An Investigation into the Suitability of Time-of-Flight Mass Spectrometry in Forensic Toxicology

by

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Summary

Liquid chromatography coupled with mass spectrometry is one of the most powerful tools in the toxicologist's arsenal to detect a wide variety of compounds from many different matrices. However, the huge number of potentially abused substances and new substances especially designed as intoxicants poses a problem in a forensic toxicology setting. Most methods are targeted and designed to cover a very specific drug or group of drugs while many other substances remain undetected.

High resolution mass spectrometry, more specifically time-of-flight mass spectrometry, represents an extremely powerful tool in analysing a multitude of compounds not only simultaneously but also retroactively. The data obtained through the time-of-flight instrument contains all compounds made available from sample extraction and chromatography, which can be processed at a later time with an improved library to detect previously unrecognised compounds without having to analyse the respective sample again.

The aim of this project was to determine the utility and limitations of time-of-flight mass spectrometry as a general and easily expandable screening method. The resolution of time-of-flight mass spectrometry allows for the separation of compounds with the same nominal mass but distinct exact masses without the need to separate them chromatographically.

To simulate the wide variety of potentially encountered drugs in such a general screening method, seven drugs (morphine, cocaine, zolpidem, diazepam, amphetamine, MDEA and THC) were chosen to represent this variety in terms of mass, properties and functional groups.

Consequently, several liquid-liquid and solid phase extractions were applied to urine samples to determine the most general suitable and unspecific extraction. Chromatography was optimised by investigating the parameters pH, concentration and gradient of the mobile phase to improve data obtained by the time-of-flight instrument. The resulting method was validated as a qualitative confirmation/identification method.

Data processing was automated using the software TargetAnalysis, which provides excellent analyte recognition according to retention time, exact mass and isotope pattern. The recognition of isotope patterns allows excellent recognition of analytes even in interference rich mass spectra and proved to be a good positive indicator.

Finally, the validated method was applied to samples received from the A&E Department of Glasgow Royal Infirmary in suspected drug abuse cases and samples received from the Scottish Prison Service, which were received from their own prevalence study targeting drugs of abuse in the prison population. The obtained data was processed with a library established in the course of this work.

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Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature _____

Printed name _____

Abbreviations

%CV	Coefficient of Variation
6-MAM	6-Monoacetylmorphine
ACN	Acetonitrile
AM-2201	1-(5-fluoropentyl)-3-(1-naphthoyl)indole
DCM	Dichloromethane
DoA	Drug of Abuse
d_x	Number of deuterated positions
EIC	Extracted Ion Chromatogram
EPI	Enhanced Product Ion
GCMS	Gas Chromatography-Mass Spectrometry
GG&C	Greater Glasgow & Clyde
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HU-210	1,1-Dimethylheptyl-11-hydroxy-tetrahydrocannabinol
IPA	Isopropylalcohol
JWH-018	1-pentyl-3-(1-naphthoyl)indole
LC	Liquid Chromatography
LLE	Liquid -Liquid Extraction
LOD	Limits of Detection
m/z	mass-to-charge ratio
MDA	3,4-Methylenedioxy-amphetamine
MDAI	5,6-Methylenedioxy-2-aminoindane
MDEA	3,4-methylenedioxy-N-ethyl-amphetamine
MDMA	3,4-methylenedioxy-methamphetamine
MeOH	Methanol
MRM	Multi Reaction Monitoring

MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MTBE	Methyl tert-butyl ether
MTS	Multi Target Screening
NHS	National Health Service
NPS	Novel Psychoactive Substance
pH	Numeric scale to specify acidity
PMA	para-Methoxyamphetamine
PMMA	para-Methoxymethamphetamine
PTRC	Pharmaca-Toxicologically Relevant Compounds
RT	Retention Time
sMRM	Sheduled Multi Reaction Monitoring
SPE	Solid Phase Extraction
TFMPP	3-Trifluoromethylphenylpiperazine
THC	(-)-trans-Δ^9-tetrahydrocannabinol
THC-COOH	11-nor-9-Carboxy-THC
TIC	Total Ion Chromatogram
Tris	Tris(hydroxymethyl)aminomethane

1 Introduction

Toxicology (from the Greek "*toxicos"* = poisonous and *"logos"* = word, reason – which is also the root of the English word logic) is the study of adverse effects of chemicals, with the recent addition of effects, such as noise and radiation, on a living organism and combines, among others, aspects of chemistry, biology and medicine. The first formalisation of toxicology as a science is attributed to Mathieu Joseph Bonaventure Orfila (1787–1853), who worked to make chemical analysis a routine part of medical forensic investigations, and his work *Traité des poisons tirés des règnes minéral, végétal et animal; ou, Toxicologie générale (1812).*

Forensic Toxicology is the study of the chemical composition, preparation and identification of alcohol, drugs (licit and illicit) and poisons. Relevant knowledge includes absorption, distribution and elimination characteristics in the body, as well as the response of the body to these substances. The most important parts of forensic toxicological analysis is the quantitative and qualitative determination of drugs and/or toxic compounds in the body, with regard, where relevant, to the potential therapeutical application and concentration of these substances.

The metabolic processes by which the body eliminates drugs and/or poisons, called biotransformations, can change the appearance of a compound considerably and these metabolic pathways must be studied separately for each individual drug or poison to determine potential analytes. Additionally, just as the pathways may (and probably will) vary for each drug so do the rates at which these biotransformations occur, which in turn has an impact on the metabolites that can be analysed. The matter is further complicated by the fact that these biotransformations can sometimes be responsible for the desired effects and/or toxicity of the administered substance – subsequently it should be mentioned that some substances, primarily poisonous substances like heavy metals such as lead, are not metabolised at all and are either excreted unchanged or accumulate in substance specific tissues.

1.1 Terminology of Drugs

The three most widespread labels used for drugs or substances that are used for recreational purposes are “Legal High”, Designer Drug and Drug of Abuse, each of which conveys a certain amount of information. While these labels are not interchangeable, more than one may apply to a certain substance or drug and in the following a short paragraph will be dedicated to each label to clarify the subtle differences and how they are used in the literature.

> ***Definition of “Legal Highs”:*** Substances which mimic the effects of traditional drugs such as cocaine, ecstasy or marijuana while not being controlled by the Misuse of Drug Act are generally referred to as “Legal Highs”.

The desired effects of “Legal Highs” are mostly along the lines of euphoria and hallucinations but the actual effects can vary significantly since most of the substances in this category have not been studied extensively, if they have been studied at all, or even undergone clinical examination. Most “Legal Highs” are either natural occurring and known substances – such as the proposed intoxicating effects of smoking certain herbs – used for recreational purposes or inappropriately used substances or drugs – such as the recreational use of cough syrup or anti-depressants. Furthermore, the term “Legal High” in a toxicological context generally refers to a new drug, whereas the novelty is more often that it is a variation of a known drug with intoxicating effects instead of being a novel compound, which is not covered by the current legislation.

Especially new drugs, either actually novel compounds or modifications of known drugs, labelled as “Legal Highs” are of (forensic) toxicological interest, as the effects on the human body upon consumption are barely known. Even though these drugs are made with specific effects or parent drugs in mind to emulate its effects, which is successful in most cases, even simple changes on a molecule can have devastating effects on the body as the metabolic rate and pathways may change accordingly. The exact nature of these changes can only be revealed by extensive analytical and clinical studies since existing models fail to sufficiently predict the complex interactions between drug and metabolism.

> ***Definition of Designer Drugs:*** "Psychotropic substances that are either synthetically changed natural compounds, modified molecular structures of existing drugs or – less commonly – completely different, designed chemicals"[1]

The term Designer Drugs does not necessarily refer to a recent discovery of these substances but rather to their recent appearance on the drug market and their recent abuse. The vast majority of these "new" Designer Drugs are actually rooted in scientific literature which is widely accessible. Primarily pharmaceutical companies, but also universities and other research facilities, have always participated in an extensive screening of structural variations of existing drugs for potential pharmaceutical agents or to gain insight into the structure-effect relationship. While these initially researched structures failed to advance to promising pharmaceutical agents or suitable research agents, they can be synthesised by those interested in them, for licit or illicit purposes, without the extensive investment into research of their own.

> ***Definition of Drugs of Abuse:*** A drug or substance is described as a Drug of Abuse when it is inappropriately used for recreational purposes or in a maladaptive pattern, which does not necessarily include dependency.

While most substances that are regarded as typical Drugs of Abuse possess psychoactive and/or mood altering properties, the label is not limited to substances used for recreational purposes. Subsequently, any drug or substance that is inappropriately used, such as the use of steroids to enhance the physical performance in sports, is classified as a Drug of Abuse.

While – technically – every use of a substance for something that it is not intended to be used for is generally regarded as being inappropriate and therefore an abuse of that substance, this traditional view of the black-and-white nature of either use or abuse can be questioned. The following model (**Figure 1.1**) has been proposed by *A Public Health Approach to Drug Control in Canada (2005)*[2] and allows a more diverse view on the matter, especially in regard to the abuse of substances.

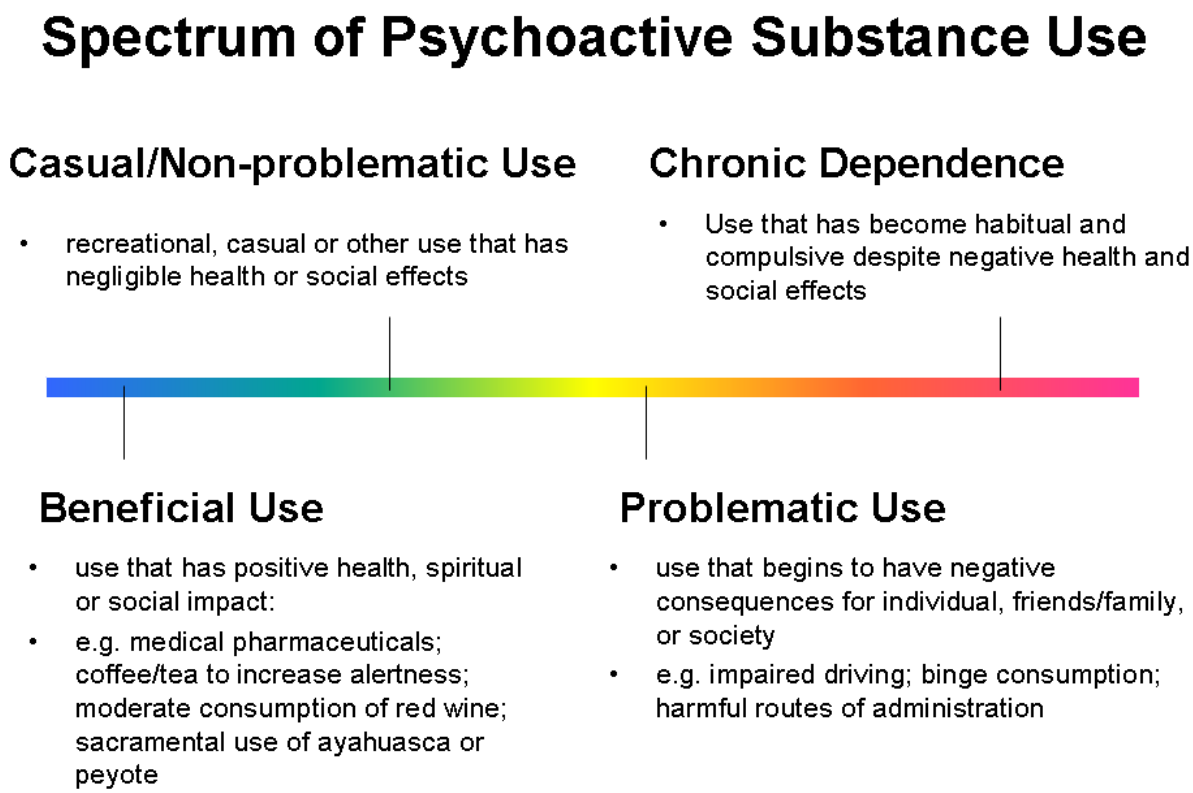


Figure 1.1 A proposed model for classification of substance abuse, mainly to be used in the context of prevention (source: *A Public Health Approach to Drug Control in Canada (2005)*)

Even though this model has certain advantages, in that it allows organising the use of substances according to their health and social effects, it is not universally applicable. The proposed instances – Beneficial Use, Casual/Non-problematic Use, Problematic Use and Chronic Dependency – work remarkably well for most legal intoxicants, such as alcohol, caffeine and nicotine, and for some drugs, such as amphetamines which are standard issue in some military branches due to their performance enhancing qualities or cannabis which has been shown to have beneficial effects in AIDS patients or cancer patients undergoing chemotherapy [3]. While this model could be applied to most – if not all – Drugs of Abuse, the inherent toxicities and/or high chances of psychological and physiological dependency would put those substances immediately onto the right side of the proposed spectrum. Additionally, the *Spectrum of Psychoactive Substance Use* doesn't take legality of the substances and drugs in question into account and is mainly a tool designed to aim prevention measures more at the right side of the spectrum.

