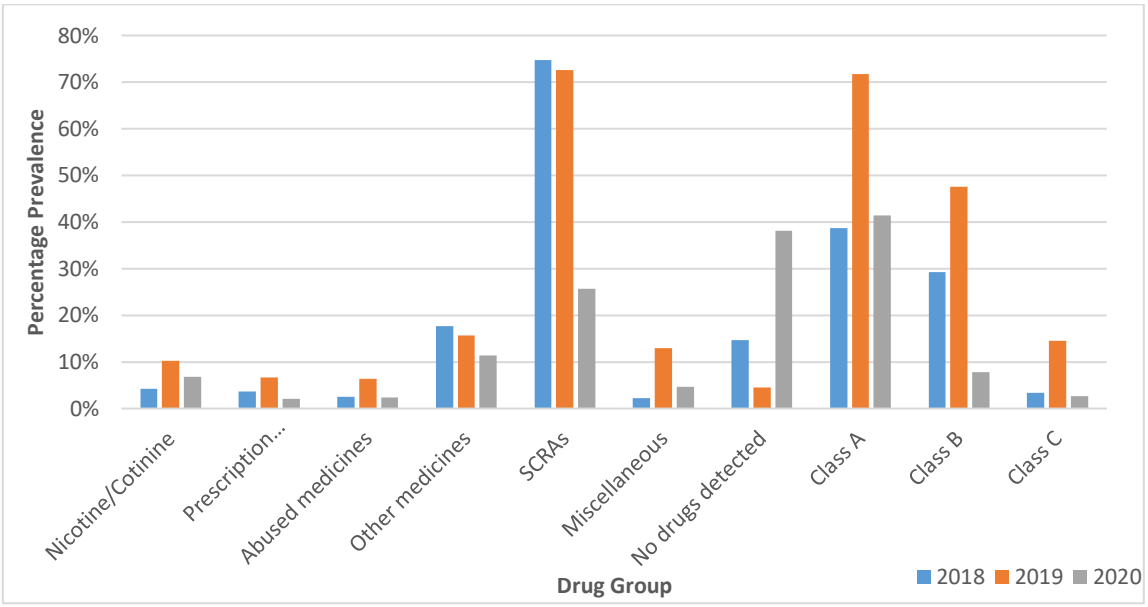

Figure 41: Bar graph showing the percentage of different drug types detected from prisons across England over the years between 2018 and 2020 based on the number of the seizures collected for drug-impregnated paper

The prevalence of drug groups detected in the drug-impregnated paper seizures from English prisons over the years 2018 to 2020 is shown in Figure 41. SCRAs were the most prevalent drug group detected in the drug-impregnated seizures from the prisons participating in this study, followed by Class A drugs. The prevalence of SCRA over the years has been decreasing between 2018 and 2020, but it is still the highest overall.

The compounds identified within the study mostly fluctuated over time, as seen in SCRAs, Class A, Class B, and Class C drugs. Some of the paper samples were the ones that the sniffer dogs positively identified for NPSs during routine searches of prison mail rooms. Because of the continuous changing nature of the NPS market, it is hard to maintain the long-term usefulness of sniffer dogs with these compounds. When a new NPS appears on the market, new sniffer dogs need to be trained for that drug, which is time-consuming and expensive. Due to the fact that not all the samples were suspected by sniffer dogs and not all prisons had sniffer dogs for drug detection, we cannot make a general statement about the effect of sniffer dogs on the fluctuation of the drugs over the years.

In the three years, of the drug-impregnated paper seizures, there were a total of 266, 241, and 144 SCRA detected seizures in 2018, 2019, and 2020, respectively. The most commonly detected SCRAs in this study were initially 5F-ADB (5F-MDMB-PINACA) (79.7%, 212 seizures) and AMB-FUBINACA (47.4%, 126 seizures) in the seizures collected in 2018 (Figure 42). According to the Advisory Council on the Misuse of Drugs (ACMD) report on SCRA (ACMD, 2020), the most reported SCRAs, which were 5F-ADB and AMB-FUBINACA in this time period, were the same as our findings. In 2019 the most prevalent compounds detected in drug-impregnated paper seizures in this study have been 5F-MDMB-PICA (68.0%, 164 seizures), 5F-ADB (40.2%, 97 seizures), 4F-MDMB-BINACA (28.2%, 68 seizures) and AMB-FUBINACA (24.9%, 60 seizures) (Figure 42). In this study, 5F-MDMB-PICA and 4F-MDMB-BINACA were detected for the first time in English prison paper seizures in 2019. In the previously mentioned ACMD report, TICTAC results from prison seizures was showing that 5F-ADB and AMB-FUBINACA were still detected in England prisons in 2019 and 4F-MDMB-BINACA and 5F-MDMB-PICA have been two of the most prevalent SCRAs in prisons in 2019 (ACMD, 2020). In 2020, MDMB-4en-PINACA (8.3%, 12 seizures) and ADB-BUTINACA (4.9%, 7 seizures) were discovered for the first time in drug-impregnated paper seizures. 5F-ADB (79.2%, 114 seizures) and 5F-MDMB-PICA (16.7%, 24 seizures) were the most prevalent SCRAs detected in 2020 drug-impregnated paper seizures in this study (Figure 42). According to the WEDINOS

Annual Report (WEDINOS, 2020) , since December 2019, the number of samples containing AMB-FUBINACA has decreased, and 5F-MDMB-PINACA was the sixth most commonly identified substance within all samples. SCRA samples are the most common in criminal justice services, particularly the prison estate and homelessness programmes (WEDINOS, 2020).

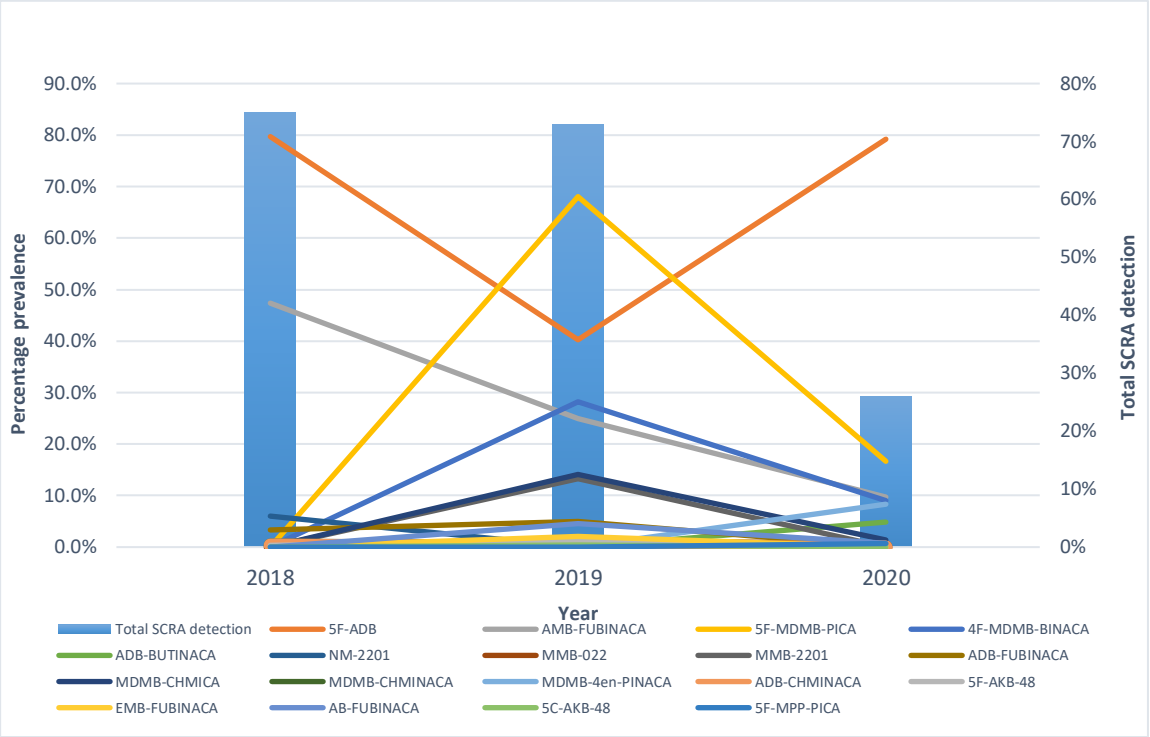

Figure 42: Line graph displaying the changes in percentage prevalence of nine SCRA types and total SCRA detection over the years in English prisons based on 266, 241 and 144 separate seizures which were believed to contain any SCRA over 2018 to 2020

Producers proceeded to modify SCRA chemical structures in order to circumvent legislation and/or avoid detection (Banister and Connor, 2018). They are continuing to develop novel structures that retain CB1 agonist action while evading increased national and international restrictions on known SCRA materials. Each year since 2016, the EMCDDA has recorded roughly ten new SCRA identifications in Europe. Many of them are simple versions of previously recognised elements that fall under the purview of the UK generic regulations (ACMD, 2020). This means that the legislation defines specific modifications of the structure (especially substituent groups in specified places in the molecule) that lead to a substance being regulated, starting with a core chemical structure that does not have to be psychoactive in and of itself (UNODC, 2015). In Figure 43, it can be seen that the core structures were similar and there were minor modifications between them over the years.

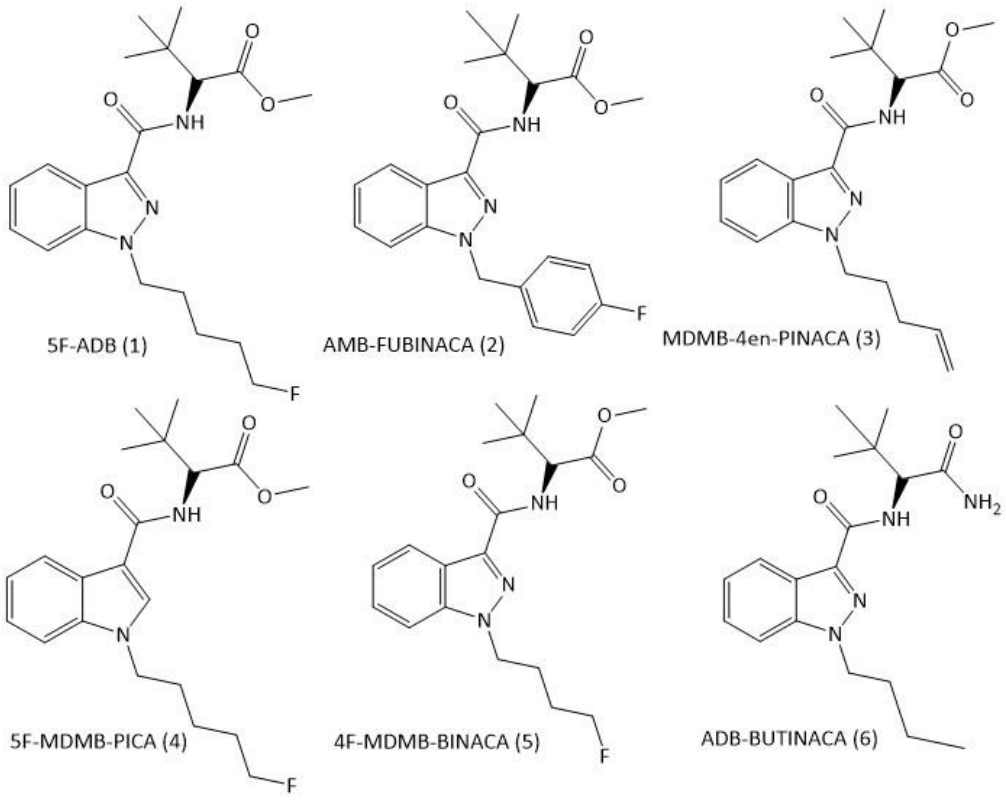

Figure 43: Structures of SCRAs detected in drug-impregnated paper seizures from English prisons between 2018 and 2020; 5F-ADB (1), AMB-FUBINACA (2) (Psychoactive Substances Act 2016) (Psychoactive Substances Act 2016) (Psychoactive Substances Act 2016) (Psychoactive Substances Act 2016) , MDMB-4en-PINACA (3), 5F-MDMB-PICA (4), 4F-MDMB-BINACA (5), ADB-BUTINACA.

The following sets of results compare the types of drugs detected in this study to the various prison types that gave samples to examine if any trends exist between drug prevalence and sex, security levels, and geographical location from 2018 to 2020. Where applicable, a Pearson Chi-square test was applied to determine whether the variables being compared were statistically independent.

5.2 Comparison by sex

Figure 44 displays a summary of the groups of drugs detected in the seizures with drug-impregnated papers from male and female-only prisons in England between 2018 and 2020, with the results separated by sex. Out of the twelve prisons providing samples for this study, two were female prisons and ten were male prisons. The estimated prison population for the two female prisons combined was calculated to be 690 using data from prison population figures in December 2020, whilst the estimated prison population for the ten male prisons combined was calculated to be 9261 (HM Prison Service, 2020). From the 1250 seizures with drug-impregnated papers

collected between 2018 and 2020, 969 evidence bags contained samples of any drug detected. Of these, 756 were supplied from the male-only prisons, whilst only 213 were from the female-only prisons. Considering the large disparity between the prison populations and the number of seizures with drugs detected between male and female prisons, any trends identified from these results will require further investigation using a larger dataset, especially for female prisons.

There were samples containing drugs from more than one group and multiple drugs from the same drug group. From Figure 44, Class A drugs and SCRAs appear to be the most prevalent drug types identified in female prisons, with 45.9% (156 seizures) of the samples out of 340 seizures analysed identified as Class A drugs and 22.1% (75 seizures) as SCRAs.

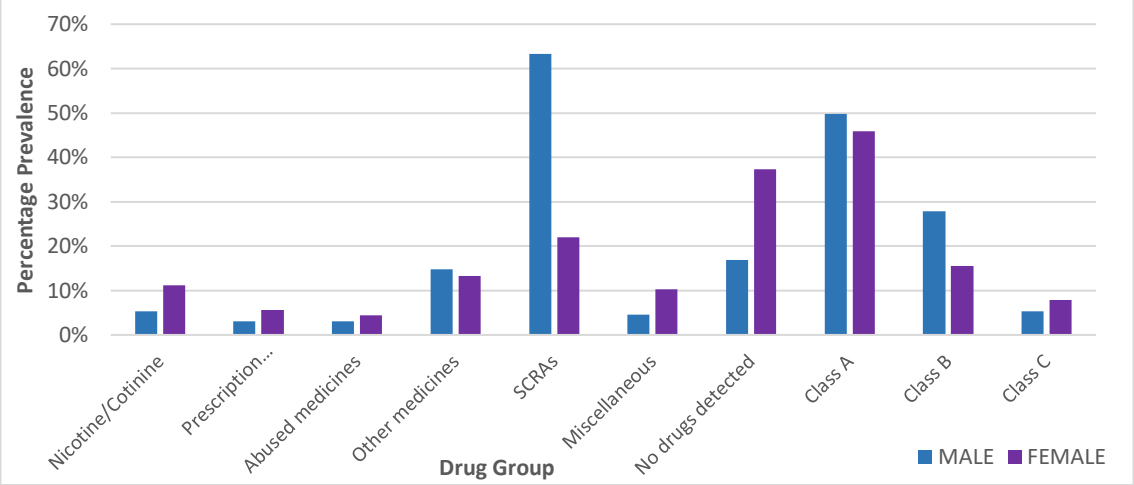

Figure 44: Bar graph showing the percentage of different drug types detected from male and female-only prisons across England between 2018 and 2020 based on the number of the seizures collected for drug-impregnated paper

Considering male prisons alone, SCRAs were the most prevalent drug group amongst male prisoners, making up 63.3% (576 seizures) of the samples in 910 seizures analysed. This is supported by a thematic study from the HM Inspectorate of Prisons, which indicated that NPS usage in adult male prisons had been increasing since 2008 and was categorised as widespread in 2013-2014 (HM Inspectorate of Prisons, 2015). The most common SCRA found was 5F-ADB, making up 63.0% (363 seizures) of the total 576 seizures that contained at least one SCRA (576 seizures). It was followed by AMB-FUBINACA (34.5%, 199 seizures) and 5F-MDMB-PICA (30.2%, 174 seizures).