1.2 Challenges for Toxicological Drug Screening

One of the challenges in forensic toxicology is the continued emergence of new drugs in the form of legal highs and designer drugs, as defined previously. The usage of these drugs can be attributed to the perceived safety of those compounds, in the case of naturally occurring compounds, the non-legislated status of novel compounds and the ability of especially designer drugs to mimic the desired effects of classical Drugs of Abuse. While legislation in the UK is attempting to catch up with the *Psychoactive Substances Bill 2016* [4], which legislates drugs according to their psychoactive effects, the mimicry of desired effects makes these drugs of potential interest to drug users even though the side effects can be unknown or quite severe. This is further complicated by the fact that some of these drugs are used to replace classical Drugs of Abuse, as they mimic similar effects, without the knowledge of the user and can make self-reported drug use unreliable.

The use of designer drugs and/or NPS has been increasingly popular in recent years and has grown beyond regional trends [5][6][7][8]. While the systematic abuse of these substances might be a recent development many if not most of these "new" drugs have been known scientifically beforehand but only recently appeared as drugs not scheduled for legislation.

Concerns have been raised on the abuse of designer drugs and NPS as health professionals have severely limited knowledge of the effects, dangers and treatments of these substances [9]. This problem is further escalated as many products contain multiple active compounds and users may be unaware of all or any of the contents they consume [8], [10].

In order to understand the difficulties in testing these drugs it is important to understand the chemical structures their similarities and differences to classical drugs of abuse. The following is a short discussion of some of the highly substituted drug groups that are part of regular screening protocols but also continue to produce new designer drugs. This poses an analytical problem as regular users may continue to use the same group of drugs for the desired effects but may change, intentionally or unwittingly, to new drugs that mimic these effects

1.2.1 Amphetamines

Amphetamine (**Figure 1.2**) is a heavily substituted drug, with many substitutes belonging to the classical Drugs of Abuse. Substitutions are generally at the hetero atom (**Figure 1.3**) or at the aromatic ring (**Figure 1.4**), usually position 3 and/or 4, or at both positions (**Figure 1.5**).

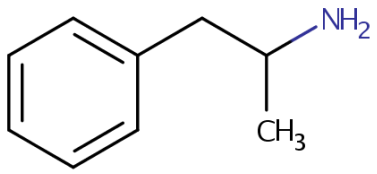


Figure 1.2 Structure of amphetamine

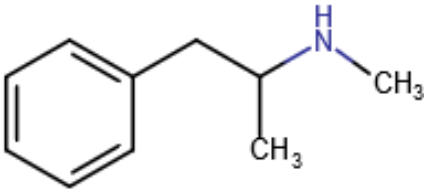


Figure 1.3 Structure of methamphetamine

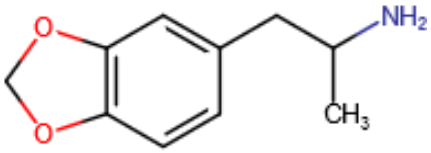


Figure 1.4 Structure of MDA

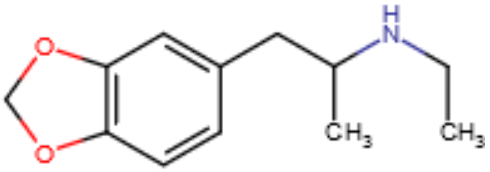


Figure 1.5 Structure of MDEA

In contrast to previously discussed amphetamine derivates, para-Methoxyamphetamine (PMA) (**Figure 1.6**) and para-Methoxy-N-methylamphetamine (PMMA) (**Figure 1.7**) do not show stimulant, euphoriant or entactogen effects [11] and have been proven to be much more dangerous for several reasons. Firstly, PMA overdose may already occur near the usual recreational dose range and the actual dosage required for a potential overdose is heavily dependent on the user and may vary significantly [12]. Furthermore, it is often sold as other amphetamines with users unknowingly ingesting the considerably more dangerous PMA [13], with more recent cases in North Ireland and Scotland [14][15] .Structurally very similar, PMMA shows similar effects and dangers to PMA and death are usually linked to mislabelled tablets [16].

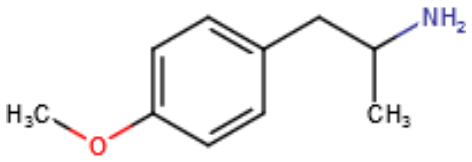


Figure 1.6 Structure of PMA

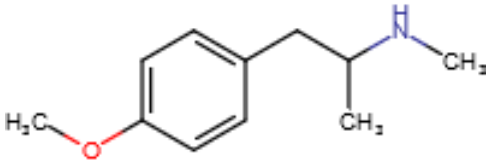


Figure 1.7 Structure of PMMA

1.2.2 Benzodiazepines

Another group of drugs with a significant number of derivates are the benzodiazepines, which encompasses several dozen drugs and metabolites, which are a result of modification on the heavily modifiable benzodiazepine base structure (**Figure 1.8**).

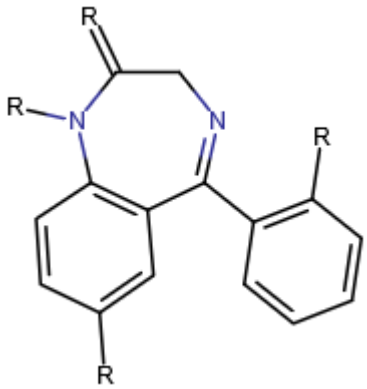


Figure 1.8 Structure of benzodiazepine with common positions for substitutions

One of the most common benzodiazepines is diazepam (**Figure 1.9**), which has numerous metabolites that are also psychoactive benzodiazepines and can be prescribed or misused independently.

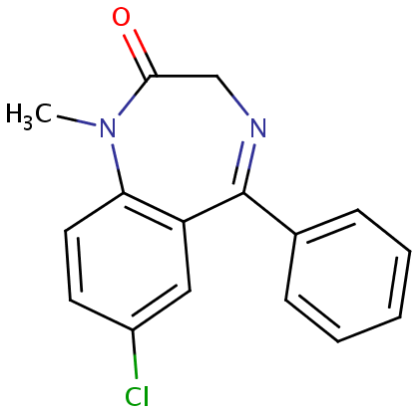


Figure 1.9 Structure of diazepam

As the effects of benzodiazepines, which include sedative, anxiolytic, anticonvulsant and muscle relaxant, are medical relevant and continue to be researched they continue to produce new derivates to be potentially abused. Furthermore, the wealth of literature available on benzodiazepines is a constant source of compounds that were previously only of academic interest. One such an example is flubromazolam (**Figure 1.10**) which has been known in the literature since the early 70s [17] and has now surfaced as an abused drug [18].

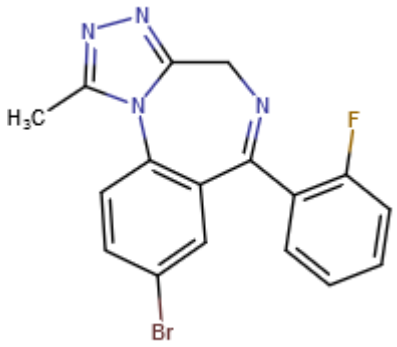


Figure 1.10 Structure of flubromazolam

1.2.3 Synthetic Cannabinoids

A further group with a significant number of analogues are the synthetic cannabinoids, which are scientifically synthesised to emulate cannabinoids, most commonly THC (**Figure 1.11**) and are of interest for the research into medicinal properties of cannabis.

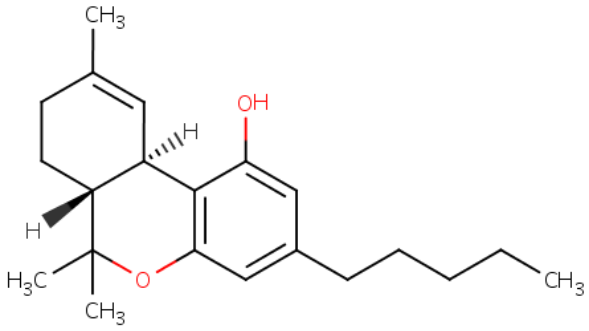


Figure 1.11 Structure of THC

Notable synthetic cannabinoids are HU-210 (**Figure 1.12**) – a structural analogue - which is a potent analgesic sharing many effects as THC, but considerably stronger, and JWH-018 (**Figure 1.13**) – which mimics the effects of THC - as one of the first synthetic cannabinoids to be under widespread legislation.

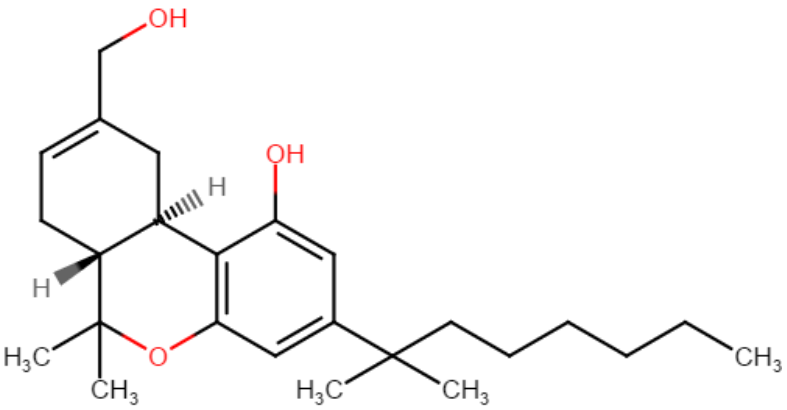


Figure 1.12 Structure of HU-210

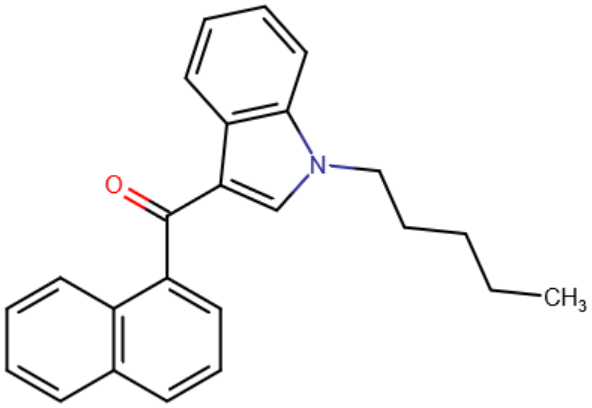


Figure 1.13 Structure of JWH-018

1.3 Development of LC-MS as an analytical method and emergence of high resolution mass spectrometry

The first publication of liquid chromatography coupled with mass spectrometry dates back to 1968 in the Russian Journal of Physical Chemistry by Victor Tal'roze. While this was regarded as an impressive feat, for it overcame severe difficulties in regard to injection of a liquid into a high vacuum, the high voltage electron impact mass spectrometer, used at the time, proved to be too sensitive to pressure changes. Hewlett Packard would then later develop the first commercially available MS interface [19] after the initial approach was improved upon by Baldwin and McLafferty in 1973 by introducing the liquid as a spray into the ionisation source. Other methods were developed around the same time by Carrol et al.

[20], McFadden et al. [21] and Thompson et al. [22], but the first interface that positively excited the LC-MS community was the Thermospray (**Figure 1.14**), as published by Blakley et al. [23].

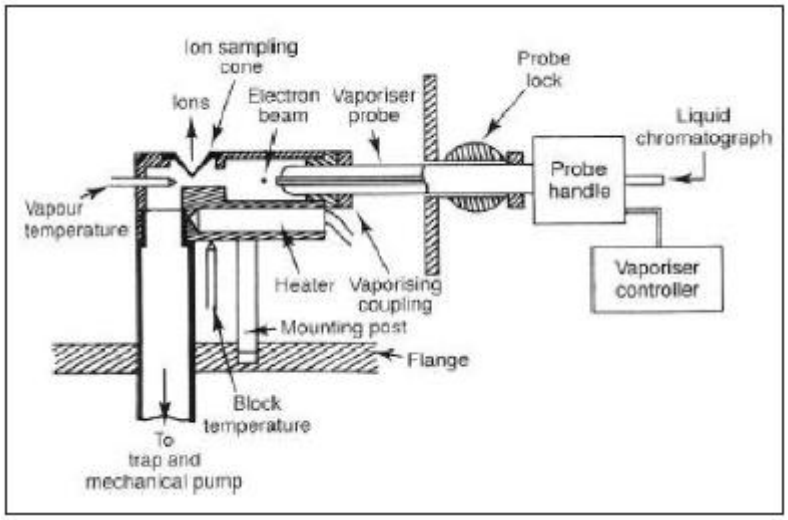


Figure 1.14 Thermospray source design by Blakley et al.

It was the first system capable of dealing with reversed phase solvent systems and compatible with LC-UV and quickly became the interface of choice.

Techniques continued to develop and culminated finally in the simultaneous development of atmospheric pressure ionisation by the two researchers Henion et al. [24] and Yamashita et al. [25]. The first publication of the resulting interfaces was from Bruins et al. [26] and demonstrated sensitivity several orders of magnitude greater than other techniques at the time.

These leaps in technology and application led to the launch of the first instrument – by Sciex in 1989 – with hardly any constraints on mobile phase composition, simple interpretation of spectrum data and no detectable fragmentation of proteins. This in turn opened the field for biochemists, drug metabolism studies and chromatographers and created the scientific field we see today.

1.4 Application of Liquid Chromatography-High Resolution Mass Spectrometry

One of the most important benefits of liquid chromatography-high resolution mass spectrometry (LC-HRMS) is the full-scan acquisition, which allows for retrospective analysis and consequently does not require any prior determination of the screened analytes. This means that, theoretically, an unlimited number of analytes can be extracted from the obtained data without sacrificing sensitivity in the process. Furthermore, the resolving power of HRMS can help to determine the elemental composition of the analytes by means of the obtained exact masses and observed isotope patterns. Thus more data is obtained from high-resolution mass spectrometry than from unit resolution data [27]. A direct comparison of the selectivity provided by single reaction monitoring (SRM) and HRMS coupled to liquid chromatography was addressed by Kaufmann et al. [28] and concluded a resolution in excess of 50,000 was routinely available for HRMS thus making it an attractive tool for the detection of trace-level amounts. The same team would later investigate the quantitative and confirmative performance of HRMS with >100 veterinary drugs and suggested that screening, quantitation and confirmation might be merged in a single step by HRMS, providing more flexibility, in contrast to the sequential steps necessary with conventional tandem mass spectrometry platforms [29].

Common fields of application for HRMS are food safety and environmental analysis as the reliable confirmation and identification can more often than not rely on trace amounts. Consequently, HRMS has been successfully used in the detection of adulterants in wine [30], [31], residues of 110 veterinary drugs in fish [32], biogenic amines in seafood [33] and marine biotoxin accumulation in shellfish [34]. Further studies concerning pesticides have demonstrated the use of HRMS to analyse the residue of 132 pesticides in agricultural products [35], surface residue of 240 pesticides [36] and the detection of over 500 pesticides in fruits/vegetables [37]. Similar studies have demonstrated the use of HRMS in the detection of mycotoxins in flour/bread [38], wheat & maize [39], beer [40] and other plant toxins in food [41].

The previously discussed benefits of HRMS data acquisition makes is a highly beneficial technique for drug discovery and has been used in quantitative bioanalysis [42], microsomal stability and plasma drug level measurement with additional investigation of selected drug metabolism via post-acquisition data mining [43].

The distinct advantages of HRMS have led to numerous recent applications in the general screening and unknown screening of large quantities of drugs, such as Dalsgaard et al. [44] who established a method for 175 compounds (psychotropic, cardiovascular, designer and abused drugs) extracted from blood by solid phase extraction and showed limits of detection ranging from 5 – 50 ng/mL. Similarly, Domínguez-Romero et al. [45] established a method for 200 multiclass sport drugs in urine with limits of detection up to < 0.1 ng/mL, which was achieved by solid phase extraction and consequent filtering of the extract. The same method was shown to be effective in the identification of non-targeted compounds by utilising in source fragmentation to identify relevant metabolites.

A method published by Marginean et al. [46] separated 23 controlled synthetic cannabinoids and 9 non-controlled positional isomers, extracted by protein precipitation, of JWH-018 to achieve a qualitative identification on HRMS data alone to be confirmed by a more discriminatory GC-MS technique.

1.5 Drug Standards

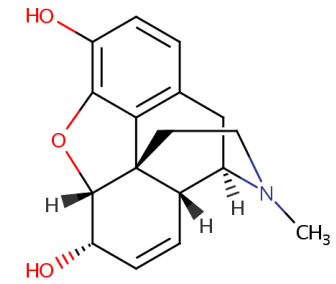
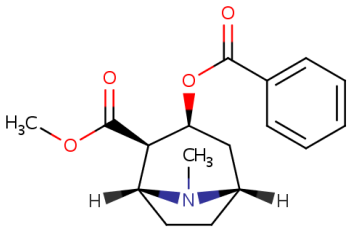
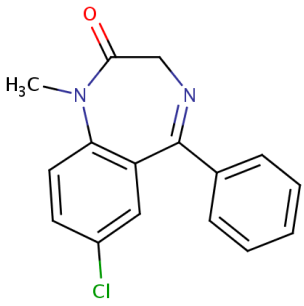
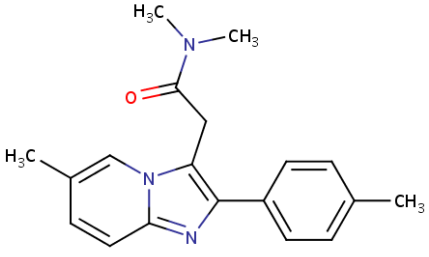
Standardised pure samples of drugs were used as drug reference standards to establish the proper methodology for analysing a particular drug, or served as a base for similar drugs should the desired drug be not available in its pure form, i.e. a newly emerged drug. Furthermore, deuterated drug standards, in which a certain number of hydrogen atoms have been exchanged with deuterium atoms, can be used to quantify the amount of this drug in a mixture as a known amount of the deuterated drug can be added to the sample without changing the amount of the original drug contained in the sample.

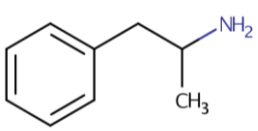
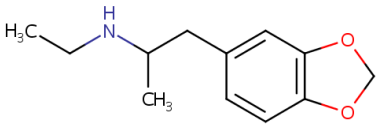
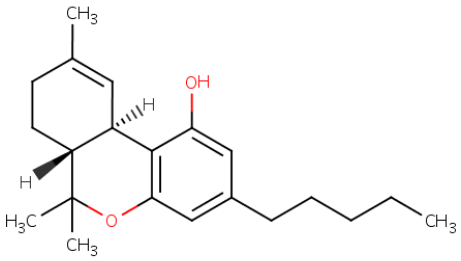
While the method development in the following chapters did not aim towards a quantitative method, it was ensured that the method would work for a wide range of different compounds. To this end, seven compounds were chosen (**Table 1.1**) to represent the spectrum of compounds to be analysed with this method: morphine, cocaine, diazepam, zolpidem, amphetamine, MDEA and THC

These drugs were chosen based on the fact that they cover a wide mass range, chemical and physical properties and are among the most abused drugs. While this certainly does not

guarantee that the method will be suitable for every drug possibly encountered, it ensures a wide application range necessary for general screening.

Table 1.1 Chosen compounds to represent the desired screening range

Structure	*Drugs + Formula*	*Exact Mass*
	Morphine $C_{17}H_{19}NO_3$	285.3377
	Cocaine $C_{17}H_{21}NO_4$	303.3529
	Diazepam $C_{16}H_{13}ClN_2O$	284.0716
	Zolpidem $C_{19}H_{21}N_3O$	307.3895

	Amphetamine $C_9H_{13}N$	135.2062
	MDEA $C_{12}H_{17}NO_2$	207.1259
	THC $C_{21}H_{30}O_2$	314.4617

1.6 Calibration

Calibration of the m/z scale of the time-of-flight mass spectrometer is achieved by external and internal calibration which refers to the process of calibration prior to analysis and simultaneously to the analysis respectively. As the calibration is essential in ensuring proper mass accuracy, the calibrant or reference compound should have a series of peaks, particularly in the mass region of interest and calibration should occur as closely as possible to the analysis to minimise the effects of instrument drift.

For this work, sodium formate was chosen as a reference compound, shown in **Figure 1.15**, as it forms a series of singly or doubly charged clusters over a wide range.

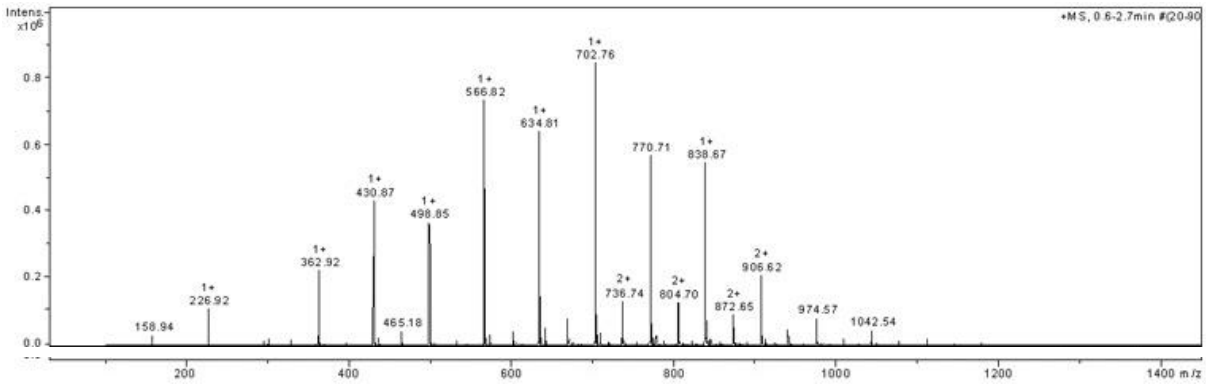


Figure 1.15 Mass range covered by sodium formate

External calibration is necessary as part of the preparation prior to running samples on the instrument and may highlight issues of maintenance. Internal calibration is achieved by introducing the reference compound at the beginning of the analysis where it does not interfere with eluting compounds and provides considerably higher accuracy.

An example of internal calibration is shown in **Figure 1.16**.

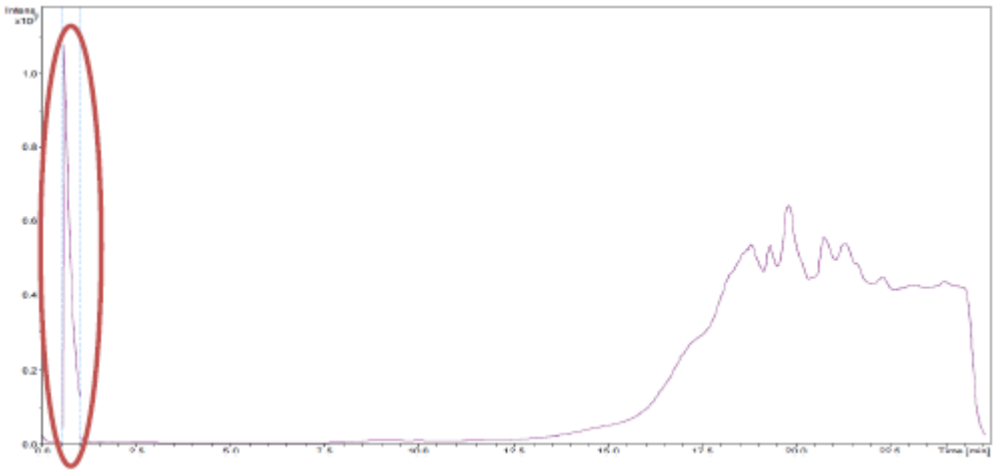


Figure 1.16 Blank urine sample with internal calibration peak between 0.5 minutes and 1 minute

The mass spectrum of the internal calibration serves the same purpose as the external calibration, but increases the accuracy of the instrument significantly, especially when processing large batch numbers**.**

1.7 Aims

The aim of this project was to develop a method for extracting and analysing a wide variety of drugs from urine, a primary matrix in forensic toxicology, and ultimately determining the utility and limitations of time-of-flight mass spectrometry as a general and easily expandable screening method, which includes practical and economic considerations.