When only female prisons were considered, Class A drugs were detected in 45.9% (156 seizures) of the samples in all 340 seizures from female prisons, with cocaine accounting for 93.5% (146

seizures) of the total seizures containing Class A drugs. It was followed by heroin (19.9%, 31 seizures) and MDMA (16.0%, 25 seizures). The literature supports heroin usage among female inmates, with the Surveying Prisoner Crime Reduction study revealing that 55 percent of male and female heroin users reported using heroin in jail (Light et al., 2013). In the same study, it was reported that 38% of men and 10% of women who reported heroin usage indicated they began using it in prison (Light et al., 2013). In the male prisons, in the 49.8% (453 seizures) of the samples in 910 seizures, the most common Class A drug detected was cocaine (45.1%, 410 seizures), which is lower than the prevalence for female prisons. MDMA and heroin followed, consisting of 17.0% (155 seizures) and 5.4% (49 seizures) of total seizures analysed. Heroin prevalence in the seizures of drug-impregnated papers was higher in female prisons. General population trends showed a similar outcome, with the EMCDDA reporting a higher proportion of men using Class A drugs compared to women (EMCDDA, 2018c). Regarding prescription drugs, their misuse is especially frequent in the female estate and might be more difficult to detect. Some inmates desire prescribed medication because of the psychotropic effect of the substance, and many will divert their prescribed medication by trading or selling it to others (HMPPS, 2019a).

To evaluate whether the prevalence of drug types was dependent on sex, the Pearson Chi-Square test was performed, with sex set as an independent variable and the different drug types identified as dependent variables. The statistical independence of each drug type was calculated separately to identify which drug types were dependent on sex. The null hypothesis was defined by the absence of any association between the drug type tested and sex, whilst the alternate hypothesis was defined by the drug type tested and sex being associated with one another. The critical values calculated that gave a result of $p > 0.05$ led to the null hypothesis being accepted, whilst the alternate hypothesis was accepted for values of $p \leq 0.05$.

Considering SCRA prevalence, X^2 (1, 1250) = 168.7, $p < 0.001$, therefore the null hypothesis was rejected, and SCRA prevalence was found to have a very highly significant dependence on sex. Likewise, Class B, nicotine/cotinine and miscallenous drug prevalence were also found to have a very highly significant correlation with sex, with X^2 (1, 1250) = 20.0 , $p < 0.001$, X^2 (1, 1250) = 12.8, $p < 0.001$ and X^2 (1, 1250) = 13.8, $p < 0.001$. Prescription medicines gave X^2 (1, 1250) = 4.3, $p = 0.038$, suggesting that there was a statistically significant association between sex and the prevalence of prescription medicines. There was no association between sex and the prevalence of Class A drugs, Class C drugs, other medicines, and abused medicines with the values, X^2 (1,

1250) = 1.5, p=0.220, X^2 (1, 1250) = 3.1, p=0.077, X^2 (1, 1250) = 0.45, p=0.503 and X^2 (1, 1250) = 1.3, p=0.249. The dependency of SCRA and prescription drug prevalence on sex further supports the literature previously mentioned.

5.3 Comparison by security level

To compare results obtained across the security level of participating prisons, only male prisons were considered. There was one category A female prison and one closed category female prison. This exclusion was done to prevent the skew of results that may have arisen as a result of the limited amount of data for female prisons and the absence of data for the prevalence of some drug types. For this comparison, data collected for the male prisons that contributed samples was separated by the security level of each prison, which were category B, providing 434 drug-impregnated paper seizures, and category C, providing 476 drug-impregnated paper seizures. Category B was the highest security level of the prisons involved, whilst category C has the lowest security level. In total, the distribution of 910 drug-impregnated paper seizures analysed between 2018 and 2020 from ten male prisons was compared. The results are displayed in Figure 45.

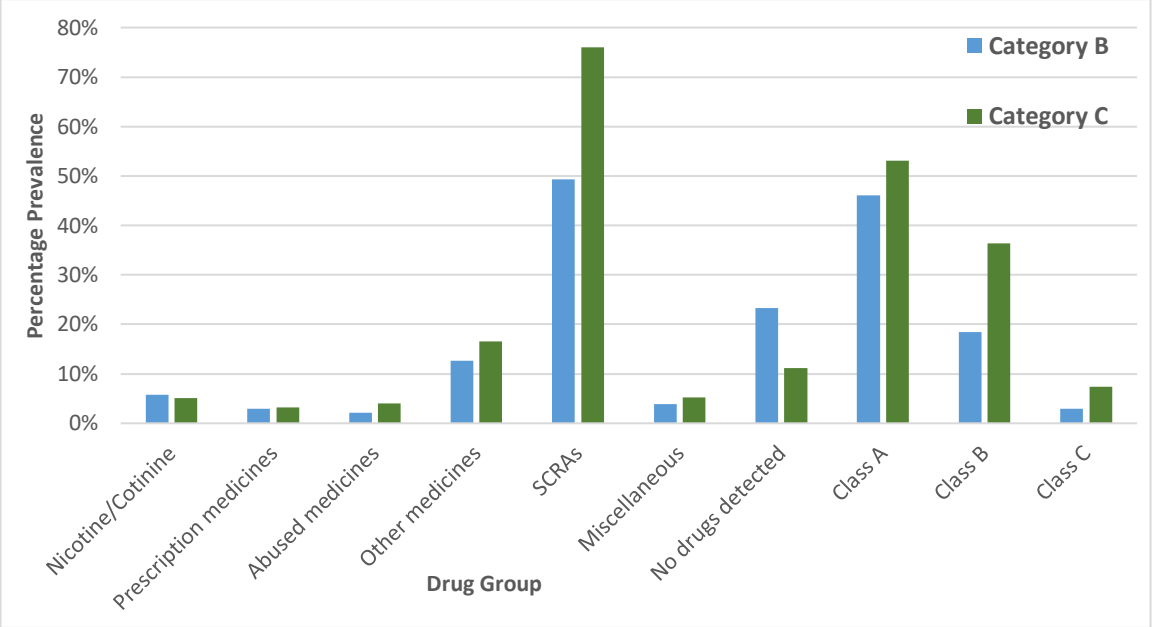

Figure 45: Bar graph showing the percentage of different drug groups detected in drug-impregnated paper seizures from ten male prisons across England between 2018 and 2020, separated by the security levels of the prisons, which ranged from Category B to C. 910 seizures of drug-impregnated papers are represented in total.

Category C prisons displayed high prevalence rates of SCRAs, Class A and Class B drugs when observed individually, with a percentage prevalence of 76.1% (362 seizures), 53.2% (253 seizures)

and 36.3% (173 seizures) of total 476 seizures, respectively. Category B male prisons appeared to have lower prevalence rates amongst SCRAs, Class A, Class B, and Class C drugs in comparison to other security levels (SCRAs with 49.3% (214 seizures), Class A with 46.1% (200 seizures), and Class B with 18.4% (80 seizures) of a total of 434 seizures in category B prisons). Previous reports have stated that the level of synthetic cannabinoid use is most severe in category C establishments (HM Inspectorate of Prisons, 2015). The nature of category C training prisons, which have large perimeters and relatively free prisoner movement, may be one explanation for this. (O'Hagan and Hardwick, 2017). In addition, men detained in a category C prison may be released on a temporary licence (ROTL) to work under very limited circumstances (Ministry of Justice, 2021). The overall lack of security in comparison to category B prisons means that there are more opportunities for drug smuggling to take place within category C prisons, resulting in higher prevalence rates of these substances.

To identify whether the prevalence of the drug types shown in Figure 45 was dependent on the security level of the male prison they originated from, a Pearson Chi-Square test was performed using the percentage prevalence of each drug type seized between 2018 and 2020. Statistical analysis was carried out on all drug groups to evaluate their level of independence in relation to prison security level. Of all the drug groups tested, only SCRAs and Class B drugs were found to have very highly significant dependence on the security of male prisons, as X^2 (1, 910) = 69.9, $p < 0.001$ and X^2 (1, 910) = 36.284, $p < 0.001$ respectively. The association between the prevalence of Class C drugs and the security level of male prisons was found to be highly significant, X2 (1, 910) = 8.627, $p = 0.003$. Class A drugs were found to have a statistically significant dependence on the security level of male prisons as X^2 (1, 910) = 4.537, $p = 0.033$. The remaining drug groups were found to have no association with security level either. These results support our findings and indicate that the higher levels of prevalence observed for SCRAs and Class B and Class C drugs in lower security male prisons.

Following these findings, it is vital to highlight that ten male prisons provided samples for this study, with four category B prisons and six category C prisons. Because of the variance in the number of prisons of each security level included in this study, more research is needed in this field employing a greater number of prisons of each security level to investigate if a bigger sample size has an impact on the significance of these findings. Future research could include samples taken from category A and category D male prisons as well as samples obtained from a broader

range of female prisons to see if any trends in drug prevalence can be found among female prisons of varied security categories.

5.3 Comparison by geographical location

To compare drug prevalence rates from drug-impregnated paper samples seized between 2018 and 2020 against the geographical location of the contributing prisons, only male prison data was used to prevent the skewing of results, as mentioned in section 5.2. The ten male prisons examined were based in five regions across England, namely Yorkshire and the Humber, South West, South East, and Eastern and West Midlands, with 910 drug-impregnated paper seizures analysed between them. Regions were used to denote location as any smaller scale classifications would have compromised the anonymity of the prisons involved, which would be in breach of the confidentiality agreement. Figure 46 shows a diagram of the results separated by region.

The diagram was created considering the number of substances detected for reasons mentioned in section 5.1. Otherwise, pie charts will not give 100% values in total.

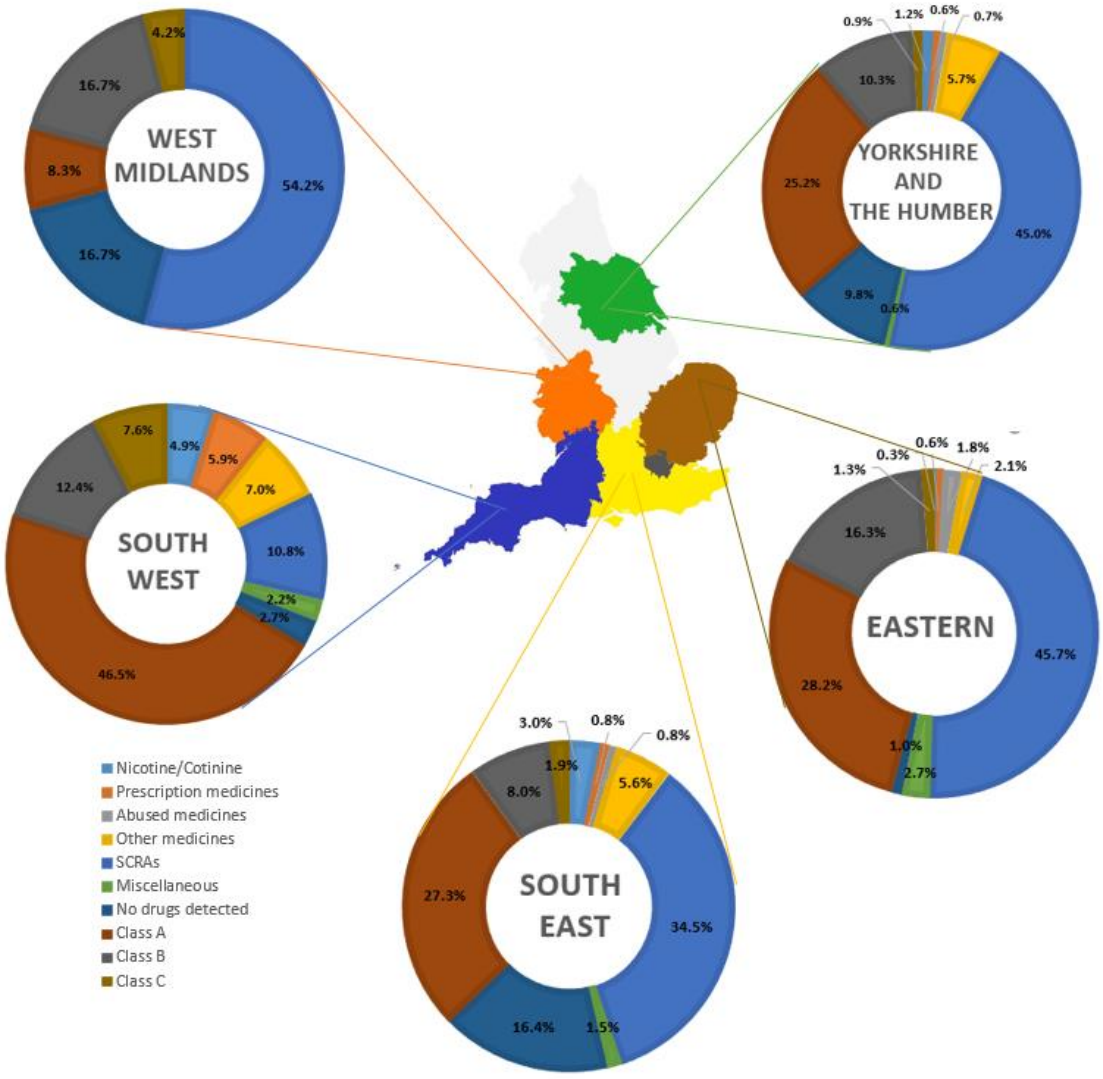

Figure 46: Diagram showing the percentage prevalence of different drug groups seized from ten male prisons across five different regions in England between 2018 and 2020, representing a total of 3922 substances detected (1790 individual papers, 910 seizures). The results for the East region reflect 773 substances, whilst the South East region, South West region and Yorkshire and Humber region and West Midlands region reflect 1647,173, 1305 and 24 substances respectively.

Figure 47 shows the detailed information about the number of seizures, number of individual papers, and number of substances detected from ten male prisons across five different regions in England between 2018 and 2020. In addition, it shows the most prevalent drug group based on the number of substances detected in each region.

Figure 47: Figure showing the detailed information about number of seizures, number of individual papers and number of substances detected from ten male prisons across five different regions in England between 2018 and 2020. In addition, it shows the most prevalent drug group based on the number of substances detected in each region.

Based on the substances detected: The prevalence of SCRAs appeared to be highest in male prisons in the West Midlands region in the west of England, making up 54.2% (13 substances) of the 24 substances detected in this region, compared to prisons in the South West region, where SCRA prevalence was 11.6 % (20 substances) of the 173 substances detected. SCRA prevalence was highest in prisons in Yorkshire and the Eastern regions of the east, with a similar proportion of the total 1305 and 773 substances detected (48.0%, 627 substances and 45.7%, 353

substances), with the prevalence in the South East region just slightly behind at 34.5% (569 substances) of the 1647 substances detected in this region. Class A drugs were the most prevalent drug group in the prisons in the South West region, making up 42.8% (74 substances) of the 173 substances detected in this region. In the east of England, Class A drug prevalence was very close to each other in the Eastern, South East, and Yorkshire regions at 28.2% (218 substances out of 773), 27.3% (450 substances out of 1647), and 20.2% (263 substances out of 1305). The highest prevalence of Class B drugs in the prisons in the west of England was found in the West Midlands region, making up 16.7% (4 substances) of the 24 substances detected in this region. In the east of England, Class B drug prevalence was higher in the Eastern region at 16.3% (126 substances) and was followed by the Yorkshire and South East regions with 11.0% (143 substances) and 8.0% (132 substances) of total substances detected in these regions. There was a lower prevalence of medication throughout all regions, and prescription medicines were more prevalent in the South West region at 6.4% (11 substances) of 173 substances detected in this region. In the West Midlands, no prescription medicines were detected. Other medicines were more prevalent in the South West region with 7.5% (13 substances out of 173), compared to prisons in Yorkshire and the South East regions, but only by a smaller margin, at 6.1% (79 substances) and 5.6% (93 substances) of the 1305 and 1647 substances detected in this region, respectively. The prevalence of nicotine/cotinine was observed to be highest in the South West region, making up 5.2% (9 substances) of the 173 substances detected in this region, while no nicotine was detected in the West Midlands region.