2 Method Development

The following chapter describes the experimental methods used in the development of the method and the consequent results obtained.

An overview is given over different approaches in extraction, liquid-liquid extraction and solid phase extraction, optimisation of mobile phase, LC conditions, relevant validation parameters and data processing.

2.1 Materials

DCM, IPA, NH_4OH, hexane, acetonitrile, methanol, ethyl acetate and MTBE were of HPLC grade, sodium bicarbonate and sodium carbonate decahydrate were of analytical grade and were all purchased from VWR International (UK). NaOH, tris-(hydroxymethyl)-aminomethane (TRIS), tartaric acid, glacial acetic acid, ammonium acetate, formic acid and HCl were of analytical grade and purchased from Sigma-Aldrich (UK).

SPE cartridges were purchased from United Chemicals Technologies (USA) (Clean Screen ZSDAU020), Agilent Technologies (USA) (Bond Elut Certify) and Phenomenex (USA) (Strata-X-C).

Certified drug reference standards and deuterated drug reference standards (morphine, cocaine, THC, MDEA, amphetamine, diazepam and zolpidem) were prepared by Cerilliant® (USA) and obtained from Sigma-Aldrich® (UK) in the concentrations of 1 mg/mL and 100 µg/mL respectively.

Synergi 4µ Fusion-RP 80Å (150 x 2.0 mm) and Gemini-C18 (4 x 2.0 mm Guard Column) were purchased from Phenomenex (USA).

2.2 Solutions

2.2.1 1M NaOH

4 g NaOH was dissolved carefully (exothermic reaction) in 80 mL of deionised water in a 200 mL beaker under constant stirring. The solution was then filled up to 100 mL with deionised water.

2.2.2 0.2M Tris Buffer

2.44 g of tris-(hydroxymethyl)-aminomethane was dissolved in 80 mL of deionised water in a 200 mL beaker under constant stirring. The solution was then filled up to 100 mL with deionised water. The solution was stored at room temperature for up to a month.

2.2.3 0.1M Phosphate Buffer pH 6

1.7g Na_2HPO_4 and 12.14g NaH_2PO_4 were dissolved in 800 mL of deionised water and pH was adjusted to pH 6 with 1M KOH. The solution was then transferred into a 1 L reagent bottle and filled up to 1 L with deionised water. Phosphate buffer was stored at room temperature for up to two weeks.

2.2.4 0.1M Acetic Acid

0.576 mL glacial acetic acid were transferred into a 100 mL volumetric flask with 80 mL deionised water. The solution was then filled up to 100 mL with deionised water.

2.2.5 Hexane/Ethyl Acetate (50:50)

For a mixture of 100 mL hexane/ethyl acetate (50:50), 50 mL of hexane were added to 50 mL of ethyl acetate and mixed.

2.2.6 DCM/IPA/NH3 (78:20:2)

Procedure should take place in a fume hood due to the high gas pressure and intense smell of NH3. 20 mL of IPA were added to 78 mL of DCM in a 100 mL reagent bottle and 2 mL of NH3 were carefully added and the bottle closed. The mixture was then thoroughly mixed to ensure mixture of all components.

2.2.7 Tartaric Acid (1 mg/mL)

100 mg of tartaric acid was dissolved in 80 mL of ethyl acetate in a beaker and the solution was then filled up to 100 mL with ethyl acetate and stored at room temperature for up to four weeks.

2.2.8 Acetic Acid (5% in Water)

5 mL of glacial acetic acid are added to 80 mL of deionised water in a volumetric flask and the solution is then filled up to 100 mL with deionised water. The solution is stored at room temperature for up to four weeks.

2.2.9 ACN + 2 % NH4OH

Procedure should take place in a fume hood due to the high gas pressure and intense smell of NH_4OH. 2 mL of NH_4OH were added to 80 mL of acetonitrile in a volumetric flask and the solution was then filled up to 100 mL with acetonitrile.

2.2.10 0.1M HCl

0.83 mL of concentrated HCl (37%) was transferred carefully into a 100 mL volumetric flask with 80 mL deionised water and then filled up to 100 mL with deionised water.

2.2.11 0.1M HCl in Methanol

0.83 mL of concentrated HCl (37%) was transferred carefully into a 100 mL volumetric flask with 80 mL methanol and then filled up to 100 mL with methanol.

2.2.12 5 % NH_4OH in Methanol

Procedure should take place in a fume hood due to the high gas pressure and intense smell of NH_4OH. 5 mL of NH_4OH was added to 80 mL of methanol in a volumetric flask and the solution was then filled up to 100 mL with methanol.

2.2.13 Blank Urine

Blank urine was collected from drug-free volunteers in house, tested to make sure the samples were negative and stored in the fridge (4 °C).

2.2.14 Standard Stock Solution

1mL of each 1 mg/mL standard solution of morphine, cocaine, zolpidem, diazepam, amphetamine, MDEA and THC were transferred into a 10 mL volumetric flask and filled up with methanol to 10 mL, which resulted in a 100 µg/mL solution for each drug. This solution was stored in the freezer at -20 °C.

2.2.15 Standard Working Solution (1 µg/mL)

50 µL of the Standard Stock Solution (100 µg/mL) was transferred to a 5 mL volumetric flask and filled up to 5 mL with methanol, which resulted in a 1 µg/mL solution for each drug. The solution was stored in the fridge at 4 °C.

2.2.16 Internal Standard Working Solution (10 µg/mL)

1mL of each 100 µg/mL standard of morphine-d3, cocaine-d3, zolpidem-d6, diazepam-d5, amphetamine-d5, MDEA-d5 and THC-d3 were transferred into a 10 mL volumetric flask and filled up with methanol to 10 mL, which resulted in a 10 µg/mL solution. The solution was stored in the fridge at 4 °C.

2.2.17 2M Ammonium Acetate

15.42 g of ammonium acetate was dissolved in 80 mL of deionised water under constant stirring and, if necessary, warming of the mixture. The solution was then filled up to its final volume of 100 mL with deionised water. The solution was stored at room temperature for up to four weeks.

2.2.18 0.1M Sodium Bicarbonate

0.84 g of sodium bicarbonate was dissolved in 80 mL of deionised water under constant stirring and, if necessary, warming of the mixture. The solution was then filled up to its final volume of 100 mL with deionised water. The solution was stored at room temperature for up to four weeks.

2.2.19 0.1M Sodium Carbonate Decahydrate

2.86 g of sodium carbonate decahydrate was dissolved in 80 mL of deionised water under constant stirring and, if necessary, warming of the mixture. The solution was then filled up to its final volume of 100 mL with deionised water. The solution was stored at room temperature for up to four weeks.

2.2.20 0.1 % Formic Acid and 2 mM Ammonium Acetate (baseline mobile phase)

1 mL of concentrated formic acid (98 - 100%) and 1 mL of 2 M ammonium acetate was added to 500 mL of deionised water (aqueous buffer) or methanol (organic solvent) in a 1 L volumetric flask and filled up to 1 L with deionised water or methanol respectively. The resulting concentration was 0.1 % formic acid and 2 mM ammonium acetate and the pH of the aqueous buffer was determined to be pH 3. Both solutions were stored at room temperature for up to two weeks.

2.2.21 0.007 % Formic Acid and 2 mM Ammonium Acetate

1 mL of 2 M ammonium acetate was added to 800 mL of deionised water (aqueous buffer) or methanol (organic solvent) in a 1 L beaker and concentrated formic acid (98 100%) was added until pH 5 was measured in the aqueous solution, resulting in the addition of 77 µL of conc. formic acid. The solution was then transferred to a 1 L volumetric flask and filled up

to 1 L with deionised water or methanol respectively. The resulting concentration was 0.007 % formic acid and 2 mM ammonium acetate. Both solutions are stored at room temperature for up to two weeks.

2.2.22 0.0013 % Formic Acid and 2 mM Ammonium Acetate

1 mL of 2 M ammonium acetate and was added to 800 mL of deionised water (aqueous buffer) or methanol (organic solvent) in a 1 L beaker and concentrated formic acid (98 100%) was added until pH 7 was measured in the aqueous solution, resulting in the addition of 13 µL of conc. formic acid. The solution was then transferred to a 1 L volumetric flask and filled up to 1 L with deionised water or methanol respectively. The resulting concentration was 0.0013 % formic acid and 2 mM ammonium acetate. Both solutions were stored at room temperature for up to two weeks.

2.2.23 Carbonate Buffer

This buffer was prepared by adding 0.9 mL of 0.1M Sodium Bicarbonate and 1.1 mL of 0.1M Sodium Carbonate Decahydrate to 500 mL of deionised water (aqueous buffer) or methanol (organic solvent) in a volumetric flask and filled up to 1 L with deionised water or methanol respectively. The pH of the aqueous buffer was determined to be pH 10. The solutions were stored at room temperature for up to two weeks.

2.2.24 0.02 % formic acid and 0.4 mM Ammonium Acetate

200 µL of concentrated formic acid (98 - 100%) and 200 µL of 2 M ammonium acetate was added to 500 mL of deionised water (aqueous buffer) or methanol (organic solvent) in a 1 L volumetric flask and filled up to 1 L with deionised water or methanol respectively. The resulting buffer concentrations were 0.02 % formic acid and 0.4 mM ammonium acetate and the pH of the aqueous buffer was determined to be pH 3. The solutions were stored at room temperature for up to two weeks.

2.2.25 0.01 % formic acid and 0.2 mM Ammonium Acetate

100 µL of concentrated formic acid (98 - 100%) and 100 µL of 2 M ammonium acetate was added to 500 mL of deionised water (aqueous buffer) or methanol (organic solvent) in a 1 L volumetric flask and filled up to 1 L with deionised water or methanol respectively. The resulting buffer concentrations were 0.01 % formic acid and 0.2 mM ammonium acetate and the pH of the aqueous buffer was determined to be pH 3. The solutions were stored at room temperature for up to two weeks.

2.2.26 0.001 % formic acid and 0.1 mM Ammonium Acetate

10 µL of concentrated formic acid (98 - 100%) and 50 µL of 2 M ammonium acetate was added to 500 mL of deionised water (aqueous buffer) or methanol (organic solvent) in a 1 L volumetric flask and filled up to 1 L with deionised water or methanol respectively. The resulting buffer concentrations were 0.001 % formic acid and 0.1 mM ammonium acetate and the pH of the aqueous buffer was determined to be pH 3. The solutions were stored at room temperature for up to two weeks.

2.2.27 0.1 % formic acid and 2 µM ammonium acetate

1 mL of concentrated formic acid (98 - 100%) and 1 mL of 2 mM ammonium acetate was added to 500 mL of deionised water (aqueous buffer) or acetonitrile (organic solvent) in a 1 L volumetric flask and filled up to 1 L with deionised water or acetonitrile respectively. The resulting concentrations were 0.1 % formic acid and 2 µM ammonium acetate and the pH of the aqueous buffer was determined to be pH 3. The solution is stored at room temperature for up to two weeks.

2.3 Instrument Parameters

Below are shown the software versions and basic nebuliser settings used as included in the software package and recommended by Bruker respectively.

Instrument	Bruker micrOTOFq
Software Package	Compass 1.3 for micrOTOF - SR1
Interface	microTOF Control Version 3.0 (Build 53)
Source	ESI+
Nebuliser	2 Bar
Dry Gas	8 L/min
Dry Temp	180 °C
Flow Rate	0.3 mL/min
Column	Synergi 4u Fusion-RP 80A 150 x 2.0 mm + Gemini-C18 4 x 2.0 mm Guard Column

Discussed are only settings which were set to deliberate values as other settings were dependent on the extensive calibration, done according to instrument specifications by qualified engineers, and were not held constant over the course of this study.

2.4 Experimental

2.4.1 Instrument Preparation

For every change in mobile phase the instrument was prepared by flushing it with the respective mobile phase at 50% aqueous buffer and 50% organic solvent for several hours

to make sure no traces of the previously used mobile phase remained within the system and contaminated the results. Afterwards, the column itself was equilibrated by setting the mobile phase mixture to 95% aqueous buffer and 5% organic solvent and letting at least five column volumes of mobile phase run through it.

2.4.2 Extraction Optimisation

2.4.2.1 Liquid-Liquid Extraction

The combination of organic solvent and aqueous buffer was optimised by investigating four different combinations. For each combination the following protocol was followed. 3mL of organic solvent was mixed with 0.5mL of aqueous buffer and 1mL of spiked urine using a vortex mixer. The sample was then centrifuged at 3000 rpm for 20 minutes to ensure phase separation and accumulation of matrix particles in a pellet. After phase separation the organic phase was extracted via a glass pipette and transferred into a 3.5 mL vial and 100 µL tartaric acid (1mg/mL in ethyl acetate) was added to prevent more volatile compounds from evaporating. Consequently, the organic solvent was evaporated under a constant stream of nitrogen at room temperature. The extract was then reconstituted in 100 µL of mobile phase and transferred to a LC vial with a 250 µL insert.

Table 2.1 below details the different solvent combinations investigated.

Table 2.1 Organic solvent and aqueous buffer combinations investigated

Combination	Organic Solvent	Aqueous Buffer
1	Dichloromethane	1M NaOH
2	Dichloromethane	tris(hydroxymethyl)aminomethane (TRIS)
3	tert-butyl methyl ether	1M NaOH
4	tert-butyl methyl ether	tris(hydroxymethyl)aminomethane (TRIS)

The results were evaluated by determining the limits of detection for the representative group of drugs detailed in **Section 1.5** for each combination in **Table 2.1**. Limits of detection have been determined by investigating the proposed extractions for five different concentrations

(4 ng/mL, 20 ng/mL, 40 ng/mL, 200 ng/mL and 400 ng/mL) achieved by spiking blank urine samples with the Standard Working Solution (Section 2.2.15) accordingly for a total sample volume of 1 mL. Additionally, 30 µL of the Internal Standard Solution (Section 2.2.16), for a final concentration of 300 ng/mL was added to all spiked samples after the extraction step to allow for the standardisation of results.

The determined limits of detection for the tested liquid-liquid extractions are displayed in **Table 2.2**.

Table 2.2 Limits of detection for all tested liquid-liquid extraction methods

Compound	**Determined Limits of Detection [ng/mL]**			
	Combination 1	***Combination 2***	***Combination 3***	***Combination 4***
Morphine	4	4	4	4
Cocaine	40	4	400	4
Zolpidem	4	4	4	4
Diazepam	4	4	4	4
Amphetamine	40	20	400	20
MDEA	20	20	400	20
THC	20	20	200	20

Combination 2 and combination 4 showed identical results and the lowest LODs, however due to practical considerations combination 4 was determined to be the optimal liquid-liquid extraction method.

2.4.2.2 Optimisation of Organic Solvent Extraction Volume

Solvent combination 4, as detailed in **Table 2.1,** was determined to be the optimum solvent combination, therefore this was used as a starting point to optimise the solvent volumes. The organic solvent volumes investigated for the extraction were 1, 2, 3 and 4 mL and followed the methodology described above (**Section 2.4.2.1**) with the respective volumes for organic solvent.

Results for the tested extraction volumes are presented in **Figure 2.1** for the volumes 1, 2, 3 and 4 mL.

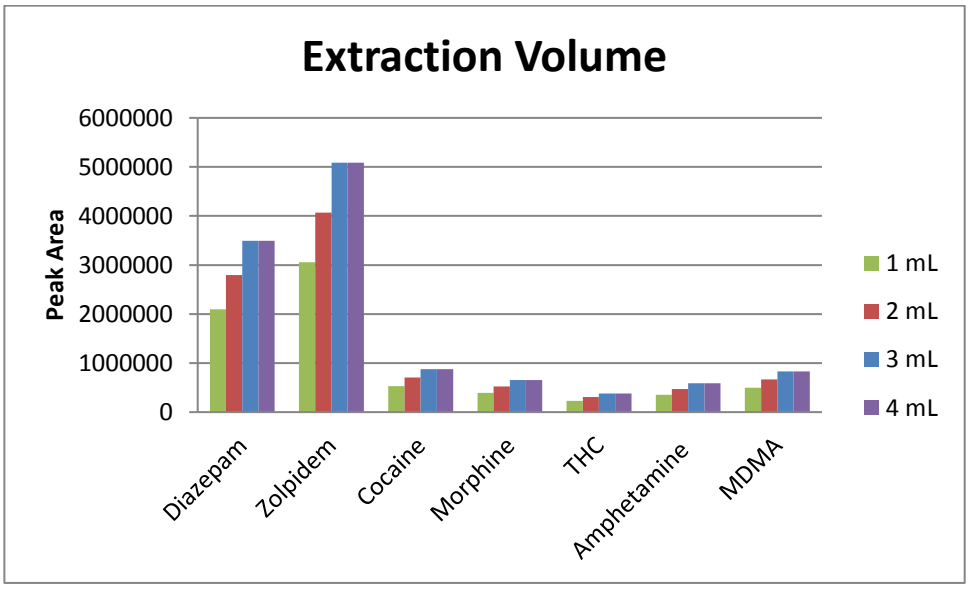


Figure 2.1 Peak area average of nine chromatograms of tested standards for different solvent volumes

Extraction with organic solvent volumes above 3 mL yielded no higher results and thus the optimal volume of organic solvent used in extraction was determined to be 3 mL.

2.4.2.3 Solid Phase Extraction

Three different solid phase extraction cartridges were investigated. Three replicates at five different concentrations were extracted using each extraction method detailed below.

2.4.2.3.1 Clean Screen Cartridge (ZSDAU020)

0.5 mL phosphate buffer (pH = 6; 100 mM) and 4 mL deionised water were added to 1 mL urine and centrifuged at 3000 rpm for 10 minutes. The cartridge was conditioned with methanol (3 mL), deionised water (3 mL) and 100 mM phosphate buffer (pH = 6; 1 mL) then the sample mixture was applied to the cartridge. After the sample had been applied, the column was washed with deionised water (3 mL), 100 mM acetic acid (1 mL) and left to dry

under full vacuum for one minute before being washed with a final application of hexane (2 mL).

The first fraction (acidic and neutral drugs) was eluted with hexane/ethyl acetate (50:50; 3 mL) and the cartridge washed again with methanol (3 mL) and dried under full vacuum for 5 minutes. A second fraction (basic drugs) was eluted with DCM/IPA/NH_4OH (78:20:2; 3 mL)

The fractions were combined and 100 µL tartaric acid solution (1mg/mL in ethyl acetate) was added to keep more volatile compounds from vaporising. The solvent was then dried under a stream of nitrogen at room temperature, reconstituted in 100 µL mobile phase and transferred to a LC vial with a 250 µL insert (injection volume: 10 µL).

2.4.2.3.2 Bond Elut Certify

The column was conditioned with methanol (2 mL) and equilibrated with 100 mM phosphate buffer (pH = 6; 2 mL). As the sample matrix was applied directly to the column without dilution, a positive displacement pipette was used to apply the sample (1 mL) gradually onto the column. Washing consisted of 5% acetic acid (1 mL), and methanol (2 mL) after which the sample was eluted with ACN + 2 % NH_4OH (3 mL).

100 µL tartaric acid (1mg/mL in ethyl acetate) were added to keep more volatile compounds from vaporising. The mixture was then dried under a stream of nitrogen, reconstituted in 100 µL mobile phase and transferred to a LC vial with a 250 µL insert (injection volume: 10 µL).

2.4.2.3.3 Strata-X-C

To 1mL of urine, pH 6.0, 100 mM phosphate buffer (0.5 mL) and deionised water (4 mL) were added before centrifuging at 3000 rpm for 10 minutes. The column was conditioned with methanol (1 mL) and equilibrated with pH 6.0, 100 mM phosphate buffer (2 mL), before the sample was applied. The column was then washed with deionised water (1 mL)

and subsequently 0.1M HCl (1 mL), this second wash fraction contains polar neutrals. Neutral and acidic compounds were washed off with 0.1M HCl in methanol (1 mL). Final drug elution was achieved with 5 % NH_4OH in methanol (1 mL).

The second wash fraction and elution fraction were analysed separately and 100 µL tartaric acid (1mg/mL in ethyl acetate) were added to each to keep more volatile compounds from vaporising. The mixture was then dried under a stream of nitrogen at room temperature, reconstituted in 100 µL mobile phase and transferred to a LC vial with a 250 µL insert (injection volume: 10 µL).

The determined limits of detection for the tested solid phase extractions are displayed in **Table 2.3** in ng/mL.

Table 2.3 Limits of detection for all tested solid phase extraction methods

Compound	**Determined Limits of Detection [ng/mL]**		
	Clean Screen	***Bond Elut Certify***	***Strata-X-C***
Morphine	4	4	20
Cocaine	4	4	4
Zolpidem	4	4	4
Diazepam	20	4	4
Amphetamine	40	20	200
MDEA	40	20	40
THC	40	20	200

As shown in Table 3.3, the best results for SPE were achieved with Bond Elut Certify cartridges with significantly lower limits of detection for diazepam, amphetamine, MDEA and THC.

2.4.3 Optimisation of Mobile Phase

The conditions of the mobile phase have considerable impact in the analysis and have a direct impact on which compounds can be detected and the quality of the resulting data, therefore it is important to optimise this parameter to suit the desired method specifications.

The following four parameters were investigated: pH of the aqueous solvent, concentration of the buffer, organic solvent and the gradient of the system.

A baseline for comparison of the mobile phase has been established by using a baseline mobile phase, which is described in detail in **Section 2.2.20**. All buffers consist of a system of formic acid and ammonium acetate, except in the investigation of pH > 7 which utilised a carbonate buffer.

2.4.3.1 pH

The first parameter investigated was the pH of the aqueous buffer solution, which can significantly influence the separation of analytes, peak shape and overall ionisation of compounds.

The original aqueous solvent of mobile phase, with a concentration of 2 mM ammonium acetate and 0.1% formic acid, was measured to have a pH of 3, consequently pH5, pH7 and pH10 were investigated for their viability in the analysis of the desired analytes. Different proportions of ammonium acetate and formic acid were used to achieve these different pH values, except for the pH 10 buffer which was a carbonate buffer system. Tests were performed with a sample concentration of 200 ng/mL and 3 separate samples were run in triplicates.

The preparation of aqueous solvents with different pH is described in **Section 2.2**.

The results for different buffer pH are presented in **Figure 2.2**. It should be noted that while for pH 7 little to no data could be acquired for Diazepam and Zolpidem - none for Cocaine, Morphine and THC - the measured peak areas were inconsequential in comparison to the significantly stronger data presented for pH 3 and pH 5 in **Figure 2.3**. Furthermore, no data

could be obtained for pH 10 as the carbonate in the solvents crystallised considerably at the spray shield and did not allow for data acquisition. Further investigations of the carbonate buffer were suspended to prevent potential damage to the instrument.

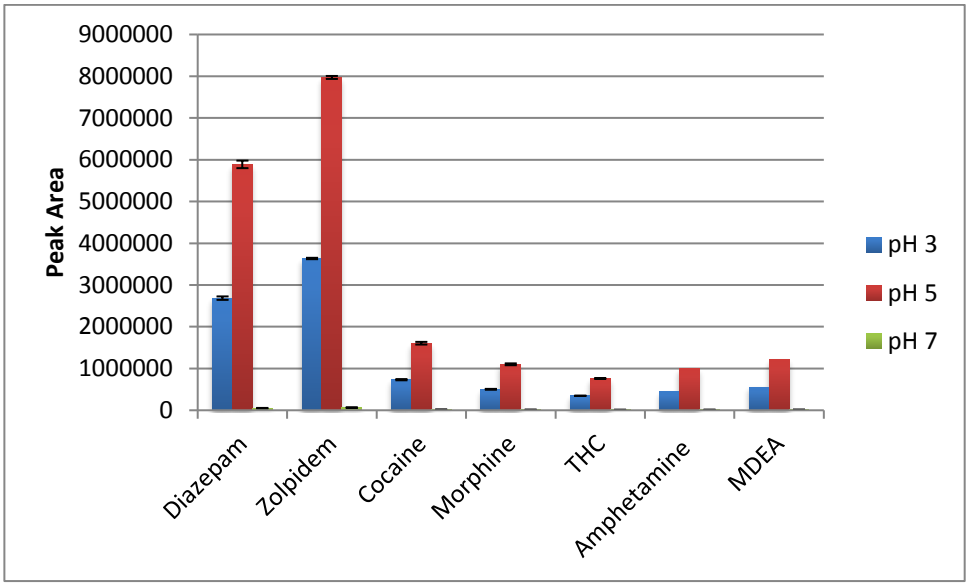


Figure 2.2 Total peak area average (c = 200 ng/mL) of nine chromatograms for representative drugs at pH 3, 5 and 7. Results were standardised with the addition of deuterated internal standards

While the change from pH 3 to pH 5 increased the response of all analysed drugs, a further increase to pH 7 yielded almost no results at all. However, as a pH of 7 is not conducive to ionisation this result is not unexpected.