Based on the seizures collected: The prevalence of SCRAs in male prisons in the west of England appeared to be highest in the West Midlands region, making up 58.8% (10 seizures) of the 17 seizures analysed in this region, compared to prisons in the South West region, where SCRA prevalence was 34.6% (9 seizures) of the 26 seizures in this region. SCRA prevalence was highest in prisons in the Eastern and Yorkshire regions in the east, presenting 88.7% (126 seizures) and 75.0% (246 seizures) of the seizures analysed in these regions (142 seizures and 328 seizures, respectively), with prevalence in the South East region just slightly behind at 46.6% (185 seizures) of the 397 seizures analysed in this region. Class A drugs were the most prevalent drug group in the prisons in the south of England, for the Eastern region, making up 78.9% (112 seizures) of the 142 seizures analysed in this region. In the east of England, Class A drug prevalence was the highest again, with the Eastern region compared to the South East and Yorkshire regions at 50.1% (199 seizures) and 38.1% (125 seizures). The highest prevalence of Class B drugs in the prisons in

the west of England was found in the South West region, making up 50.0% (13 seizures) of the 26 seizures analysed in this region. In the east of England, Class B drug prevalence was higher in the Eastern region at 47.9% (68 seizures) and was followed by Yorkshire and South East regions with 27.4% (90 seizures) and 19.6% (78 seizures) of total seizures analysed in these regions. There was a lower prevalence of medication throughout all regions, and prescription medicines were more prevalent in the South West region at 23.1% (6 seizures) of the 26 seizures analysed in this region. In the West Midlands, no prescription medicines were detected. Other medicines were more prevalent in the South West region with 30.8% (8 seizures), compared to prisons in Yorkshire and the South East regions, but only by a smaller margin, at 16.5% (54 seizures) and 14.9% (59 seizures) of the seizures analysed in these regions. The prevalence of nicotine was observed to be highest in the South West region, making up 23.1% (6 seizures) of the seizures analysed in this region, while no nicotine was detected in the West Midlands region.

The Pearson Chi-Square test was applied to these results to evaluate whether the prevalence of the different drug groups was associated with the regional location of the male prisons drug-impregnated samples were seized from. Each drug group was tested separately to obtain individual X^2 and p values. Except for SCRAs, Class A, Class B, and other medicines, the statistical analysis software could not calculate p values for other drug groups. It might be due to non-homogenous data distribution through the regions of prisons (see Figure 47). In comparison, SCRAs, Class A and Class B, were found to have very highly significant associations with the location of seizure, with X^2 (4,910) = 115.9, $p<0.001$, X^2 (4, 910) = 76.4, $p<0.001$ and X^2 (4, 910) = 48.3, $p<0.001$ respectively. The association for the other medicine drug group with the geographical location of the prison was highly significant, with X^2 (4, 910) = 12.6 $p=0.014$.

In spite of these findings, no significant overall trends were found among drug group prevalence in the north, south, and east of the country. According to this study, in the north and south, SCRA was the most prevalent, Class A was the second most prevalent drug group, and percentage prevalence values were very close to each other. The most prevalent drug category were SCRAs in all prisons in the east and north regions of the UK. In the west and south regions of the UK, both Class A and SCRAs were seen as the most prevalent, though not in all prisons in these regions. There is no data for the SCRA prevalence in the general population for different regions, so we cannot compare the SCRA prevalence data of this study with the general population data. According to Office for National Statistics (ONS) data from the CSEW on the extent and trends of

illicit drug use (Office for National Statistics, 2020), reported use of any Class A drug was the highest in the South West region. It is followed by the South East, the Yorkshire and the Humber, the Eastern and the West Midlands, respectively. In this study, on the other hand, based on impregnated seizures collected from English prisons, Class A drug prevalence was the highest in the Eastern region and was followed by the South West, the South East, the Yorkshire and the Humber and the West Midlands regions, respectively. For Class B drug prevalence data from CSEW, it was the highest in the South West region, followed by the South East and the Eastern regions. In the Yorkshire and the Humber and the West Midlands regions, Class B drug use was lower than that of other regions. Similarly, in this study, based on the drug-impregnated seizures collected from English prisons, Class B drug prevalence was the highest in the South West region. It was followed by the Eastern, the Yorkshire and the Humber, the South East and the West Midlands regions. Although drug prevalence trends were observed in individual regions of the country, the lack of prisons included in the results meant that it was highly unlikely that these results were reflections of trends in location. In addition, there is a lack of research about drug prevalence trends in prisons with respect to different locations, and therefore there is little literature to support or refute these findings. Furthermore, the seizure size, which ranged from 17 seizures for the West Midlands region to 397 seizures for the South East, had an impact on the observed results (Figure 47). In particular, out of the ten male prisons examined, only one was located in the West Midlands. For each region, two prisons were located in the South West, South East, and Eastern regions, and three prisons were located in the Yorkshire region. As a result, it is considerably more likely that the results obtained for these regions represent individual prison tendencies rather than regional trends. A larger number of samples collected from several prisons in various locations of the country would provide more information about potential trends in drug prevalence and prison location.

5.5 Analysis of drug prevalence over multiple years

Of the 12 prisons participating in this study, one prison had been contributing drug-impregnated paper seizures for analysis since 2018, over three years. This meant that it was possible to monitor drug prevalence over time. The name of the prison will not be revealed to protect its anonymity. However, it was located in the South East region of England and housed male prisoners only. One of the prisons, referred to as Prison A, was able to provide drug-impregnated samples from 371 seizures across 2018-2020. For the comparison of drug prevalence, nine drug groups containing

the most commonly used active compounds were considered: nicotine, other medicines, abused medicines, and prescription medicines, miscellaneous, SCRAs, Class A, Class B, and Class C drugs. In total, Prison A contributed 777 samples over the 3-year period. The drug prevalence was calculated for each individual seizure for each prison, and the results were plotted against the time at which the seizures were taken into possession for analysis. Over-time drug prevalence results for Prison A can be seen in Figure 48. It is worth mentioning that any drug prevalence trends identified will not be representative of the entire prison population but will only reflect the drug situation in the prison being examined.

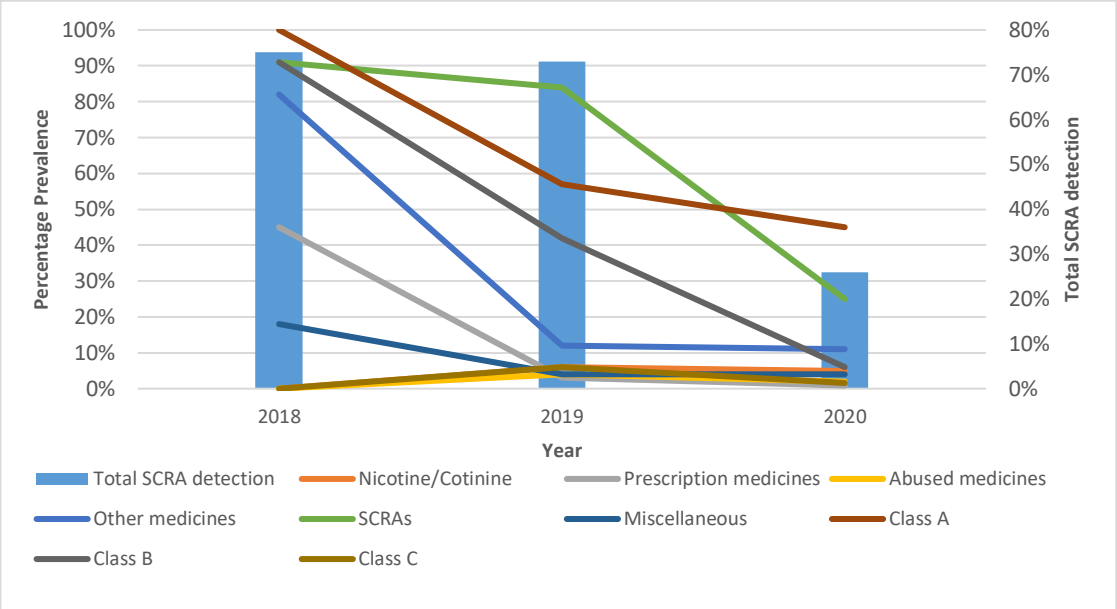

Figure 48: Line graph displaying the changes in percentage prevalence of nine drug types in Prison A based on 371 separate seizures (770 individual paper samples) over 2018 to 2020 and bar graph displaying the changes in percentage prevalence of SCRA in all prisons over the years.

The prevalence of synthetic cannabinoids appears to have decreased steadily over the three years (2018-2020) in Prison A. In Figure 48, the right y-axis shows the percentage prevalence of nine drug types in Prison A between 2018 and 2020, and the left y-axis shows the percentage prevalence of total SCRA detection in all prisons over three years. From Figure 48, it appears as though the prevalence of synthetic cannabinoids in Prison A and total SCRA detection in all prisons were at their highest point over the three years (2018-2020) in 2018, after the Psychoactive Substances Act 2016 (PSA). Producing, supplying, importing, or exporting psychoactive compounds, once known as "legal highs," became an offence under the act (Psychoactive Substances Act 2016). As a result of the act's passing, there is a decline in SCRA prevalence; hence,

the act may have influenced the availability of NPS in prisons. This is likely, given the Home Office discovered in a review of the PSA dating back to November 2018 that the act had resulted in an increase in the price of NPS and a decrease in their availability as they were more difficult to source (Home Office, 2018). However, because drug analysis data for these prisons is only accessible from 2016 onwards, it is difficult to assess the extent to which the PSA played a role in SCRA prevalence without further historical data.

The decrease in SCRAs is contrary to findings reported by the Ministry of Justice, whereby psychoactive substances are the most prevalent drug type in prison and were present in 51% of all positive samples, overtaking cannabis by a large margin from 2018 to 2019 (Ministry of Justice, 2019). Potential NPS-containing paper samples seized between 2018 and 2020 will be helpful to accurately evaluate the prevalence of psychoactive substances in prisons together with the SCRA findings alone, which provide information on the prevalence of NPS. As a result, SCRAs are likely to be more prevalent in prisons than reported in this study, with the EMCDDA reporting that one in every eight prisoners has used synthetic cannabinoids (EMCDDA, 2018c).

Because most research has focused on illicit drugs, there is not much evidence accessible about prescription drug prevalence in prisons throughout time. This is mostly because prescription medication is known to circulate within prison populations, as inmates are often supplied medication from prison pharmacies for medical problems. As a result, determining whether prescription medication seized was legally prescribed or distributed for abuse is challenging. Changes to prison policy have been implemented to ensure that prescription medication is correctly and securely supplied to prisoners who require it, while decreasing the possibility of corruption within prison pharmacies, as stated in the Prison Drugs Strategy 2019 (HMPPS, 2019a).

Class A drug prevalence appears to have decreased over the past three years in Prison A. It was still prevalent after SCRAs, consistent with the overall trend observed in the analysis of all prisons participating in this study. According to the HMPPS Annual Digest 2019/20 report (Ministry of Justice, 2020), Class A drug finds in UK prisons were high compared to other drug types, which supports our findings, but it has been increasing between 2018 and 2020 . Figure 48 shows that Class A drug prevalence has been decreasing over time in this time period in Prison A. It can be the prevalence trends only reflecting the drug situation in the prison examined. In comparison, from 2018 to 2020, nicotine prevalence grew somewhat. This follows the completion in July 2018 of all closed prisons in England and Wales becoming smoke-free, a process that began in stages

beginning in September 2015 (Action on Smoking and Health, 2018). The rise in prevalence could be attributed to an increase in the use of nicotine patches among inmates as a result of the smoke ban or to the introduction of e-cigarettes and vapes in prisons to help prisoners quit smoking.

In summary, drug testing data from seized drug-impregnated paper samples from prisons has been shown to be a good indicator of drug prevalence, highlighting trends that are supported by nationally published statistics. The analytical method employed overcomes the limits of other well-established methods, such as prisoner surveys. The findings also show that drug prevalence is influenced to some extent by security level and sex, variables that have not before been studied in this context. This finding has the potential to influence the direction of future studies into drug prevalence in prisons. From this study, SCRAs were found to be the most prevalent drug group in English prisons based on drug-impregnated papers seized between 2018 and 2020. Regarding differences in sex, SCRAs had a higher prevalence in male prisons, while female prisons showed a higher prevalence of Class A drugs. In addition, male prisons with a lower security level (category C) were observed to have a higher prevalence of Class B, Class C, and abused prescription drugs in comparison to category B prisons, which had a higher prevalence of nicotine. Finally, although drug prevalence trends were observed in individual regions of the country, the lack of prisons included in the results meant that it was highly unlikely that these results were reflections of trends in location.

This study's findings have revealed new information about drug use in prison. These findings may have significance as police information and could be used to assist drug policymakers in prisons, specifically in the establishment of treatment plans targeting the most commonly misused substances in each prison. Furthermore, the spreadsheets used to record data on the identity of samples collected from each prison can be forwarded to the participating prisons, giving them information about the samples being seized. This information could aid in the establishment of smuggling routes for drugs into prisons, allowing prison officials to focus more attention on these areas. Information on the distribution of drug prevalence across England's regions may aid in the detection of drug-trafficking organisations that contribute to the supply of drugs within prisons.

5.6 Polydrug seizures

Polydrug use refers to the use of more than one drug or type of drug by a person, either concurrently or sequentially. It includes the use of both illegal drugs and legal substances such as alcohol and medications, and most drug users will use more than one substance on occasion

(EMCDDA, 2022a). Polydrug usage can be classified into three categories: (1) the combination of medications to produce a cumulative or novel effect; (2) the use of one drug to reduce the effects of another; and (3) the substitution of one drug for another due to low cost, availability, or fashion. Polydrug abuse has become a major public health issue. Some of the most serious outcomes of polydrug usage are toxicity, overdose, and death (Hernández-Serrano et al., 2016).

Depending on the specific combination of substances, the specific short- and long-term effects related to polysubstance usage will vary; nonetheless, there are some basic risks involved with polysubstance misuse. These consist of greater severity of adverse effects; acute health issues: and complications as a result of concurrent mental health issues (American Addiction Centres, 2022). When more than one drug is used at the same time, the effects become even more unpredictable. In addition to the elements described above, the effect of drug mixing is determined by the medications that are mixed together. Combining medicines with similar physical effects (for example, two or more stimulants or two or more depressants) is particularly risky. This is because it has a greater impact on regular brain and body functioning (National Drug and Alcohol Research Centre, 2014).

Drug toxicity can be increased by interactions between several medications taken close together in time. Some psychoactive substances' effects can increase the dangerous use of other compounds. Alcohol intoxication, for example, can alter judgments regarding the amount of opioids consumed or the danger of lowered tolerance following treatment or prison. Likewise, mixing cocaine and alcohol can enhance toxicological risks. The use of multiple substances can also increase the chance of an accident or injury (EMCDDA, 2022a).

In this study, polydrug seizures were identified in the impregnated paper samples. This analysis was made with respect to individual paper samples because most evidence bags included more than one sample and, in some samples, different substances were detected.

In total, there were 2499 individual paper samples that were analysed. In 756 of them, no drugs were detected.

- In 829 of them, one type of drug was detected,
- in 466 of them, two types of drug were detected,
- in 293 of them, three types of drug were detected,
- in 105 of them, four types of drug were detected,

- in 36 of them, five types of drug were detected,
- in 11 of them, six types of drug were detected and,
- in 3 of them, seven types of drug detected.

295 individual paper samples included only SCRA type drugs. Multiple SCRAs were seen in 155 individual paper samples. Only Class A, only Class B, only Class C, only nicotine, only miscellaneous, only other medicines, only prescribed medicines, and only abused medicines were detected in 269, 23, 16, 27, 3, 29, 6 and 6 individual paper samples respectively. SCRA + Class A drugs were seen in 224 individual paper samples. SCRA + Class A + Class B were seen in 174 individual paper samples. SCRA + Class B drugs were seen in 65 individual paper samples. Class A + Class B drugs were seen in 31 individual paper samples.

From 1790 individual paper samples from male prisons, 716 of them (40%) included polydrug, and from 709 individual paper samples from female prisons, 198 of them (28%) included polydrug. Polydrug use was more prevalent in male prisons.

6. DISCUSSION

NPS use is an emerging problem for prisoners and prison staff in English prisons, and approaches are needed to prevent NPS smuggling into prisons. Recently, impregnated postal letters have become one of the routes to bring drugs into prison. Analysis of drugs from seized drug-impregnated papers from prisons has been demonstrated to be a good indicator of drug prevalence and trends in drug use, supported by nationally published statistics. Generally, prisoner surveys and rMDT results are used to provide detailed and specific prevalence data for drug use in prisons. Prisoner surveys generally provide self-reported data and it may not be clear whether prisoners are reliable respondents. Detection of NPS may be a challenge with conventional screening methods because of insufficient information about their chemical composition due to being produced in clandestine laboratories without quality and control. Following their detection and control by law, newer versions of NPS that have modifications to their chemical structure to avoid legislation are manufactured. This makes it difficult to monitor and regulate these types of drugs. With rMDT tests, urinalysis may not provide a positive result for NPS used by the prisoner due to the distinct chemical composition of NPS, making its detection difficult. In addition, unknown levels of dose and metabolism of the newly emerged NPS may make it more difficult.

The analytical method conducted for this research overcomes the limitations of other methods. Mass spectrometric methods have been preferred over conventional screening methods such as immunoassays. Colorimetric tests or immunoassays have many advantages such as being fast, sensitive, inexpensive, and easy, but they lack specificity that can cause false positive results due to cross reactivity with other non-targeted compounds of similar chemical structure. Chromatographic methods coupled to mass spectrometry have been mostly used for identification due to their high separation capability and identification power. Moreover, NPS use changes very quickly, and the addition of new NPS MS data to the database and amendment of the mass spectrometric identification technique according to new versions of NPS in toxicological samples can be done very easily compared to conventional methods.

For the identification and analysis of drug-impregnated paper samples from English prisons, a reference standard-based spectral library containing MS data of NPS and related compounds was developed to be used with a Q-TOF-LC/MS based screening method. Q-TOF-LC/MS method was used in this study because of its high specificity, sensitivity, and ability to detect even a few traces

of the substance compared to conventional screening methods. Due to its detection capability of trace amounts, contamination might have been detected whether it was impregnated or not with that drug. For instance, for the prescription drugs or some drug metabolites, paper samples such as cards or letters might have been contaminated by the people's hands who wrote and sent them. Moreover, cross contamination due to prison staff who handle lots of paper samples and bulk drugs may occur in postal rooms of prisons. Storage conditions of paper samples and other drug samples seized in prisons can cause contamination of papers as well because they might be stored in large bags with many dozens of samples. For metabolites detected in some of the samples, sweat on the hands that produced contamination may cause metabolite formation from those drugs on the paper. These types of contamination can produce only a trace amount of drug on the paper samples and might be detected with this method even though they may not have actually been impregnated on paper.

For the samples including photos in this study, there was mostly no detection of a drug. The reason could be the texture of photos, which is different than plain paper, and that it may not be soaked with the drugs. The drug solution may just change the colour or appearance of the photo paper. Due to the change in colour of the photograph (which seems like a stain), it may be thought that the sample has been impregnated with a drug. It can be stated that the texture and type of paper have an effect on the impregnation of the drugs. Moreover, every colour change on the paper may not mean that the sample is infused with drugs. Visual inspection can give false information.

After method development, suspected drug-impregnated paper samples from prisons in England were screened in order to confirm the applicability of the method for use with real-world samples. In this study, 1250 drug-impregnated paper seizures were collected from 12 English prisons between 2018 and 2020 and screened with this method. From these 1250 seizures, 2499 individual paper samples were prepared, and 5107 substances were detected in all samples. The English prisons providing samples for this study included 10 male and 2 female prisons. There were four different security levels for the participating prisons, ranging from category A, B, C, and closed, and their geographical location was changing across six different regions in England. To analyse the data collected, the detected substances were divided into nine different groups with respect to the type of active compounds identified. The groups were: nicotine/cotinine, abused medicines (non-prescription), prescription medicines, other medicines (consisting of drugs not

abused and over-the-counter drugs), synthetic cannabinoid receptor agonists (SCRAs), miscellaneous (misc.), Class A drugs, other Class B drugs (excluding cannabis, SCRAs, and prescription), Class C drugs, and no drugs detected. “Miscellaneous” included any samples that did not fit into the above groups, such as caffeine. “No drugs detected” was used to indicate samples in which no active compounds were identified after the run on the Q-TOF LC/MS. Over the course of this study, no new active compounds were identified. he results were compared based on various variables such as gender, security level, and geographical location, and the data was statistically analysed to determine whether the variables were dependent on one another. Statistical analysis was carried out using Minitab software in order to carry out a Pearson Chi-square test on each drug type against sex, security level, and geographical location. Within each drug group, the total percentages represent the sum of all the groups detected in the seizure. Because a single seizure or individual paper sample may contain various types of drugs, sum totals will include certain seizures or individual paper samples more than once. In the analysis of the results and percentage calculations, this case needs to be considered. In this study, between 2018 and 2020, SCRAs were found to be the most prevalent drug group detected in drug-impregnated paper seizures from English prisons, making up 52.1% (651 seizures) of all the seizures (1250 seizures). It was followed by Class A and Class B drugs, making up 48.7% (609 seizures) and 24.5% (306 seizures) of all seizures. In 281 (22.5%) of the 1250 seizures, no active compounds were detected. Between 2018 and 2020, 5F-ADB was the most common SCRA detected in drug-impregnated individual paper samples by 67.7% (784 individual paper samples) of the all 1157 SCRA detected individual paper samples, followed by AMB-FUBINACA (30.3%, 351 individual paper samples), 5F-MDMB-PICA (23.5%, 272 individual paper samples) and 4F-MDMB-BINACA (10.0%, 116 individual paper samples). Class A drugs were the second prevalent drug group, consisting of 40.4% (1010 individual paper samples out of 2499 individual paper samples). The most common Class A drug detected was cocaine with 87.7% (913 individual paper samples) and followed by MDMA (24.9%, 252 individual paper samples) and heroin (12.3%, 124 individual paper samples). Other Class B drug group was the next prevalent drug group detected in 463 individual paper samples (18.5%) of all 2499 drug impregnated individual paper samples. Ketamine was the most common Class B drug detected by 88.8% (411 individual paper samples) of the 463 individual paper samples analysed in this group, and it was followed by amphetamine (14.0%, 65 individual paper samples) and codeine (3.7%, 17 individual paper samples). When the general population’s trends for drug use were compared to the findings in this study, they showed similar outcomes.

The prevalence of drug use in the general population might be a good indicator of the prevalence of drug use in the prison environment.

SCs were by far the most prevalent NPS group observed, followed by synthetic cathinones, synthetic opioids, novel benzodiazepines, and stimulants to a lesser extent. According to the literature, most NPS, particularly SCs, are smuggled into prisons via paper and herbal matrices, primarily via mail systems (Vaccaro et al., 2022). SCs were also the most prevalent drug type in English prisons in this study. Pregabalin (n=16, 1.28%), gabapentin (n=33, 2.64%) and etizolam (n=2, 0.16%) were seen over the three years in 1250 paper seizures, but the percentages were very small. Maybe it is because pregabalin, gabapentin, and etizolam are drugs that can be obtained illicitly within the prison environment because they are prescription drugs. Prison staff and healthcare professionals need to be more careful about these drugs. It is critical that safe prescription routines take place in prisons. Prison doctors are well aware of the risk of medication abuse in a variety of contexts. Such abuse can be directly related to the bullying and misuse that exists in some prisons. It may also expose inmates to direct and unforeseen injury as a result of unsupervised use of prescribed and non-prescribed medicines (Bicknell, 2013). In the UK, gabapentin and pregabalin will be reclassified as class C controlled compounds as of April 1, 2019. Pregabalin and gabapentin repeat dispensing will be prohibited as a result of the reclassification (Torjesen, 2019).

The drugs identified reflect drug use patterns within the prison system. The presented data on drug use among inmates typically reflects the entire prison population, regardless of the type of sentence. Even though many drug users cease or limit their drug usage when they reach prison, some continue to use - some may even begin to use drugs there (Montanari et al., 2014). Inmates have reportedly been observed smoking, chewing, and licking the letters, according to anecdotal observations by prison employees (Ford and Berg, 2018). These compounds may commonly be vaped because in some evidence bags collected in this study, vape cartridges and burned papers were seen. Because of the preparation procedures for papers illicitly soaked with SCs, drug concentrations may vary greatly from one location to the next, making it plausible that the compounds discovered here were more concentrated in some areas. According to the literature, the inhomogeneous distribution of drug concentrations across the paper may enhance the danger of overdose if users consume paper sections marked by greater SC concentrations, so-called "hot-

spots." This issue is especially concerning when papers contain both SCs and other NPSs (Giorgetti et al., 2022).

When the changes in the prevalence of different types of SCRAs over the years were analysed, it was observed that the number of 5F-ADB and AMB-FUBINACA detected paper seizures was sharply decreasing from 2018 to 2019. In the second half of 2018, the production and export of 5F-ADB and AMB-FUBINACA were under control by law in the People's Republic of China. Therefore, it can be seen that the availability of these SCRAs decreased in the English prisons due to regulation changes for these drugs at the border. There is evidence that chemical businesses based mostly in China, but maybe also in other countries such as India, promote the synthesis and dissemination of novel substances. In China, 32 NPS were legally prohibited in August 2018. There were eight SCRAs among them, including 5F-ADB and AMB-FUBINACA (EMCDDA, 2018a, UNODC, 2018a). According to this study, on the other hand, there was a sharp increase in the detection of 5F-MDMB-PICA and 4F-MDMB-BINACA in the drug-impregnated paper seizures. The detection of new alternatives with a similar structure to one of the banned SCRA (5F-ADB) by the People's Republic of China, which were 5F-MDMB-PICA and 4F-MDMB-BINACA, was increased in 2019. MDMB-4en-PINACA was firstly seen in 2020 drug-impregnated paper samples, which had a similar core structure and minor modifications compared to banned SCRAs. The legal situation of NPS in the People's Republic of China has a significant influence on the prevalence of SCRAs in the general population and also the prison population. According to our research observations, the new versions of SCRAs produced following the legislation generally had similar chemical structures with slight alterations to the core structure. Therefore, the chemical composition of new SCRAs might be predicted before they become common in the UK market. In addition, by following the legislation on SCRAs in the People's Republic of China, the new alternatives to SCRAs may be predicted and controlled early.

The majority of seizures with no obvious staining or contamination test negative. The ones without stain giving positive results may be because of the contamination because all samples were stored together and prison staff dealing with these samples may have been contaminated when handling the samples (without changing gloves, putting them together with bulk drugs). For those with stains but negative results, the sender of the letter may have spilled coffee or something completely unrelated to a drug on the letter, which may have caused the stain. These samples may have come to be intercepted and taken as suspicious due to a Rapiscan test result,

stopped by sniffer dogs or looked a bit dodgy. Rapiscan tests can give false positive results due to being hypersensitive even if there is a trace amount of substance on there (contamination).

The use of each smuggling route and the traffic flowing along it will vary from time to time and from place to place (Blakey, 2008). The means of supply may change due to improvements in searching during visits and inside the prisons. When insufficient searching is done in a prison during visits, smuggling a drug by a visitor can be very easy. Drug supply with a post or parcel by impregnating letters has become popular because of the low chance of being intercepted by prison staff. However, in some prisons, the letters were photocopied and the copy was given to the prisoner (not the original one) to prevent this method of supply (Pooran, 2022).

Drug prevalence was observed over sex by analysing male and female-only prisons in this study. Two of the prisons that provided paper seizures for this study were female, and ten of the prisons that provided paper seizures for this study were male. The large difference between the estimated populations of these prisons with respect to sex showed that the results for the prevalence of drugs based on sex need further investigation with a larger dataset for female prisons. According to these study findings, the most prevalent drug detected in drug-impregnated paper seizures in the male prisons was SCRAs by 63.3% (576 seizures) of all the 910 seizures from the male prisons between 2018 and 2020. 5F-ADB was the most prevalent SCRA detected in drug-impregnated paper seizures from male prisons, making up 63.0% (363 seizures out of all 576 SCRA detected seizures). In this study, for female prisons, the most common drug group was observed to be different compared to male prisons. Class A drugs were most commonly detected in the drug-impregnated paper seizures, consisting of 45.95% (156 seizures) of all 340 samples from only female prisons. The most prevalent Class A drug detected in impregnated paper seizures from female prisons was found to be cocaine in 93.5% (146 seizures) of all 156 Class A detected seizures. Heroin and MDMA followed with 19.9% (31 seizures) and 16.0% (25 seizures) of all 156 Class A detected seizures in female prisons. Prevalence of NPS, in which SCRAS were the most often seized category by the police and border forces, was higher in male adults than female adults according to CSEW statistics on drug misuse in 2018/19 datasets (Home Office, 2019) which was similar to findings of this study. Considering these results, the change in the prevalence data for different sex groups may be helpful for the management of drug detection and drug treatment plans in female and male prisons.

When comparing drug prevalence data with respect to security levels of prisons, female-only prisons (one Category A and one closed category) were excluded due to the disparity between prison populations and the number of collected seizures in order not to get skewed results. For category C male prisons, SCRAs were the most prevalent drug group detected in drug-impregnated seizures, making up 76.1% (362 seizures) of the total 476 seizures in this category. It was followed by Class A and Class B drugs, with a percentage prevalence of 53.2% (253 seizures) and 36.3% (173 seizures) of a total of 476 seizures for this category of prison. For the higher security level category B prisons, the prevalence of SCRAs, Class A and Class B drugs detected in drug-impregnated paper seizures between 2018 and 2020 was 49.3% (214 seizures), 46.1% (200 seizures) and 18.4% (80 seizures) respectively. It could be understood that a security level category may have a significant effect on the prevalence of drug groups in prisons. Smuggling drugs to category C prisons might be easier due to a lack of security compared to category B prisons. According to the findings of this study, the prevalence data may be used for security and detection measurements and for drug smuggling prevention to catch it before it becomes common in prison.