2.4.3.2 Buffer Concentration

A change in the concentration of the aqueous solvent buffer, while simultaneously keeping a constant pH, affects the polarity and ionic strength of the mobile phase. Consequently, this mainly affects the peak shape but can in some cases even affect the retention of analytes on the stationary phase.

Starting from the original concentration of 2 mM ammonium acetate and 0.1 % formic acid, several dilutions with 0.02 % formic acid and 0.4 mM ammonium acetate, 0.01 % formic acid and 0.2 mM ammonium acetate and 0.001 % formic acid and 0.1 mM ammonium acetate were investigated. Preparation of the mobile phases is detailed **Section 2.2**.

Figure 2.3 shows the results of 4 buffers.

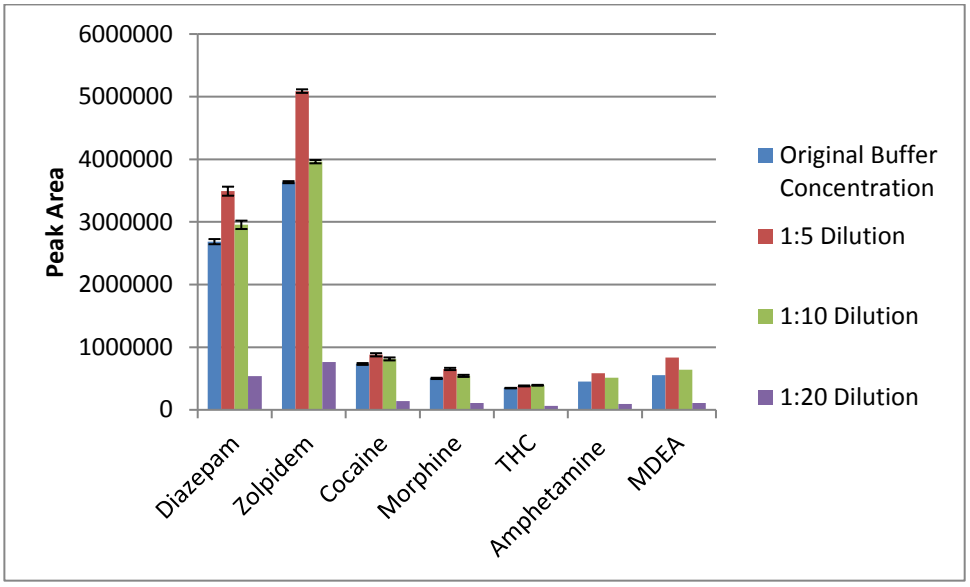


Figure 2.3 Total peak area average of nine chromatograms for representative drugs at three buffer concentrations

A change from the original concentration of 0.1 % formic acid and 2 mM ammonium acetate to 0.02 % formic acid and 0.4 mM ammonium acetate increased the overall response of all drugs while a further decrease of the concentration to 0.01 % formic acid and 0.2 mM ammonium acetate did only yield a slight increase in the response over the original concentration. Further reduction of the concentration to 0.001 % formic acid and 0.1 mM ammonium acetate resulted in a significant drop in the overall response of all drugs.

2.4.3.3 Gradient

The percentage of the stronger solvent - methanol or acetonitrile in this case - is in reverse phase chromatographic methods gradually increased to reduce the overall retention window, reduce peak tailing and increase peak sharpness. While a gradient program can be of considerable complexity, including sudden steps, which increase the percentage of the organic solvent, or different slopes at different times to target or create certain retention windows, the investigated gradients have been kept fairly simple to accommodate a wide variety of expected and unexpected analytes.

Three gradient variations were investigated. Firstly, a simple linear gradient (Gradient A) from 95% aqueous solvent to 5% aqueous solvent over 27.5 minutes as presented in **Figure 2.4**. The decreasing gradient was followed by an immediate return to 95% aqueous solvent over 0.5 minutes and a three-minute isocratic plateau to re-equilibrate the column for the next sample.

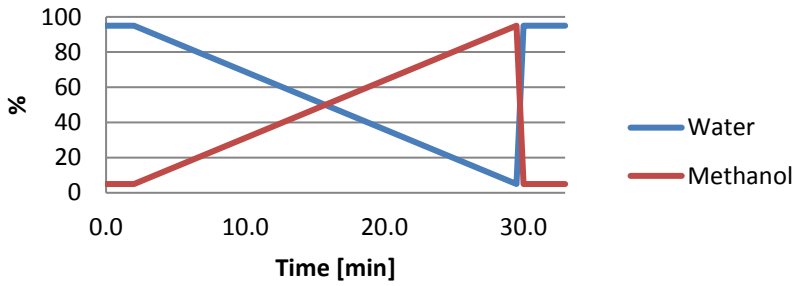


Figure 2.4 Intervals and display of the linear Gradient A

The second tested gradient (Gradient B shown in **Figure 2.5**) included a very steep step from 95% aqueous solvent to 55% aqueous solvent in one minute and a consecutive slower decline from 55% aqueous solvent to 5% aqueous solvent over 23.5 minutes. This gradient was paced in that way as almost all of the desired analytes eluted when the gradient had reached a higher percentage of organic solvent. Consequently, Gradient B favoured the elution of these analytes as it shifts the focus of elution from 45% organic solvent to 95% organic solvent over the same time frame as Gradient A. Since the slope of the Gradient has consequently been decreased in Gradient B, it is aimed at the prevention of having too many analytes co elute at the same time. While high resolution mass spectrometry can resolve a high amount of co eluting analytes, co elutes can contaminate the individual mass spectra which are an important part of identification by the software. Additionally, the initial low organic percentage allows the elution of analytes that are favoured by these conditions, however as most analytes prefer a higher organic percentage the focus was put on the organic elution part of the gradient.

This was followed by a short three-minute plateau, to ensure elution of all compounds, a quick return to 95% aqueous solvent over 0.5 minutes and again a short plateau at 95% aqueous solvent over 3 minutes to re-equilibrate the column.

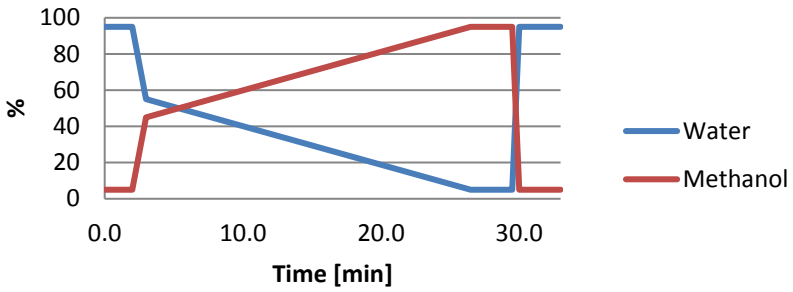


Figure 2.5 Intervals and display of the linear Gradient B

The final tested gradient (Gradient C shown in **Figure 2.6**) was very similar to Gradient B with a significantly increased gradient from 55% aqueous solvent to 5% aqueous solvent over just 12 minutes in contrast to 23.5 minutes for Gradient B. The overall time was shortened to decrease the time necessary to analyse multiple samples in succession while simultaneously achieving the same quality of analyte elution as previously established.

The mixture was again switched quickly from 5% aqueous solvent to 95% aqueous solvent in 0.5 minutes with an equilibration period of 6.5 minutes. The re-equilibration period is significantly greater in Gradient C as in previous gradients, 6.5 minutes compared to 3 minutes respectively.

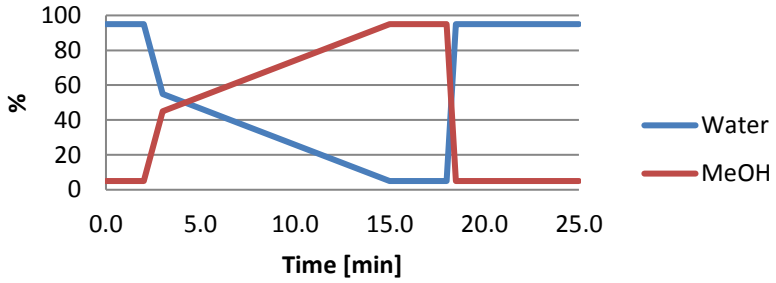


Figure 2.6 Intervals and display of the linear Gradient C

The results for gradients B and C are shown in **Figure 2.7** and **Figure 2.8** with the background (TIC) and the individual EIC's.

The change from gradient B (**Figure 2.7**) to C (**Figure 2.8**) did not significantly alter the distance between the different peaks, but succeeded in shifting the retention times into an earlier window. This made a shorter run time possible and allowed the analysis of more samples in less time.

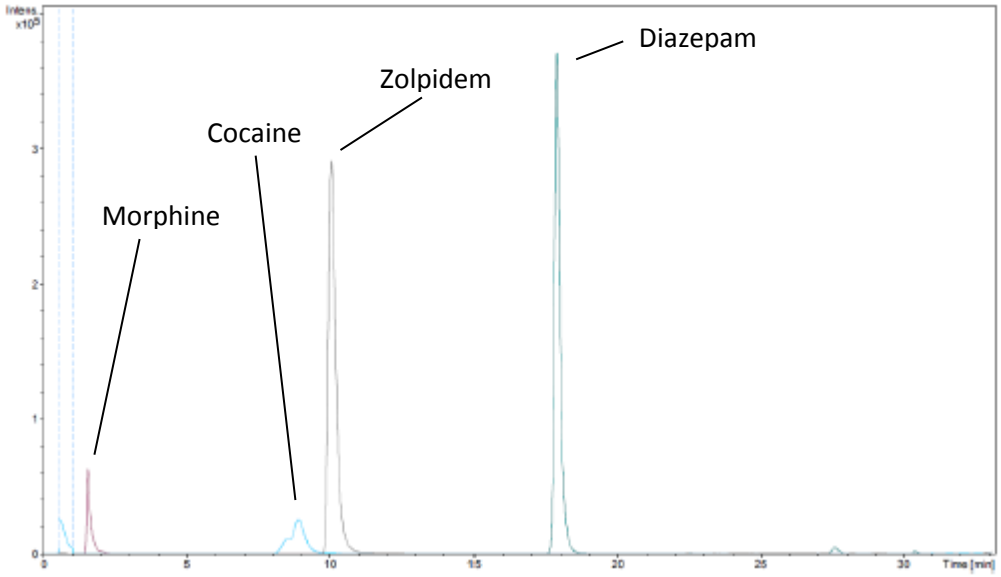


Figure 2.7 Chromatogram containing morphine, cocaine, zolpidem and diazepam (from left to right) using MeOH as the organic component of the mobile phase and Gradient B

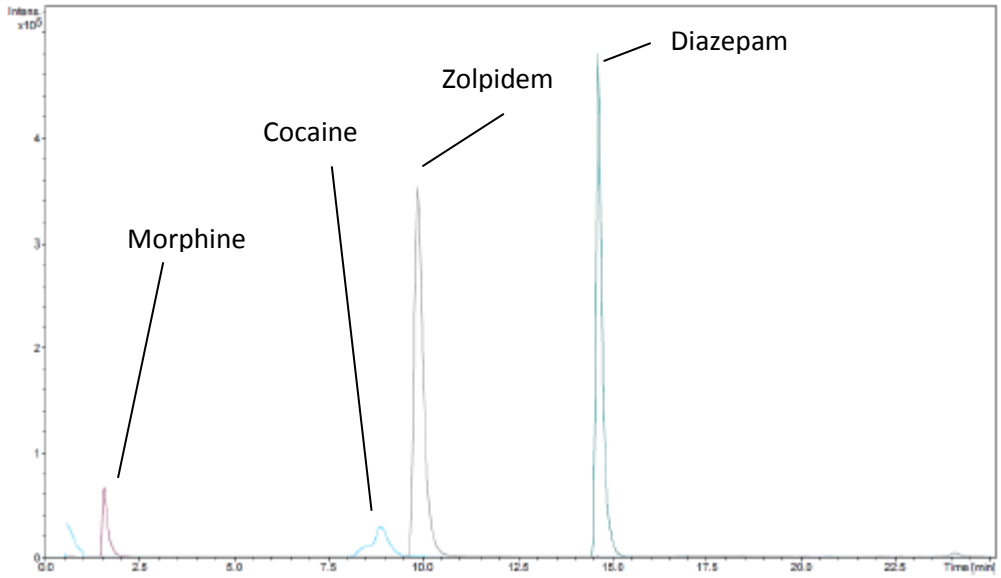


Figure 2.8 Chromatogram containing morphine, cocaine, zolpidem and diazepam (from left to right) using MeOH as the organic component of the mobile phase and Gradient C

2.5 Qualitative Confirmation/Identification Validation

Validation is the process of performing a set of experiments that reliably estimates the efficacy and reliability of an analytical method or modification to a previously validated method. The aim of this procedure was to establish objective evidence towards the method's capability of performing at set standards and to identify the limitations under normal operating conditions.