Drug prevalence analysis was conducted for the prisons located in five different regions in England. Data from only 10 male prisons was used to prevent skewing of the results. In order to compromise the anonymity of the prisons participating in this study, which would be in breach of the confidentiality agreement regions were used to denote the locations as any smaller scale classifications. SCRAs were the most prevalent drug group detected in drug-impregnated paper seizures between 2018 and 2020 in the west of England, making up 58.8% (10 seizures out of 17) in the West Midlands and 34.6% (9 seizures out of 26) in the South West region. When the prevalence in the east of England was analysed, the most prevalent drug group was SCRAs by 88.7% (126 seizures) and 75.0% (246 seizures) of the seizures analysed in the Eastern and Yorkshire regions (142 seizures and 328 seizures, respectively). In the south of England, Class A drug group was the most prevalent in the Eastern region, consisting of 78.9% (112 seizures) of the total 142 seizures in this region. In the east of England, Class A drug prevalence in Eastern region (78.9%, 112 seizures) was the highest among the South East and Yorkshire regions (50.1%, 199 seizures and 38.1%, 125 seizures). Despite these findings, no significant overall trends were found among drug group prevalence in the north, south, and east of the country. Although drug prevalence trends were observed in individual regions of the country, the lack of prisons included in the results meant that it was highly unlikely that these results were reflections of trends in

location. To the best of the author's knowledge, there is inadequate research about drug prevalence trends in prisons with respect to different locations, and thus there is little literature to support or refute these findings. Moreover, the seizure size, which ranged from 17 seizures for the West Midlands region to 397 seizures for the South East, may have had an influence on the observed results. In particular, out of the ten male prisons examined, only one prison was located in the West Midlands. For each region, two prisons were located in the South West, South East, and Eastern, and three prisons were located in the Yorkshire region. Consequently, it is considerably more likely that the results obtained for these regions might indicate individual prison tendencies rather than regional trends. A homogeneously distributed number of seizures collected from different prisons in various locations of the country would provide more information about potential trends in drug prevalence and prison location.

The goal of this research is to help rapid detection and definite identification for analysis of seized drug-impregnated papers in English prisons and to check the association between drug prevalence and different security levels, different geographical locations and sex between 2018 and 2020. To the best of the author's knowledge, it is the first comprehensive study reporting analysis of seized drug-impregnated papers in English prisons with respect to different security levels, different geographical locations and sex between 2018 and 2020. Ford and Berg (Ford and Berg, 2018) reported the first analytical confirmation that the letters sent to inmates are being used to smuggle NPS into UK prisons. According to their findings, they detected NPS on the analysed letters which contained the stimulants ethylphenidate, methiopropamine, and methoxiphenidaine, the sedative etizolam, and the third generation SCRAs 5F-AKB-48, AB-FUBINACA and MDMB-CHMICA. Other substances detected were the Class A drug cocaine, Class B drug methylphenidate and the cutting agents lignocaine, benzocaine and procaine. However, their sample set was very limited, including only five drug-impregnated letters that may not give an overall use of these drugs in English prisons. There were other studies (Norman et al., 2020, Antonides et al., 2019, Norman et al., 2021b, Antonides et al., 2021, Norman et al., 2021a) from a research group from the University of Dundee in Scotland. They reported the detection, quantitation, and pharmacological evaluation of SCRAs in impregnated papers from Scottish prisons using different methods. However, their research was focused on Scottish prisons. There were no reported detections of English prisons from this research group. When compared with our study findings, the most notable difference between detections in Scottish and English prisons was that 5F-MDMB-PICA has not been detected in any 2018 paper seizures in English prisons,

although it was one of the detected SCRA in Scottish prisons at the end of 2018. In both Scottish and English prisons, 5F-ADB and AMB-FUBINACA were detected in 2018 paper seizures. 4F-MDMB-BINACA was first detected in 2019 in impregnated paper seizures in both Scottish and English prisons. MDMB-4en-PINACA was first detected in 2019 in Scottish prisons, while it was detected for the first time in English prisons in 2020. ADB-BUTINACA was first detected in impregnated paper seizures in English prisons in 2020, whereas it was detected for the first time in Scottish prisons in 2021.

This research confirms that NPS being brought into UK prisons via drug-impregnated letters posted to prisoners still remains a problem. The author of this thesis hopes that the proposed research and approach will motivate policymakers as well as serve as a basis for future study on NPS detection. Furthermore, accurate data on the prevalence, use, and effects of the drugs is critical in assessing the better management of the issues associated with the drugs, such as adverse health effects on drug users and potential safety and security concerns the drugs may pose to other prisoners, prison staff, and the prison environment as a whole.

7. CONCLUSION & FUTURE WORK

This research was conducted in order to build a spectral database and to develop a qualitative Q-TOF LC/MS method to identify and analyse the variety of emerging NPS and related compounds in seized impregnated papers from English prisons between 2018 and 2020. In this study, 1,250 suspected drug-impregnated paper seizures from twelve English prisons (ten male and two female) located in the South East, South West, West Midlands, East, and Yorkshire and Humber regions, including category A, category B, category C, and closed category prisons, were collected and screened. From these seizures, 2499 individual paper samples were prepared and 5107 substances were detected. The research findings show that SCRA was the most prevalent drug group detected in drug-impregnated paper seizures in English prisons between 2018 and 2020 and was followed by Class A drugs, Class B drugs, other medicines, nicotine/cotinine, miscellaneous, Class C drugs, prescription medicines, and abused medicines, respectively. With respect to differences in sex, SCRAs had a higher prevalence in male prisons, whereas female prisons presented a higher prevalence of Class A drugs. Furthermore, male prisons with lower security levels (category C) appeared to have a higher prevalence of Class B, Class C, and abused prescription drugs compared to category B prisons, which had a higher prevalence of nicotine. Finally, although drug prevalence trends were observed in individual regions of the country, the lack of prisons included in the results meant that it was highly unlikely that these results were reflections of trends in location.

The outcomes of this study have provided new information regarding drug use in prison. These findings may be significant as police information and could be utilised to aid drug policymakers in prisons, particularly in the development of treatment plans addressing the most commonly misused drugs in each prison. Additionally, the datasheets on the identity of samples obtained from each prison can be given to the involved prisons, providing them with data about the samples being seized. This analysis would help in the identification of drug smuggling routes into jails, enabling prison staff to pay more attention to these sites. Information on the distribution of drug prevalence across England's regions may aid in the detection of drug-trafficking organisations that contribute to the supply.

Using a larger and more homogeneous dataset to estimate drug prevalence in prisons

This research was used for assessing drug prevalence and provided findings that were mainly dependent on sample size. Therefore, it is essential that future research maintain a sample size homogenously distributed over the different variables to allow for a better evaluation. Thus, future research should include more prisons to provide samples for both a larger range of security levels and geographical locations, possibly even expanding into jails across the UK instead of focusing only on English prisons. The regular monitoring of drug prevalence in these prisons will aid in the determination of long-term trends in drug prevalence across the country.

Updating the spectral database and the analytical method according to the newly emerging NPS

It is important to continuously follow the varying drug trends, mainly for NPS, and integrate new compounds into analytical techniques as rapidly as possible in order to catch positive results within the population. This may help to prevent the future appearance and spread of NPS in prisons.

Detection and quantification of the possible environmental contaminants on the impregnated papers

It is important to detect contamination and quantify the level of contamination on paper samples to understand whether they are actually impregnated on the papers or not. An analysis can be made by using a reference standard of the compound that is believed to contaminate paper samples to contaminate samples from 1) several pieces of blank paper, 2) prison paper for which the analysed sample had no drugs detected, and 3) an unstained far corner of a piece of paper from which possible contamination substances were detected.

8. APPENDICES

Appendix 1: Statistical Analysis

Sex

Chi-Square Test for Association: Sex, SCRAs

Rows: Sex Columns: SCRA

	0	1	All
Female	265	75	340
	162.9	177.1	
Male	334	576	910
	436.1	473.9	
All	599	651	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	168.661	1	0.000
Likelihood Ratio	175.510	1	0.000

Chi-Square Test for Association: Sex, NICOTINE/COTININE

Rows: Sex Columns: NICOTINE/COTININE

	0	1	All
Female	302	38	340
	316.34	23.66	
Male	861	49	910
	846.66	63.34	
All	1163	87	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	12.822	1	0.000
Likelihood Ratio	11.747	1	0.001

Chi-Square Test for Association: Sex, CLASS A

Rows: Sex Columns: CLASS A

	0	1	All
Female	184	156	340
	174.4	165.6	
Male	457	453	910
	466.6	443.4	
All	641	609	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	1.505	1	0.220
Likelihood Ratio	1.507	1	0.220

Chi-Square Test for Association: Sex, CLASS B

Rows: Sex Columns: CLASS B

	0	1	All
Female	287	53	340
	256.8	83.2	

Male	657	253	910
	687.2	222.8	
All	944	306	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	19.973	1	0.000
Likelihood Ratio	21.328	1	0.000

Chi-Square Test for Association: Sex, CLASS C

Rows: Sex Columns: CLASS C

	0	1	All
Female	313	27	340
	319.60	20.40	
Male	862	48	910
	855.40	54.60	
All	1175	75	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	3.120	1	0.077
Likelihood Ratio	2.956	1	0.086

Chi-Square Test for Association: Sex, MISCALLENOUS

Rows: Sex Columns: MISCALLENOUS

	0	1	All
Female	305	35	340
	319.06	20.94	
Male	868	42	910
	853.94	56.06	
All	1173	77	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	13.808	1	0.000
Likelihood Ratio	12.554	1	0.000

Chi-Square Test for Association: Sex, OTHER MEDICINES

Rows: Sex Columns: OTHER MEDICINES

	0	1	All
Female	295	45	340
	291.3	48.7	
Male	776	134	910
	779.7	130.3	
All	1071	179	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	0.448	1	0.503
Likelihood Ratio	0.454	1	0.500

Chi-Square Test for Association: Sex, PRESCRIPTION MEDICINES

Rows: Sex Columns: PRESCRIPTION MEDICINES

	0	1	All
Female	321	19	340
	327.22	12.78	
Male	882	28	910
	875.78	34.22	
All	1203	47	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	4.314	1	0.038
Likelihood Ratio	3.993	1	0.046

Chi-Square Test for Association: Sex, ABUSED MEDICINES

Rows: Sex Columns: ABUSED MEDICINES

	0	1	All
Female	325	15	340
	328.30	11.70	
Male	882	28	910
	878.70	31.30	
All	1207	43	1250

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	1.328	1	0.249
Likelihood Ratio	1.264	1	0.261

Prison Category

Chi-Square Test for Association: Prison Cat, SCRAs

Rows: Prison Cat Columns: SCRA

	0	1	All
Category B	220	214	434
	159.3	274.7	
Category C	114	362	476
	174.7	301.3	
All	334	576	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	69.879	1	0.000
Likelihood Ratio	70.748	1	0.000

Chi-Square Test for Association: Prison Cat, NICOTINE/COTININE

Rows: Prison Cat Columns: NICOTINE/COTININE

	0	1	All
Category B	409	25	434
	410.63	23.37	
Category C	452	24	476
	450.37	25.63	
All	861	49	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	0.230	1	0.632
Likelihood Ratio	0.230	1	0.632

Chi-Square Test for Association: Prison Cat, CLASS A

Rows: Prison Cat Columns: CLASS A

	0	1	All
Category B	234	200	434
	218.0	216.0	
Category C	223	253	476
	239.0	237.0	
All	457	453	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	4.537	1	0.033
Likelihood Ratio	4.541	1	0.033

Chi-Square Test for Association: Prison Cat, CLASS B

Rows: Prison Cat Columns: CLASS B

	0	1	All
Category B	354	80	434
	313.3	120.7	
Category C	303	173	476
	343.7	132.3	
All	657	253	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	36.284	1	0.000
Likelihood Ratio	37.024	1	0.000

Chi-Square Test for Association: Prison Cat, CLASS C

Rows: Prison Cat Columns: CLASS C

	0	1	All
Category B	421	13	434
	411.11	22.89	
Category C	441	35	476
	450.89	25.11	
All	862	48	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	8.627	1	0.003
Likelihood Ratio	8.995	1	0.003

Chi-Square Test for Association: Prison Cat, MISCELLANEOUS

Rows: Prison Cat Columns: MISCELLANEOUS

	0	1	All
Category B	417	17	434
	413.97	20.03	
Category C	451	25	476
	454.03	21.97	
All	868	42	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	0.919	1	0.338
Likelihood Ratio	0.926	1	0.336

Chi-Square Test for Association: Prison Cat, OTHER MEDICINES

Rows: Prison Cat Columns: OTHER MEDICINES

	0	1	All
Category B	379	55	434
	370.09	63.91	
Category C	397	79	476
	405.91	70.09	
All	776	134	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	2.784	1	0.095
Likelihood Ratio	2.800	1	0.094

Chi-Square Test for Association: Prison Cat, PRESCRIPTION MEDICINES

Rows: Prison Cat Columns: PRESCRIPTION MEDICINES

	0	1	All
Category B	421	13	434
	420.65	13.35	
Category C	461	15	476
	461.35	14.65	
All	882	28	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	0.018	1	0.892
Likelihood Ratio	0.019	1	0.892

Chi-Square Test for Association: Prison Cat, ABUSED MEDICINES

Rows: Prison Cat Columns: ABUSED MEDICINES

	0	1	All
Category B	425	9	434
	420.65	13.35	
Category C	457	19	476
	461.35	14.65	
All	882	28	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	2.800	1	0.094
Likelihood Ratio	2.874	1	0.090

Geographical Location

Chi-Square Test for Association: Geo_Loc, SCRAs

Rows: Geo_Loc Columns: SCRA

	0	1	All
Eastern	16	126	142
	52.12	89.88	
South East	212	185	397
	145.71	251.29	
South West	17	9	26
	9.54	16.46	
West Mid	82	246	328
	120.39	207.61	
Yorkshire	7	10	17
	6.24	10.76	
All	334	576	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	115.877	4	0.000
Likelihood Ratio	122.412	4	0.000

Chi-Square Test for Association: Geo_Loc, NICOTINE/COTININE

Rows: Geo_Loc Columns: NICOTINE/COTININE

	0	1	All
Eastern	140	2	142
	134.35	7.65	
South East	371	26	397
	375.62	21.38	
South West	20	6	26
	24.60	1.40	
West Mid	313	15	328
	310.34	17.66	
Yorkshire	17	0	17
	16.08	0.92	

All	861	49	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF
Pearson	22.829	4
Likelihood Ratio	18.664	4

1 cell(s) with expected counts less than 1.
Chi-Square approximation probably invalid.
2 cell(s) with expected counts less than 5.

Chi-Square Test for Association: Geo_Loc, CLASS A

Rows: Geo_Loc Columns: CLASS A

	0	1	All
Eastern	30	112	142
	71.31	70.69	
South East	198	199	397
	199.37	197.63	
South West	11	15	26
	13.06	12.94	
West Mid	203	125	328
	164.72	163.28	
Yorkshire	15	2	17
	8.54	8.46	
All	457	453	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	76.444	4	0.000
Likelihood Ratio	80.997	4	0.000

Chi-Square Test for Association: Geo_Loc, CLASS B

Rows: Geo_Loc Columns: CLASS B

	0	1	All
Eastern	74	68	142
	102.52	39.48	
South East	319	78	397
	286.63	110.37	
South West	13	13	26
	18.77	7.23	
West Mid	238	90	328
	236.81	91.19	
Yorkshire	13	4	17
	12.27	4.73	
All	657	253	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	48.250	4	0.000
Likelihood Ratio	45.706	4	0.000

1 cell(s) with expected counts less than 5.

Chi-Square Test for Association: Geo_Loc, CLASS C

Rows: Geo_Loc Columns: CLASS C

	0	1	All
Eastern	134	8	142
	134.51	7.49	
South East	378	19	397
	376.06	20.94	
South West	17	9	26
	24.63	1.37	
West Mid	317	11	328
	310.70	17.30	
Yorkshire	16	1	17
	16.10	0.90	
All	862	48	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF
Pearson	47.459	4
Likelihood Ratio	24.271	4

1 cell(s) with expected counts less than 1.
Chi-Square approximation probably invalid.
2 cell(s) with expected counts less than 5.

Chi-Square Test for Association: Geo_Loc, MISCALLENOUS

Rows: Geo_Loc Columns: MISCALLENOUS

	0	1	All
Eastern	127	15	142
	135.45	6.55	
South East	378	19	397
	378.68	18.32	
South West	24	2	26
	24.80	1.20	
West Mid	322	6	328
	312.86	15.14	
Yorkshire	17	0	17
	16.22	0.78	
All	868	42	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF
Pearson	18.603	4
Likelihood Ratio	18.023	4

1 cell(s) with expected counts less than 1.
Chi-Square approximation probably invalid.
2 cell(s) with expected counts less than 5.