While validating a method in a forensic toxicological setting, it should be acknowledged that performance can vary in day-to-day analysis due to the complexity of the components involved. The evaluated validation parameters serve as an estimate of a method's actual performance.

2.5.1 Validation

Validation of the in **Section 2.4.2.1.** determined method was performed according to the guidelines presented in Standard Practices for Method Validation in Forensic Toxicology Appendix D, published by the Scientific Working Group for Forensic Toxicology (SWGTOX)[47].

2.5.2 Selectivity

For the analysis with high resolution mass spectrometry, the potential interference for each particular analyte must be determined individually to prevent the occurrence of false positive results in more complex and real samples.

- To determine any selectivity issues from matrix for individual analytes, three blank urine samples – made up by combining blank urine from 10 different sources – were prepared and spiked with a single standard (c = 300 ng/mL). The spiked urine sample was then extracted according to the previously established method and analysed. This procedure was repeated for all drugs and standards used in the method.

- The selectivity between analytes or internal standards was determined by spiking a blank urine sample in triplicate – made up by combining blank urine from 10 different sources – with one analyte and internal standards (c = 300 ng/mL). The experiment was repeated for each analyte contained in the method and extraction was conducted according to the previously established protocol.

- Furthermore, three blank urine samples – made up by combining blank urine from 10 different sources – were spiked with internal standards and a mixture containing

all relevant analytes (c = 300 ng/mL) and were extracted according to the established method.

Two specific issues of selectivity were identified between the chosen standards and respective internal standards as shown in **Figure 2.9**. The shown interference was between the pure standards cocaine-d_3 and zolpidem at 13.1 min and between the pure standards zolpidem-d_6 and THC at 14.4 min.

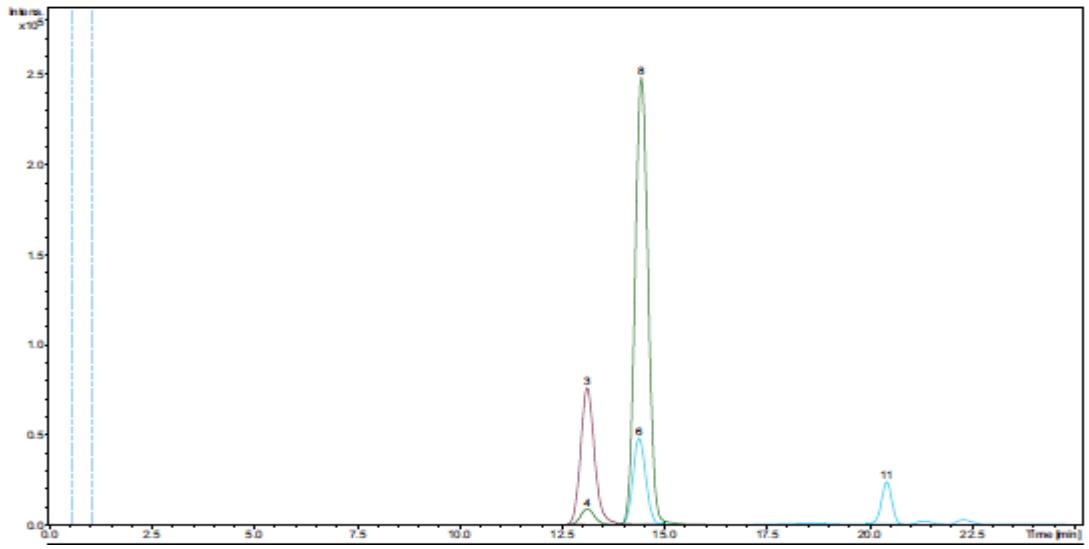


Figure 2.9 Observed selectivity issues in the chromatograms of zolpidem (green) and THC (blue) by the internal standards cocaine-d_3 and zolpidem-d_6 respectively

Furthermore, it has been found that a peak that was regularly recognised by the software according to its exact mass as AM-2201 as shown in **Figure 2.10.**

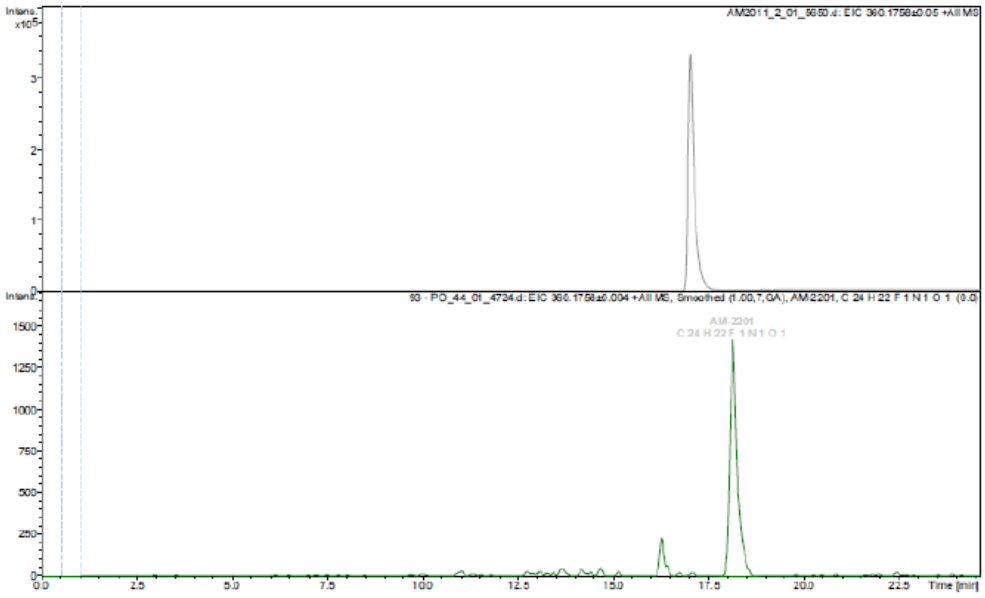


Figure 2.10 Regularly observed exact mass match (bottom) with corresponding standard peak (top) for AM-2201

2.5.3 Matrix Effects

Overall matrix effects were assessed by investigating the matrix effects for the chosen internal standards to represent the range of different substances.

Blank urine was collected from 10 different sources and three samples from each source were spiked with internal standard and standard mix solution (c = 300 ng/mL) and consequently extracted according to the previously established method. The results were consequently compared with samples extracted from pure drugs (c = 300 ng/mL).

The ionisation suppressions/enhancements have been estimated for the established method by investigating and comparing different sources of urine and comparison of the observed deviations from samples extracted from pure standards.

Figure 2.11 depicts the results for the individual standards, morphine, cocaine, zolpidem, diazepam, amphetamine MDMA and THC, which were extracted from spiked blank urine injected in triplicate.

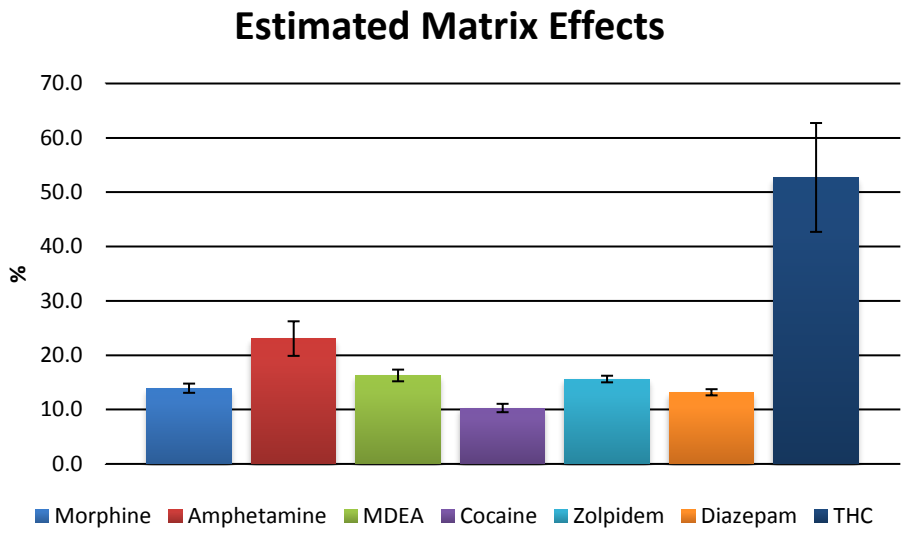


Figure 2.11 Assessment of matrix effects

Figure 2.11 shows the percentage the signal deviated in the matrix from the pure standard and while a general matrix enhancement was determined, the degree of the effect varied depending on the individual urine sample. THC, while also showing matrix enhancement in some cases, could not be detected in several samples, which would suggest heavy matrix suppression.

2.5.4 Limits of Detection

The limits of detection have been determined and discussed previously in **Section 2.4.2.1**.

2.5.5 Exact Mass Library

The software TargetAnalysis utilises a separate user-generated library which contains the chemical formula, name and retention time of the desired analytes.

Each analyte and standard was analysed individually and a library was established by adding the chemical formulae and respective experimental retention times of the analytes into a comma separated value file (.csv) which takes the form of "m/z (M+H), rt, formula, name".

While it allows for the addition of the exact mass of the compound, the actual value used by the program is calculated internally from the chemical formula and takes precedence over the manual entry.

2.5.6 Data processing

The processing of data is not only an integral part of any analytical method as it is designed to target the specifics of any method, but it also is a powerful tool in method development. How the obtained data is processed has an influence mainly on the quality and scope of results and determines the occurrence of false positive or negative results, which is of particular importance forensic toxicological considerations.

The instrument accompanying software to analyse different aspects of the acquired data provides several tools in data extraction and processing. Several variations of data processing have been investigated and are presented in the following.

TargetAnalysis processes the obtained data by generating extracted ion chromatograms (EIC) for every analyte contained in the library and comparing the parameters defined in the library to the ones obtained from the EIC. Matches are rated in regard to acceptable deviations defined prior to processing and the output is demonstrated in **Figure 2.12**.