Chi-Square Test for Association: Geo_Loc, OTHER MEDICINES

Rows: Geo_Loc Columns: OTHER MEDICINES

	0	1	All

Eastern	129	13	142
	121.09	20.91	
South East	338	59	397
	338.54	58.46	
South West	18	8	26
	22.17	3.83	
West Mid	274	54	328
	279.70	48.30	
Yorkshire	17	0	17
	14.50	2.50	
All	776	134	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF	P-Value
Pearson	12.569	4	0.014
Likelihood Ratio	14.443	4	0.006

2 cell(s) with expected counts less than 5.

Chi-Square Test for Association: Geo_Loc, PRESCRIPTION MEDICINES

Rows: Geo_Loc Columns: PRESCRIPTION MEDICINES

	0	1	All
Eastern	139	3	142
	137.63	4.37	
South East	386	11	397
	384.78	12.22	
South West	20	6	26
	25.20	0.80	
West Mid	320	8	328
	317.91	10.09	
Yorkshire	17	0	17
	16.48	0.52	
All	882	28	910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF
Pearson	36.428	4
Likelihood Ratio	17.103	4

2 cell(s) with expected counts less than 1.
Chi-Square approximation probably invalid.
3 cell(s) with expected counts less than 5.

Chi-Square Test for Association: Geo_Loc, ABUSED MEDICINES

Rows: Geo_Loc Columns: ABUSED MEDICINES

	0	1	All
Eastern	132	10	142
	137.63	4.37	
South East	388	9	397
	384.78	12.22	
South West	26	0	26
	25.20	0.80	
West Mid	319	9	328
	317.91	10.09	
Yorkshire	17	0	17

		16.48	0.52
All		882	28 910

Cell Contents
Count
Expected count

Chi-Square Test

	Chi-Square	DF
Pearson	9.847	4
Likelihood Ratio	9.305	4

2 cell(s) with expected counts less than 1.
Chi-Square approximation probably invalid.
3 cell(s) with expected counts less than 5.

Appendix 2: Drug groups used in this study

Prescription Medicines	**Abused Medicines**	**Class A**	**Class B**	**Class C**	**SCRAs**	**Miscallenous**	**Other Medicines**
Amitriptyline Mirtazapine Trazodone Hydroxyzine Haloperidol Carbamazepine Venlafaxine Desvenlafaxine Procyclidine Lamotrigine Sertraline Flupentixol	Quetiapine Glaucine Dextromethorphan Methoxphenidine	Schedule 1 Cocaine MDMA 2C-B Mescaline PMMA 5-MeO-DALT Schedule 2 Morphine Oxymorphone Oxycodone Meperidine Hydrocodone Heroin Methadone	Schedule 1 Ketamine Mephedrone Amphetamine Ethylone Methaqualone Methylone Schedule 5 Codeine Dihydrocodeine	Schedule 1 Etizolam Flubromazolam Flubromazepam Schedule 3 Buprenorphine Pregabalin Gabapentin Tramadol Diazepam Schedule 4 Alprazolam	5F-ADB AMB-FUBINACA NM-2201 ADB-FUBINACA ADB-CHMINACA ADB-BUTINACA 4F-MDMB-BINACA 5F-MDMB-PICA 5F-AKB-48 MMB-2201 MDMB-CHMICA EMB-FUBINACA AB-FUBINACA 5C-AKB-48 MDMB-4en-PINACA 5F-MPP-PICA MMB-022 MDMB-CHMINACA	Diphenylguanidine Levamisole Phenacetin Lidocaine	Mebeverine Cetirizine Paracetamol Caffeine Metacetamol Promethazine Diphenhydramine

Appendix 3: Data

Data used in this research includes confidential information and cannot be shared by the public. Detailed spreadsheet for the data may be shared upon request. Requests to access these datasets should be directed to Asena Avci Akca.

Appendix 4: Database

Name	Formula	Mass	RT	CAS Number	ChemSpider Number	IUPAC name
2C-B	C10H14BrNO2	259.0208	5.948	66142-81-2	88978	2-(4-bromo-2,5-dimethoxyphenyl)ethanamine
2C-I	C10H14INO2	307.0069	6.359	69587-11-7	8442670	2-(4-iodo-3-methoxyphenyl)-2-methoxyethanamine
3-Acetamidophenol	C8H9NO2	151.0633	3.83	103-90-2	1906	N-(4-Hydroxyphenyl)acetamide
4F-MDMB-BINACA	C19H26FN3O3	363.1958	9.16	2390036-46-9	71117201	Methyl (S)-2-(1-(4-fluorobutyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate
4-MEC (4-Methylethcathinone)	C12H17NO	191.131	4.975	1225617-18-4	25630091	(RS)-2-ethylamino-1-(4-methylphenyl)propan-1-one
5C-AKB-48 (5-Chloro-APINACA)	C23H30ClN3O	399.2077	10.583	2160555-52-0	95533410	N-((3s,5s,7s)-adamantan-1-yl)-1-(5-chloropentyl)-1H-indazole-3-carboxamide
5F-AB-PINACA	C18H25FN4O2	348.1962	8.298	1800101-60-3	29763723	N-[(2S)-1-amino-3-methyl-1-oxobutan-2-yl]-1-(5-fluoropentyl)indazole-3-carboxamide
5F-ADB (5F-MDMB-PINACA)	C20H28FN3O3	377.2115	9.45	1715016-75-3	32741709	Methyl (S)-2-[1-(5-fluoropentyl)-1H-indazole-3-carboxamido]-3,3-dimethylbutanoate
5F-AKB-48	C23H30FN3O	383.2373	10.4	1400742-13-3	29339965	N-(adamantan-1-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide
5F-MDMB-PICA	C21H29FN2O3	376.2162	9.136	1971007-88-1	68003951	Methyl (2S)-2-[[1-(5-fluoropentyl)indole-3-carbonyl]amino]-3,3-dimethylbutanoate
5F-MN-24 (5F-NNE1)	C24H23FN2O	374.1794	9.112	1445580-60-8	29341632	1-(5-Fluoropentyl)-N-(naphthalen-1-yl)-1H-indole-3-carboxamide
5F-MPP-PICA	C24H27FN2O3	410.2006	8.872		71117173	Methyl 2-[[1-(5-fluoropentyl)indole-3-carbonyl]amino]-3-phenyl-quinolin-8-yl 1-(5-fluoropentyl)indazole-3-carboxylate
5F-NPB-22	C22H20FN3O2	377.154	8.99	1445579-79-2	30922492	
5-methoxy-DALT (5-MeO-DALT)	C17H22N2O	270.1732	5.55	928822-98-4	21106245	N-[2-(5-methoxy-1H-indol-3-yl)ethyl]-N-prop-2-enylprop-2-en-1-amine
6-Acetylmorphine	C19H21NO4	327.1471	4.15	2784-73-8	4575434	(4R,4aR,7S,7aR,12bS)-9-hydroxy-3-methyl-2,3,4,4a,7,7a-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinolin-7-yl acetate
7-Aminoclonazepam	C15H12ClN3O	285.0669	5.508	4959-17-5	163665	7-Amino-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one
AB-CHMINACA	C20H28N4O2	356.2212	9.477	1185887-21-1	30646774	N-[(2S)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)indazole-3-carboxamide
AB-FUBINACA	C20H21FN4O2	368.1649	8.613	1185282-01-2	28537614	N-[(2S)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]indazole-3-carboxamide
AB-PINACA	C18H26N4O2	330.2056	9.077	1445752-09-9	28537615	N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-pentyl-1H-indazole-3-carboxamide
ACP (Zopiclone Degradation Product)	C5H5ClN2	128.0141	3.82	1072-98-6	59561	2-Amino-5-chloropyridine
ADB-BUTINACA	C18H26N4O2	330.2056	8.873	2682867-55-4	81407832	N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-butyl-1H-indazole-3-carboxamide
ADB-CHMINACA	C21H30N4O2	370.2369	9.742		48059556	N-[(2S)-1-Amino-3,3-dimethyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)indazole-3-carboxamide
ADB-FUBINACA	C21H23FN4O2	382.1805	8.887	1185282-00-1	29763706	N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide
AKB-48 (APINACA)	C23H31N3O	365.2467	10.88	1345973-53-6	28189076	N-(1-adamantyl)-1-pentylindazole-3-carboxamide
Alfentanil	C21H32N6O3	416.2536	7.42	71195-58-9	46451	N-{1-[2-(4-Ethyl-5-oxo-4,5-dihydro-1H-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-4-piperidinyl}-N-phenylpropanamide
Alprazolam	C17H13ClN4	308.0829	7.97	28981-97-7	2034	8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine
AM-1220	C26H26N2O	382.2045	7.69	137642-54-7	26231035	[1-[[(2R)-1-methyl-2-piperidyl]methyl]indol-3-yl]-(1-naphthyl)methanone
AM-2201	C24H22FNO	359.1685	9.59	335161-24-5	24751884	[1-(5-fluoropentyl)indol-3-yl]-naphthalen-1-ylmethanone

AM-2233	C22H23IN2O	458.0855		444912-75-8	8401836	1-[(N-methylpiperidin-2-yl)methyl]-3-(2-iodobenzoyl)indole
AM-694	C20H19FINO	435.0495	9.18	335161-03-0	8064843	[1-?(5-?fluoropentyl)-?1H-?indol-?3-?yl](2-?iodophenyl)-?methanone
Amantadine	C10H17N	151.1361	5.047	768-94-5	2045	1-Adamantanamine
AMB-FUBINACA	C21H22FN3O3	383.1645	9.251	1745016-76-4	32741679	methyl (2S)-2-{[1-[(4-fluorophenyl)methyl]indazole-3-carbonyl]amino}-3-methylbutanoate
Amfetamine	C9H13N	135.1048	4.212	51-64-9	5621	(2S)-1-Phenyl-2-propanamine
Amisulpride	C17H27N3O4S	369.1722	4.4	71675-85-9	2074	4-Amino-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(ethylsulfonyl)-2-methoxybenzamide
Amitriptyline	C20H23N	277.1831	7.71	50-48-6	2075	3-(10,11-Dihydro-5H-dibenzo[a,d][7]annulen-5-ylidene)-N,N-dimethyl-1-propanamine
Amlodipine	C20H25ClN2O5	408.1452	7.743	88150-42-9	2077	(RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate
APICA (2NE1)	C24H32N2O	364.2515	10.52	1345973-50-3	29341717	N-(1-adamantyl)-1-pentylindole-3-carboxamide
Aripiprazole	C23H27Cl2N3O2	447.148	7.97	129722-12-9	54790	7-[4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butoxy]-3,4-dihydro-1H-quinolin-2-one
Atenolol	C14H22N2O3	266.163	3.41	29122-68-7	2162	2-{4-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl}acetamide
Azacyclonol	C18H21NO	267.1623	6.37	115-46-8	14952	Diphenyl(piperidin-4-yl)methanol
BB-22 (QUCHIC)	C25H24N2O2	384.1838	10.16	1400742-42-8	29339967	1-(cyclohexylmethyl)-1H-indole-3-carboxylic acid 8-quinolinyl ester
Benperidol	C22H24FN3O2	381.1853	6.37	2062-84-2	15521	1-{1-[4-(4-Fluorophenyl)-4-oxobutyl]-4-piperidinyl}-1,3-dihydro-2H-benzimidazol-2-one
Benzoylecgonine	C16H19NO4	289.1314	4.97	519-09-5	395095	(1R,2R,3S,5S)-3-(Benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid
Bisoprolol	C18H31NO4	325.2253	6.47	66722-44-9	2312	1-{4-[(2-Isopropoxyethoxy)methyl]phenoxy}-3-(isopropylamino)-2-propanol
Bromazepam	C14H10BrN3O	315.0007	7.22	1812-30-2	2347	7-Bromo-5-(2-pyridinyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Buprenorphine	C29H41NO4	467.3036	7.75	52485-79-7	559124	(5alpha,14beta,18R)-17-(Cyclopropylmethyl)-18-[(2S)-2-hydroxy-3,3-dimethyl-2-butanyl]-6-methoxy-18,19-dihydro-4,5-epoxy-6,14-ethenomorphinan-3-ol
Caffeine	C8H10N4O2	194.0804	4.6	58-08-2	2424	1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione
Carbamazepine	C15H12N2O	236.095	7.4	298-46-4	2457	5H-Dibenzo[b,f]azepine-5-carboxamide
Cetirizine	C21H25ClN2O3	388.1554	8.133	83881-51-0	2577	(±)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1- piperazinyl]ethoxy]acetic acid
Chlorcyclizine	C18H21ClN2	300.1393	7.7	82-93-9	2609	1-[(4-Chlorophenyl)(phenyl)methyl]-4-methylpiperazine
Chlordiazepoxide	C16H14ClN3O	299.0825	8.14	58-25-3	10248513	7-Chloro-N-methyl-5-phenyl-3H-1,4-benzodiazepin-2-amine 4-oxide
Chloroquine	C18H26ClN3	319.1815	4.39	54-05-7	2618	N~4~-(7-Chloro-4-quinolinyl)-N~1~,N~1~-diethyl-1,4-pentanediamine
Chlorpheniramine	C16H19ClN2	274.1237	6.56	132-22-9	2624	3-(4-Chlorophenyl)-N,N-dimethyl-3-(2-pyridinyl)-1-propanamine
Chlorpromazine	C17H19ClN2S	318.0958	8.019	50-53-3	2625	3-(2-Chloro-10H-phenothiazin-10-yl)-N,N-dimethyl-1-propanamine
Citalopram	C20H21FN2O	324.1638	6.75	59729-33-8	2669	1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile
Clobazam	C16H13ClN2O2	300.0666	7.74	22316-47-8	2687	7-Chloro-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4(3H,5H)-dione
Clomipramine	C19H23ClN2	314.155	8.27	303-49-1	2699	3-(3-Chloro-10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethyl-1-propanamine
Clonazepam	C15H10ClN3O3	315.0411	7.47	1622-61-3	2700	5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2H-1,4-benzodiazepin-2-one

Clopidogrel Metabolite 1 (SR26334, clopidogrel carboxylic acid)	C15H14ClSNO2	307.0434	5.95	144750-42-5	8037099	(2S)-(2-Chlorophenyl)(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetic acid
Clozapine	C18H19ClN4	326.1298	7.18	5786-21-0	10442628	8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine
Cocaine	C17H21NO4	303.1471	5.31	50-36-2	10194104	Methyl (1R,2R,3S,5S)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate
Codeine	C18H21NO3	299.1521	3.59	76-57-3	4447447	(5alpha,6alpha)-3-Methoxy-17-methyl-7,8-didehydro-4,5-epoxymorphinan-6-ol
Colchicine	C22H25NO6	399.1682	6.73	64-86-8	5933	N-[(7S)-1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide
Coumachlor	C19H15ClO4	342.0659	8.85	81-82-3	10443016	3-[1-(4-chlorophenyl)-3-oxobutyl]-2-hydroxychromen-4-one
Cyclizine	C18H22N2	266.1783	7.04	82-92-8	6470	1-(Diphenylmethyl)-4-methylpiperazine
Dehydroaripiprazole	C23H25Cl2N3O2	445.1324	7.89	129722-25-4	8290042	7-{4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]butoxy}-2(1H)-quinolinone
Demoxepam	C15H11ClN2O2	286.0509	7.15	963-39-3	10441314	7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one 4-oxide
Desalkylflurazepam	C15H10ClFN2O	288.0466	8.02	2886-65-9	4381	7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Desethylamiodarone	C23H25I2NO3	616.9924	9.507	83409-32-9	94581	(2-butyl-1-benzofuran-3-yl)-[4-[2-(ethylamino)ethoxy]-3,5-diiodophenyl]methanone
Desipramine	C18H22N2	266.1783	7.72	50-47-5	2888	3-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)-N-methyl-1-propanamine
Dextromethorphan	C18H25NO	271.1936	6.79	125-71-3	13109865	(9alpha,13alpha,14alpha)-3-Methoxy-17-methylmorphinan
Diazepam	C16H13ClN2O	284.0716	8.54	439-14-5	2908	7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Diclofenac	C14H11Cl2NO2	295.0167	8.98	15307-86-5	2925	{2-[(2,6-Dichlorophenyl)amino]phenyl}acetic acid
Dihydrocodeine	C18H23NO3	301.1678	3.64	125-28-0	4447600	(5alpha,6alpha)-3-Methoxy-17-methyl-4,5-epoxymorphinan-6-ol
Diltiazem	C22H26N2O4S	414.1613	7.1	42399-41-7	35850	(2S,3S)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate
Diphenhydramine	C17H21NO	255.1623	6.79	58-73-1	2989	2-(Diphenylmethoxy)-N,N-dimethylethanamine
Diphenylguanidine	C13H13N3	211.111	4.536	102-06-7	7313	1,2-diphenylguanidine
Dipipanone	C24H31NO	349.2406	7.89	467-83-4	12766	4,4-Diphenyl-6-(1-piperidinyl)-3-heptanone
Domperidone	C22H24ClN5O2	425.1619	6.42	57808-66-9	3039	5-Chloro-1-{1-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl]-4-piperidinyl}-1,3-dihydro-2H-benzimidazol-2-one
Donepezil	C24H29NO3	379.2147	6.44	120014-06-4	3040	2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one
Dothiepin	C19H21NS	295.1395	7.37	113-53-1	4445580	(3Z)-3-(Dibenzo[b,e]thiepin-11(6H)-ylidene)-N,N-dimethyl-1-propanamine
Doxepin	C19H21NO	279.1623	7.06	1668-19-5	3046	3-(Dibenzo[b,e]oxepin-11(6H)-ylidene)-N,N-dimethyl-1-propanamine
Doxylamine	C17H22N2O	270.1732	5.62	469-21-6	3050	(RS)-N,N-dimethyl-2-(1-phenyl-1-pyridin-2-yl-ethoxy)-ethanamine
Duloxetine	C18H19NOS	297.1187	7.56	116539-59-4	54822	(+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine
EDDP	C20H23N	277.1831	6.76	30223-73-5	23254962	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EG-018	C28H25NO	391.1936	10.91		30922490	naphthalen-1-yl(9-pentyl-9H-carbazol-3-yl)methanone
EMB-FUBINACA	C22H24FN3O3	397.1802	9.58	2365470-94-4	67169331	ethyl (2S)-2-[[1-[(4-fluorophenyl)methyl]indazole-3-carbonyl]amino]-3-methylbutanoate
Ethylone (bk-MDEA)	C12H15NO3	221.1052	4.26	1112937-64-0	21106271	(RS)-1-(1,3-benzodioxol-5-yl)-2-(ethylamino)propan-1-one
Etizolam	C17H15ClN4S	342.0706	8.02	40054-69-1	3191	4-(2-Chlorophenyl)-2-ethyl-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine
Fenazepam (Phenazepam)	C15H10BrClN2O	347.9665	8.32	51753-57-2	36657	7-?bromo-?5-?(2-?chlorophenyl)-?1,?3-?dihydro-?2H-?1,?4-?benzodiazepin-?2-?one

Fentanyl	C22H28N2O	336.2202	6.54	437-38-7	3228	N-Phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]propanamide
Flubromazepam	C15H10BrFN2O	331.9961	7.948	2647-50-9	10441497	7-Bromo-5-(2-fluorophenyl)-1,3-dihydro-1,4-benzodiazepin-2-one
Flubromazolam	C17H12BrFN4	370.0229	4.969	612526-40-6	10684757	8-bromo-6-(2-fluorophenyl)-1-methyl-4H-[1,2,4]triazolo[4,3-a] [1,4]benzodiazepine
Flunitrazepam	C16H12FN3O3	313.0863	7.538	1622-62-4	3263	5-(2-Fluorophenyl)-1-methyl-7-nitro-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Fluoxetine	C17H18F3NO	309.1341	7.93	54910-89-3	3269	N-Methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]-1-propanamine
Flupentixol	C23H25F3N2OS	434.164	8.88	2709-56-0	4445173	2-(4-{(3Z)-3-[2-(Trifluoromethyl)-9H-thioxanthen-9-ylidene]propyl}-1-piperazinyl)ethanol
Flurazepam	C21H23ClFN3O	387.1514	6.85	17617-23-1	3276	7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one
FUB-PB-22	C25H19FN2O2	398.1431	9.41	1800098-36-5	29763736	quinolin-8-yl 1-[(4-fluorophenyl)methyl]-1H-indole-3-carboxylate
Gabapentin	C9H17NO2	171.1259	3.78	60142-96-3	3328	[1-(Aminomethyl)cyclohexyl]acetic acid
Glaucine	C21H25NO4	355.1784	6.09	475-81-0	15883	(6aS)-1,2,9,10-Tetramethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline
Glibenclamide	C23H28ClN3O5S	493.1438	8.91	10238-21-8	3368	5-Chloro-N-(2-{4-[(cyclohexylcarbamoyl)sulfamoyl]phenyl} ethyl)-2-methoxybenzamide
Gliclazide	C15H21N3O3S	323.1304	8.12	21187-98-4	3356	N-(Hexahydrocyclopenta[c]pyrrol-2(1H)-ylcarbamoyl)-4-methylbenzenesulfonamide
Haloperidol	C21H23ClFNO2	375.1401	7.14	52-86-8	3438	4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone
Heroin	C21H23NO5	369.1576	5.31	561-27-3	4575379	(5alpha,6alpha)-17-Methyl-7,8-didehydro-4,5-epoxymorphinan-3,6-diyl diacetate
Hydrocodone	C18H21NO3	299.1521	3.89	125-29-1	4447623	(5alpha)-3-Methoxy-17-methyl-4,5-epoxymorphinan-6-one
Hydroxychloroquine	C18H26ClN3O	335.1764	4.11	118-42-3	3526	2-[{4-[(7-Chloro-4-quinolinyl)amino]pentyl}(ethyl)amino]ethanol
Hydroxyzine	C21H27ClN2O2	374.1761	7.83	68-88-2	3531	2-(2-{4-[(4-Chlorophenyl)(phenyl)methyl]-1-piperazinyl}ethoxy)ethanol
Imipramine	C19H24N2	280.194	7.64	50-49-7	3568	3-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethyl-1-propanamine
JWH-018 (AM-678)	C24H23NO	341.178	10.18	209414-07-3	8558143	(1-?pentyl-?1H-?indol-?3-?yl)-?1-?naphthalenyl-?methanone
JWH-073	C23H21NO	327.1623	9.91	208987-48-8	8647081	(1-?butyl-?1H-?indol-?3-?yl)-?1-?naphthalenyl-?methanone
JWH-081	C25H25NO2	371.1885	10.38	210179-46-7	8722599	(4-?methoxy-?1-?naphthalenyl)(1-?pentyl-?1H-?indol-?3-?yl)-?methanone
JWH-122	C25H25NO	355.1936	10.49	619294-47-2	24623066	(4-?methyl-?1-?naphthalenyl)(1-?pentyl-?1H-?indol-?3-?yl)-?methanone
JWH-200 (WIN-55225)	C25H24N2O2	384.1838	8.84	103610-04-4	8221134	[1-?[2-?(4-?morpholinyl)ethyl]-?1H-?indol-?3-?yl]-?1-?naphthalenyl-?methanone
JWH-250	C22H25NO2	335.1885	9.89	864445-43-2	23256117	1-?(1-?pentyl-?1H-?indol-?3-?yl)-?2-?(2-?methoxyphenyl)-?ethanone
Ketamine	C13H16ClNO	237.092	5.17	6740-88-1	3689	2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone
Lamotrigine	C9H7Cl2N5	255.0079	5.63	84057-84-1	3741	6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine
Levamisole	C11H12N2S	204.0721	3.86	14769-73-4	25037	(6S)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3]thiazole
Levetiracetam	C8H14N2O2	170.1055	3.81	102767-28-2	390096	(2R)-2-(2-oxopyrrolidin-1-yl)butanamide
Lidocaine	C14H22N2O	234.1732	4.715	137-58-6	3548	2-(diethylamino)-
Loprazolam	C23H21ClN6O3	464.1364	6.943	61197-73-7	2298440	(2Z)-6-(2-Chlorophenyl)-2-[(4-methyl-1-piperazinyl)methylene]-8-nitro-2,4-dihydro-1H-imidazo[1,2-a][1,4]benzodiazepin-1-one
Lorazepam	C15H10Cl2N2O2	320.0119	7.861	846-49-1	3821	7-chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-1,4-benzodiazepin-2-one

Lormetazepam	C16H12Cl2N2O2	334.0276	8.135	848-75-9	12750	7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1-methyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Loxapine	C18H18ClN3O	327.1138	7.518	02/10/1977	3827	8-chloro-6-(4-methylpiperazin-1-yl)benzo[b][1,4]benzoxazepine
Lurasidone	C28H36N4O2S	492.2559	9.01	367514-87-2	184739	(3aR,4S,7R,7aS)-2-{(1R,2R)-2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-ylmethyl] cyclohexylmethyl}hexahydro-4,7-methano-2H-isoindole-1,3-dione
MDMB-4en-PINACA	C20H27N3O3	357.2052	9.685	2504100-70-1	71117180	Methyl 3,3-dimethyl-2-[(1-pent-4-enylindazole-3-
MDMB-CHMICA	C23H32N2O3	384.2413	10.032	1971007-95-0	34450863	Methyl (2S)-2-{[1-(cyclohexylmethyl)-1H-indol-3-yl]formamido}-3,3-dimethylbutanoate
MDMB-CHMINACA	C22H31N3O3	385.2365		1185888-32-7	32055574	Methyl (2S)-2-{[1-(cyclohexylmethyl)-1H-indazol-3-yl]formamido}-3,3-dimethylbutanoate
Mebeverine	C25H35NO5	429.2515	7.010	630-20-3	3891	4-{Ethyl[1-(4-methoxyphenyl)-2-propanyl]amino}butyl 3,4-dimethoxybenzoate
Medazepam	C16H15ClN2	270.0924	8.53	06/12/2898	3901	7-Chloro-1-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepine
Meloxicam	C14H13N3O4S2	351.0348	7.8	71125-38-7	10442740	4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide.
Memantine	C12H21N	179.1674	6.84	19982-08-2	3914	3,5-dimethyladamantan-1-amine
Meperidine (Pethidine)	C15H21NO2	247.1572	5.76	57-42-1	3918	Ethyl 1-methyl-4-phenyl-4-piperidinecarboxylate
Mephedrone (4-Methylmethcathinone, 4-MMC)	C11H15NO	177.1154	4.77	1189805-46-6	21485694	2-(methylamino)-1-(4-methylphenyl)propan-1-one
Meprobamate	C9H18N2O4	218.1267	6.257	57-53-4	3924	[2-(carbamoyloxymethyl)-2-methyl-pentyl] carbamate
Mescaline	C11H17NO3	211.1208	4.669	54-04-6	3934	2-(3,4,5-trimethoxyphenyl)ethanamine
Metacetamol	C8H9NO2	151.0633	3.642	621-42-1	11626	N-(3-Hydroxyphenyl)acetamide
Metamfetamine (Deoxyephedrine)	C10H15N	149.1205	4.25	537-46-2	1169	(2S)-N-Methyl-1-phenyl-2-propanamine
Metformin	C4H11N5	129.1014	0.75	657-24-9	3949	N,N-Dimethylimidodicarbonimidic diamide
Methadone	C21H27NO	309.2093	7.71	76-99-3	3953	6-(Dimethylamino)-4,4-diphenyl-3-heptanone
Methaqualone	C16H14N2O	250.1106	7.776	72-44-6	6055	2-Methyl-3-(2-methylphenyl)-4(3H)-quinazolinone
Methoxphenidine	C20H25NO	295.1936	6.634	127529-46-8	52085156	(±)-1-[1-(2-methoxyphenyl)-2-phenylethyl]piperidine
Methylendioxymetamfetamine (MDMA)	C11H15NO2	193.1103	4.41	42542-10-9	1556	1-(1,3-Benzodioxol-5-yl)-N-methyl-2-propanamine
Methylenedioxyamfetamine (MDA)	C10H13NO2	179.0946	4.37	4764-17-4	1555	1-(1,3-Benzodioxol-5-yl)-2-propanamine
Methylenedioxyethylamfetamine (MDEA)	C12H17NO2	207.1259	4.75	82801-81-8	94775	1-(1,3-Benzodioxol-5-yl)-N-ethyl-2-propanamine
Methylone (bk-MDMA)	C11H13NO3	207.0895	3.82	186028-79-5	21106350	1-(1,3-benzodioxol-5-yl)-2-(methylamino)propan-1-one
Methylphenidate (Ritalin)	C14H19NO2	233.1416	5.54	113-45-1	4015	Methyl phenyl(2-piperidinyl)acetate
Metoclopramide	C14H22ClN3O2	299.1401	4.704	364-62-5	4024	4-amino-5-chloro-N-(2-(diethylamino)ethyl)-2-methoxybenzamide
Metoprolol	C15H25NO3	267.1834	5.48	37350-58-6	4027	1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]-2-propanol
Mianserin	C18H20N2	264.1627	6.93	24219-97-4	4040	2-Methyl-1,2,3,4,10,14b-hexahydrodibenzo[c,f]pyrazino[1,2-a]azepine
Midazolam	C18H13ClFN3	325.0782	8.019	59467-70-8	4047	8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-a][1,4]benzodiazepine
Mirtazapine	C17H19N3	265.1579	5.89	61337-67-5	4060	2-Methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-a]pyrido[2,3-c][2]benzazepine
MMB-022	C20H26N2O3	342.1943	9.215			methyl (1-(pent-4-en-1-yl)-1H-indole-3-carbonyl)-L-valinate

MMB-2201	C20H27FN2O3	362.2006	8.884	1616253-26-9	30922499	(S)-Methyl 2-(1-(5-fluoropentyl)-1H-indole-3-carboxamido)-3-methylbutanoate
Moclobemide	C13H17ClN2O2	268.0979	5.09	71320-77-9	4087	4-Chloro-N-[2-(4-morpholinyl)ethyl]benzamide
Morphine	C17H19NO3	285.1365	2.23	57-27-2	4450907	(5alpha,6alpha)-17-Methyl-7,8-didehydro-4,5-epoxymorphinan-3,6-diol
Naloxone	C19H21NO4	327.1471	3.74	465-65-6	4447644	(5alpha)-17-Allyl-3,14-dihydroxy-4,5-epoxymorphinan-6-one
Naproxen	C14H14O3	230.0943	8.268	22204-53-1	137720	(2S)-2-(6-Methoxy-2-naphthyl)propanoic acid
N-desmethyltramadol	C15H23NO2	249.1729	5.495	73806-55-0	171856	1-(3-methoxyphenyl)-2-(methylaminomethyl)cyclohexan-1-ol
N-Desmethylvenlafaxine	C16H25NO2	263.1885	6.53	1088-11-5	2890	7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Nefazodone	C25H32ClN5O2	469.2245	7.891	83366-66-9	4294	1-(3-[4-(3-chlorophenyl)piperazin-1-yl]propyl)-3-ethyl-4-(2-phenoxyethyl)-1H-1,2,4-triazol-5(4H)-one
Nefopam	C17H19NO	253.1467	6.1	13669-70-0	4295	5-Methyl-1-phenyl-3,4,5,6-tetrahydro-1H-2,5-benzoxazocine
Nicotine	C10H14N2	162.1157	1.57	54-11-5	80863	3-[(2S)-1-Methyl-2-pyrrolidinyl]pyridine
Nitrazepam	C15H11N3O3	281.08	7.49	146-22-5	4350	7-Nitro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
NM-2201	C24H22FNO2	375.1635	10	837122-21-7	30922478	naphthalen-1-yl 1-(5-fluoropentyl)-1H-indole-3-carboxylate
Norchlordiazepoxide	C15H12ClN3O	285.0669	7.617	7722-15-8	10441296	7-chloro-4-hydroxy-5-phenyl-3H-1,4-benzodiazepin-2-imine
Norclobazam	C15H11ClN2O2	286.0509	7.434	22316-55-8	80921	8-Chloro-1-phenyl-1H-1,5-benzodiazepine-2,4(3H,5H)-dione
Norclozapine (N-desmethylclozapine)	C17H17ClN4	312.1142	7.011	6104-71-8	14126465	8-chloro-11-piperazin-1-yl-5H-dibenzo[b,e][1,4]diazepine
Norsertraline (Desmethylsertraline)	C16H15Cl2N	291.0582	8.151	87857-41-8	102729	(1S,4S)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydronaphthalen-1-amine
Nortriptyline	C19H21N	263.1674	7.77	72-69-5	4384	3-(10,11-Dihydro-5H-dibenzo[a,d][7]annulen-5-ylidene)-N-methyl-1-propanamine
Olanzapine	C17H20N4S	312.1409	5.56	132539-06-1	10442212	2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine
Omeprazole	C17H19N3O3S	345.1147	7.498	73590-58-6	4433	5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methanesulfinyl]-1H-benzimidazole
Orphenadrine	C18H23NO	269.178	7.209	83-98-7	4440	N,N-Dimethyl-2-[(2-methylphenyl)(phenyl)methoxy]ethanamine
Oxazepam	C15H11ClN2O2	286.0509	7.89	604-75-1	4455	7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Oxcarbazepine	C15H12N2O2	252.0899	6.76	28721-07-5	31608	10-Oxo-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide
Oxycodone	C18H21NO4	315.1471	3.76	76-42-6	4447649	(5alpha)-14-Hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-one
Oxymorphone	C17H19NO4	301.1314	2.59	76-41-5	4447650	(5alpha)-3,14-Dihydroxy-17-methyl-4,5-epoxymorphinan-6-one
Paracetamol	C8H9NO2	151.0633	3.21	103-90-2	1906	N-(4-Hydroxyphenyl)acetamide
Phenacetin	C19H20FNO3	329.1427	7.48	61869-08-7	39888	(3S,4R)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine
Phenacetin	C10H13NO2	179.0946	6.19	62-44-2	4590	N-(4-Ethoxyphenyl)acetamide
Phentermine	C10H15N	149.1205	4.96	122-09-8	4607	2-Methyl-1-phenyl-2-propanamine
Pholcodine	C23H30N2O4	398.2206	2.34	509-67-1	4470854	(5alpha,6alpha)-17-Methyl-3-[2-(4-morpholinyl)ethoxy]-7,8-didehydro-4,5-epoxymorphinan-6-ol
PMMA	C11H17NO	179.131	2.249	22331-70-0	81951	1-(4-Methoxyphenyl)-N-methylpropan-2-amine
Pregabalin	C8H17NO2	159.1259	3.6	148553-50-8	4589156	(3S)-3-(aminomethyl)-5-methylhexanoic acid
Prochlorperazine	C20H24ClN3S	373.138	8.511	58-38-8	4748	2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10H-phenothiazine
Procyclidine	C19H29NO	287.2249	7.392	77-37-2	4750	1-Cyclohexyl-1-phenyl-3-(1-pyrrolidinyl)-1-propanol

Promethazine	C17H20N2S	284.1347	7.33	60-87-7	4758	N,N-Dimethyl-1-(10H-phenothiazin-10-yl)-2-propanamine
Propoxyphene (Dextropropoxyphene)	C22H29NO2	339.2198	7.58	469-62-5	9696	(2S,3R)-4-(Dimethylamino)-3-methyl-1,2-diphenyl-2-butanyl propionate
Propranolol	C16H21NO2	259.1572	6.7	525-66-6	4777	1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol
Pyribenzamine	C16H21N3	255.1736	6.38	91-81-6	5385	N,N-dimethyl-N-(phenylmethyl)-N-pyridin-2-ylethane-1,2-diamine
Quetiapine	C21H25N3O2S	383.1667	7.29	111974-69-7	4827	2-{2-[4-(dibenzo[b,f][1,4]thiazepin-11-yl)piperazin-1-yl]ethoxy}ethanol
Quinine	C20H24N2O2	324.1838	6.1	130-95-0	84989	(8alpha,9R)-6'-Methoxycinchonan-9-ol
Quinoline	C9H7N	129.0579	5.698	91-22-5	6780	1-Benzopyridine
RCS-4 Hydroxymethyl	C21H23NO2	321.1729	9.55	1345966-78-0	24769418	2-(4-methoxyphenyl)-1-(1-pentyl-indol-3-yl)methanone
Risperidone	C23H27FN4O2	410.2118	6.34	106266-06-2	4895	3-{2-[4-(6-fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl}-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one
Ropinirole	C16H24N2O	260.1889	4.41	91374-21-9	4916	4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one
SDB-006	C21H24N2O	320.1889	9.445	695213-59-3	1665835	N-benzyl-1-pentyl-1H-indole-3-carboxamide
Sertraline	C17H17Cl2N	305.0738	8.17	79617-96-2	61881	(1S,4S)-4-(3,4-Dichlorophenyl)-N-methyl-1,2,3,4-tetrahydro-1-naphthalenamine
Sildenafil	C22H30N6O4S	474.2049	7.62	139755-83-2	5023	5-{2-Ethoxy-5-[(4-methyl-1-piperazinyl)sulfonyl]phenyl}-1-methyl-3-propyl-1,4-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one
SKF525A (Proadifen?)	C23H31NO2	353.2379	8.49	302-33-0	4741	2-Diethylaminoethyl 2,2-diphenylpentanoate
Strychnine	C21H22N2O2	334.1681	4.433	57-24-9	389877	Strychnidin-10-one
STS-135	C24H31FN2O	382.242	10.09	1354631-26-7	28189067	N-(Adamantan-1-yl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide
Sulpiride	C15H23N3O4S	341.1409	3.062	15676-16-1	5162	N-[(1-ethylpyrrolidin-2-yl)methyl]-2-methoxy-5-sulfamoylbenzamide
Tadalafil	C22H19N3O4	389.1376	7.9	171596-29-5	99301	(6R,12aR)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione
Tapentadol	C14H23NO	221.178	5.597	175591-23-8	8013742	3-[(1R,2R)-3-(dimethylamino)-1-ethyl-2-methylpropyl]phenol
Temazepam	C16H13ClN2O2	300.0666	8.07	846-50-4	5198	7-Chloro-3-hydroxy-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Tetrahydrocannabinol (THC)	C21H30O2	314.2246	10.063	03/08/1972	15266	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol
THJ-018	C23H22N2O	342.1732	10.36	1364933-55-0	29341702	1-naphthalenyl(1-pentyl-1H-indazol-3-yl)-methanone
THJ-2201	C23H21FN2O	360.1638	9.771	1801552-01-1	30646749	[1-(5-Fluoropentyl)-1H-indazol-3-yl](1-naphthyl)methanone
Timolol	C13H24N4O3S	316.1569	5.45	26839-75-8	31013	(2S)-1-[(2-Methyl-2-propanyl)amino]-3-{[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy}-2-propanol
Tolbutamide	C12H18N2O3S	270.1038	7.6	64-77-7	5304	N-(Butylcarbamoyl)-4-methylbenzenesulfonamide
Tramadol	C16H25NO2	263.1885	5.44	27203-92-5	31105	(1R,2R)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol
Tramadol Metabolite 1 (O-desmethyltramadol)	C15H23NO2	249.1729	4.44	73986-53-5	115703	3-{2-[(dimethylamino)methyl]-1-hydroxycyclohexyl}phenol
Trazodone	C19H22ClN5O	371.1513	6.55	19794-93-5	5332	2-{3-[4-(3-Chlorophenyl)-1-piperazinyl]propyl}[1,2,4]triazolo[4,3-a]pyridin-3(2H)-one
Triazolam	C17H12Cl2N4	342.0439	7.85	28911-01-5	5355	8-Chloro-6-(2-chlorophenyl)-1-methyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine
Trifluoperazine	C21H24F3N3S	407.1643	8.703	117-89-5	5365	10-[3-(4-methylpiperazin-1-yl)propyl]-
Trihexyphenidyl	C20H31NO	301.2406	7.67	144-11-6	5371	1-Cyclohexyl-1-phenyl-3-(1-piperidinyl)-1-propanol
Trimeprazine (Alimemazine)	C18H22N2S	298.1504	7.469	84-96-8	5373	N,N,2-trimethyl-3-phenothiazin-10-yl-propan-1-amine

Trimethoprim	C14H18N4O3	290.1379	4.305	738-70-5	5376	5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine
Trimipramine	C20H26N2	294.2096	7.88	739-71-9	5382	3-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N,2-trimethyl-1-propanamine
Vardenafil	C23H32N6O4S	488.2206	7.73	224789-15-5	99300	2-{2-Ethoxy-5-[(4-ethyl-1-piperazinyl)sulfonyl]phenyl}-5-methyl-7-propylimidazo[5,1-f][1,2,4]triazin-4(1H)-one
Varenicline	C13H13N3	211.111	3.839	249296-44-4	4470510	7,8,9,10-Tetrahydro-6,10-methano-6H-pyrazino[2,3-h] [3]benzazepine
Venlafaxine	C17H27NO2	277.2042	6.49	93413-69-5	5454	1-[2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol
Venlafaxine Metabolite 1 (O-Desmethylvenlafaxine)	C16H25NO2	263.1885	5.09	93413-62-8	111300	4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl)ethyl]phenol
Vortioxetine	C18H22N2S	298.1504	8.306	508233-74-7	8141643	1-{2-[(2,4-Dimethylphenyl)sulfanyl]phenyl}piperazine
Warfarin	C19H16O4	308.1049	8.43	81-81-2	10442445	4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one
Zaleplon	C17H15N5O	305.1277	6.97	151319-34-5	5517	N-[3-(3-Cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-N-ethylacetamide
Zolpidem	C19H21N3O	307.1685	6.23	82626-48-0	5530	N,N-Dimethyl-2-[6-methyl-2-(4-methylphenyl)imidazo[1,2-a]pyridin-3-yl]acetamide
Zopiclone	C17H17ClN6O3	388.1051	5.41	43200-80-2	5533	6-(5-Chloro-2-pyridinyl)-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-5-yl 4-methyl-1-piperazinecarboxylate
Zuclopenthixol	C22H25ClN2OS	400.1394	8.79	53772-83-1	4470984	cis-(Z)-2-(4-(3-(2-chloro-9H-thioxanthen-9-ylidene)propyl)piperazin-1-yl)ethanol

TICTAC:

5C-AKB-48, 5F-AB-PINACA, 5F-ADB, 5F-AKB-48, 5F-MDMB-PICA, 5F-MN-24, 5F-MPP-PICA, 5F-NPB-22, AB-CHMINACA, AB-FUBINACA, AB-PINACA, ADB-CHMINACA, ADB-FUBINACA, AKB-48 (APINACA), AM-1220, AM-2201, AM-2233, AM-694, AMB-FUBINACA, APICA (2NE1), BB-22 (QUCHIC), EG-018, EMB-FUBINACA, FUB-PB-22, JWH-018 (AM-678) , JWH-073, JWH-081, JWH-122, JWH-200 (WIN-55225), JWH-250, , MDMB-CHMICA, MDMB-CHMINACA, MMB-022, MMB-2201, NM-2201, SDB-006, STS-135, THJ-018, THJ-2201

ASI:

2C-B, 2C-I, 3-Acetamidophenol, 4-MEC (4-Methylethcathinone, 5-methoxy-DALT (5-MeO-DALT), 6-Acetylmorphine, 7-Aminoclonazepam, ACP (Zopiclone Degradation Product), Alfentanil, Alprazolam, Amantadine, Amfetamine, Amisulpride, Amitriptyline, Amlodipine, Aripiprazole, Atenolol, Azacyclonol, Benperidol, Benzoylecgonine, Bisoprolol, Bromazepam, Buprenorphine, Caffeine, Carbamazepine, Cetirizine, Chlorcyclizine, Chlordiazepoxide, Chloroquine, Chlorpheniramine, Chlorpromazine, Citalopram, Clobazam, Clomipramine, Clonazepam, Clopidogrel Metabolite 1 (SR26334), Clozapine, Cocaine, Codeine, Colchicine, Coumachlor, Cyclizine, Dehydroaripiprazole, Demoxepam, Desalkylflurazepam, Desethylamiodarone, Desipramine, Dextromethorphan, Diazepam, Diclofenac, Dihydrocodeine, Diltiazem, Diphenhydramine, Diphenylguanidine, Dipipanone, Domperidone, Donepezil, Dothiepin, Doxepin, Doxylamine, Duloxetine, EDDP, Ethylone (bk-MDEA), Etizolam, Fenazepam (Phenazepam), Fentanyl, Flunitrazepam, Fluoxetine, Flupentixol, Flurazepam, Gabapentin, Glaucine, Glibenclamide, Gliclazide, Haloperidol, Heroin, Hydrocodone, Hydroxychloroquine, Hydroxyzine, Imipramine , Ketamine, Lamotrigine, Levamisole, Levetiracetam, Lidocaine, Loprazolam, Lorazepam, Lormetazepam, Loxapine, Lurasidone, Mebeverine, Medazepam, Meloxicam, Memantine, Meperidine (Pethidine), Mephedrone (4-Methylmethcathinone, 4-MMC), Meprobamate, Mescaline, Metacetamol, Methamphetamine (Deoxyephedrine),

Metformin, Methadone, Methaqualone, Methoxphenidine, Methylenedioxymethamphetamine (MDMA), Methylenedioxyamphetamine (MDA), Methylenedioxymethamphetamine (MDEA), Methylone (bk-MDMA), Methylphenidate (Ritalin), Metoclopramide, Metoprolol, Mianserin, Midazolam, Mirtazapine, Moclobemide, Morphine, Naloxone, Naproxen, N-desmethyltramadol, N-Desmethylvenlafaxine, Nefazodone, Nefopam, Nicotine, Nitrazepam, Norchlordiazepoxide, Norclobazam, Norclozapine (N-desmethylclozapine), Norsertraline (Desmethylsertraline), Nortriptyline, Olanzapine, Omeprazole, Orphenadrine, Oxazepam, Oxcarbazepine, Oxycodone, Oxymorphone, Paracetamol, Phenacetin, Phentermine, Pholcodine, PMMA, Pregabalin, Prochlorperazine, Procyclidine, Promethazine , Propoxyphene (Dextropropoxyphene), Propranolol, Pyribenzamine, Quetiapine, Quinine, Quinoline, RCS-4 Hydroxymethyl, Risperidone, Ropinirole, Sertraline, Sildenafil, SKF525A, Strychnine, Sulpiride, Tadalafil, Tapentadol, Temazepam, Tetrahydrocannabinol (THC), Timolol, Tolbutamide, Tramadol, Tramadol Metabolite 1 (O-desmethyltramadol), Trazodone, Triazolam, Trifluoperazine , Trihexyphenidyl, Trimeprazine (Alimemazine), Trimethoprim, Trimipramine, Vardenafil, Varenicline, Venlafaxine, Venlafaxine Metabolite 1 (O-Desmethylvenlafaxine), Vortioxetine, Warfarin, Zaleplon , Zolpidem , Zopiclone, Zuclopenthixol

4F-MDMB-BINACA (Krotulski et al., 2019)

MDMB-4en-PINACA (Antonides et al., 2021)

ADB-BUTINACA (Kronstrand et al., 2022)

Flubromazolam (World Health Organization, 2021)

Flubromazepam (Abouchedid et al., 2018)

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