#	Found	Compound Name	Reg.No.	Mol.Formula	PMI	d RT[min]	Err[ppm]	Err[mDa]	mSigma	Area	Intens.	RT,exp.[min]	RT,meas.[min]	m/z,calc.	m/z,meas.
1	+	Morphine	000	C 17 H 19 N 1 O 3	+	-2.05	4.5	1.3	13.7	489408	15953	0.00	2.05	286.1438	286.1425
2	+	AMP	000	C 9 H 13 N 1	+	-8.62	14.1	1.9	10.4	31782	1245	0.00	8.62	136.1121	136.1102
3	+	MDEA	000	C 12 H 17 N 1 O 2	+	-9.40	6.4	1.3	7.1	53786	2950	0.00	9.40	208.1332	208.1319
4	+	Cocaine	000	C 17 H 21 N 1 O 4	+	-10.60	8.2	2.5	6.4	833741	28697	0.00	10.60	304.1543	304.1518
5	+	Zolpidem	000	C 19 H 21 N 3 O 1	+	-12.14	1.2	0.4	12.0	3800999	165852	0.00	12.14	308.1757	308.1754
6	+	Diazepam	000	C 16 H 13 Cl 1 N 2 O 1	+	-16.48	-6.2	-1.8	19.3	4198432	288496	0.00	16.48	285.0789	285.0807
7	+	THC	000	C 21 H 30 O 2	+	-20.64	10.8	3.4	10.3	199056	20882	0.00	20.64	315.2319	315.2284

Figure 2.12 Result of a spiked urine sample processed with TargetAnalysis

The resulting EIC's generated by the software are shown overlapped and stacked in **Figure 2.13** and individually in **Figure 2.14.**

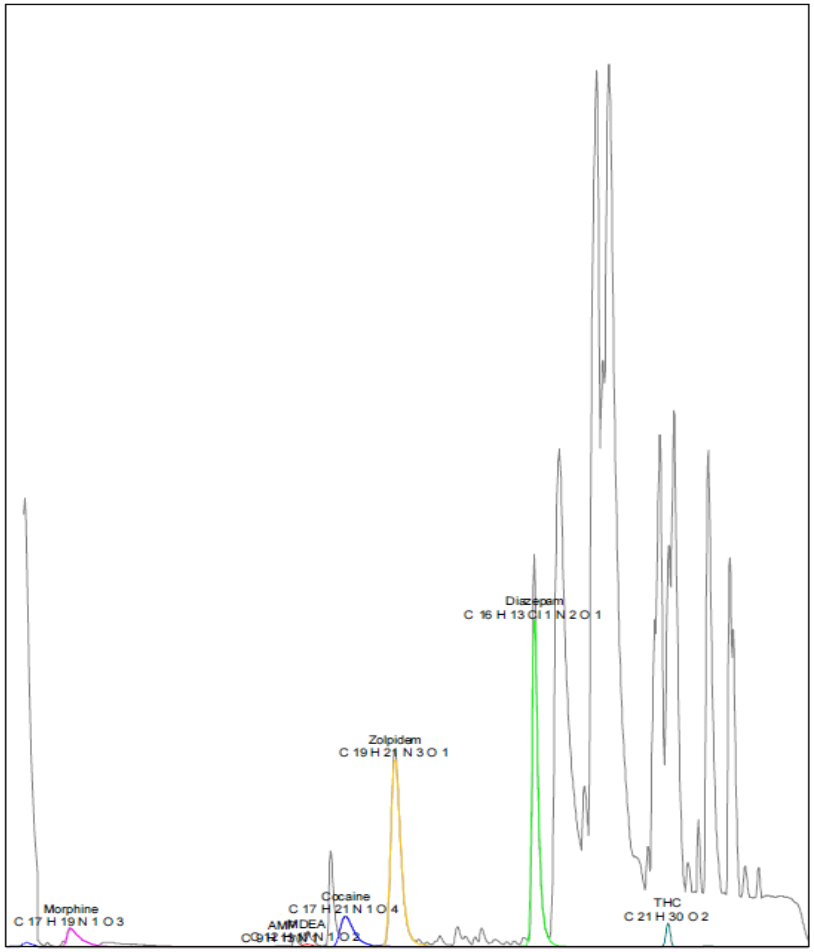


Figure 2.13 Peaks as identified by TargetAnalysis and TIC

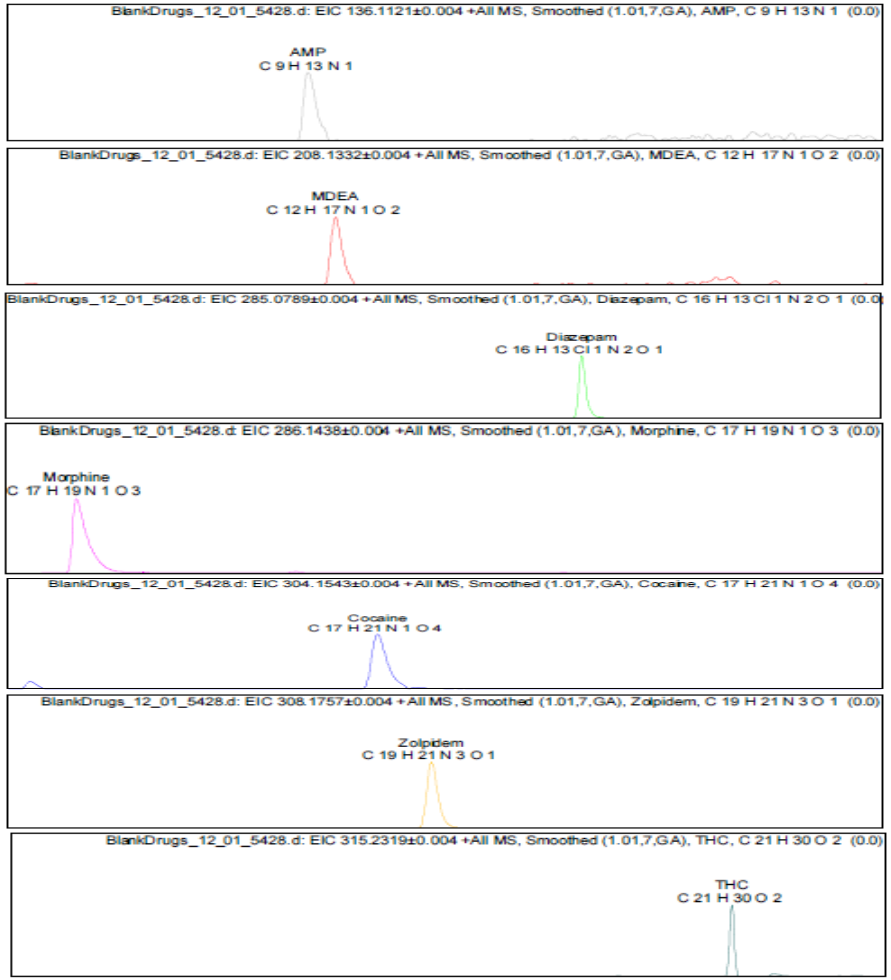


Figure 2.14 Individual extracted ion chromatogram

2.6 Discussion & Conclusion

2.6.1 Optimisation of Extraction

The organic solvents used in the investigated liquid-liquid extraction are differing on a very practical point, their density. Experimentally this means that DCM, which is heavier than water, collects as the lower phase, while MTBE, which is lighter than water, collects in the upper phase. This is mainly of practical consideration as MTBE is far easier to extract and to keep free of contamination from the aqueous phase. DCM needs to be extracted through the aqueous phase and thus requires much more care to prevent contamination from the aqueous phase. Additionally, it is considerably easier to remove MTBE through vaporisation than DCM which makes this particular step during the extraction process less time consuming.

Comparison of the liquid-liquid extraction for urine shows that the extractions with Tris show generally lower limits of detection with 4 ng/mL for morphine, cocaine, zolpidem and diazepam and 20 ng/mL for amphetamine, MDEA and THC regardless of organic solvent used. Extractions with sodium hydroxide show significantly higher limits of detection with 40 ng/mL for cocaine and amphetamine with sodium hydroxide and DCM and 400 ng/mL for cocaine, amphetamine and MDEA and 200 ng/mL for THC with sodium hydroxide and MTBE.

Investigation of solid phase extraction cartridges showed the lowest limits of detection for the Bond Elut Certify cartridges with 4 ng/mL for morphine, cocaine, zolpidem and diazepam and 20 ng/mL for amphetamine, MDEA and THC. The Clean Screen cartridges showed slightly higher limits of detection for diazepam with 20 ng/mL and amphetamine, MDEA and THC with 40 ng/mL. Significantly higher limits of detection have been found for the Strata-X-C cartridges with 20 ng/mL for morphine, 40 ng/mL for MDEA and 200 ng/mL for amphetamine and THC.

Investigation of the organic solvent volume needed to make optimal use of the liquid-liquid extraction (**Figure 2.1**) showed 3 mL of organic solvent to be the most effective volume. Even though a higher volume of organic solvent (4 mL) has been tested it did not increase

the performance of the extraction as it showed the same results as with 3 mL of organic solvent.

Matrix effects have been determined not for every drug contained in the library, but for the seven representative drugs to allow a general assessment of matrix effects in the established method for a wide variety of drugs differing in mass, retention time, solubility and chemical properties. Furthermore, the assessment of matrix effects for each drug contained in the library was considered to be economically unfeasible.

Consequently, for the extraction of urine case samples the extraction with MTBE and Tris was chosen. Extractions with Tris showed in general a cleaner chromatogram as extractions with NaOH, as the background noise and TIC (Total Ion Chromatogram) were significantly lower in extractions using Tris. Furthermore, the variability between samples and injections has been assessed for both extractions utilising Tris to determine any significant difference in the %CV between both extractions. Both extraction variants, using DCM or MTBE with Tris, proved to be extremely close together with average %CV between different injection of the same extraction of 5.8 % and 6.1 % for morphine, 14.2 % and 12.4 % for amphetamine, 4.5 % and 4.3 % for MDEA, 7.2 % and 6.1 % for cocaine, 4.7 % and 5.3 % for zolpidem, 5.4 % and 4.1 % for diazepam and 20.4 % and 17.7 % for THC respectively. %CV values between samples were overall higher, but similarly close for extraction with DCM or MTBE with 14.6 % and 13.9 % for morphine, 32.9 % and 32.1 % for amphetamine, 15.8 % and 16.3 % for MDEA, 11.4 % and 10.3% for cocaine, 15.1 % and 15.6 % for zolpidem, 15.4 % and 13.2 % for diazepam and 47.5 % and 52.7% for THC respectively. While the same limits of detection were determined for the extraction with MTBE and Tris as for DCM and Tris, the considerable easier practical considerations in the extraction process provided by using MTBE, as discussed previously, made this method preferable.

2.6.2 Mobile Phase Optimisation

2.6.2.1 pH

The change of the pH of the aqueous buffer solvent proved to be unsuccessful for pH 7 and pH 10. In the case of pH 7, it is most likely that the absence of considerable amounts of free

protons (H^+) did not favour the ionisation of the compounds as the ion source, in positive ionisation mode, yields ions in the form of M+H+. Consequently, while a very limited amount of data could be obtained, the detected response for all seven analytes was either extremely low (diazepam, zolpidem) or remained entirely undetected (morphine, cocaine, amphetamine, MDEA and THC).

The analysis of the pH 10 buffer, achieved by a carbonate buffer, resulted in a completely different problem as the carbonate salts that make up the buffer in the aqueous solvent, while readily dissolving during preparation, were not volatile enough and crystallised again in the spray chamber physically blocking this and making the acquisition of data impossible.

The only viable tested pH values of the aqueous solvent were pH 3 and pH 5. The recorded response is significantly higher with the pH 5 buffer than with the pH 3 buffer for all analytes and therefore this pH was chosen for the final method.

2.6.2.2 Buffer Concentration

The next investigated parameter was the concentration of the aqueous solvent buffer which was varied from the original concentration of 0.1 % formic acid and 2 mM ammonium acetate to 0.02 % formic acid and 0.4 mM ammonium acetate, 0.01 % formic acid and 0.2 mM ammonium acetate and 0.001 % formic acid and 0.1 mM ammonium acetate. The lowest tested concentration, 0.001 % formic acid and 0.1 mM ammonium acetate, resulted in very low responses from the analytes with especially low responses for amphetamine and THC. While 10% dilution, with 0.01 % formic acid and 0.2 mM ammonium acetate, shows a slight increase in response over the original concentration the 5% dilution, with 0.02 % formic acid and 0.4 mM ammonium acetate, showed a significant increase in response over the original concentration.

Similar limits of detection were achieved by Daalsgard et al. [44] – with 5 ng/mL for diazepam, cocaine and zolpidem, and 50 ng/mL for different amphetamines and morphine – while utilising SPE extraction of blood and a mobile phase of water/ACN and formic acid. Good results were shown for the extraction of synthetic cannabinoids by protein precipitation by Marginean et al. [46], with limits of detection ranging from 7-50 ng/mL

while utilising a water/MeOH mobile phase with formic acid and ammonium formate. Dimínguez-Romero [45] has shown considerable improved limits of detection, with many compounds significantly below 1 ng/mL, by using solid phase extraction followed by filtration of the extracts of urine samples.

2.6.2.3 Gradient

Changes in the gradient have been investigated for both solvents as alternatives for the organic phase of the mobile phase and methanol, as seen in **Figure 2.7** to **Figure 2.8**, suffer the same interference from a high background throughout the whole chromatogram with the same deteriorating consequences as discussed before. In contrast, methanol as the organic phase of the mobile phase reacted as intended to the changes in the gradient with an overall reduced retention time window and reduced run length which in turn saves time in analysing whole batches of samples.

2.6.3 Validation

2.6.3.1 Selectivity

The main purpose of this qualitative confirmation/identification validation is to identify potential sources for contamination from the matrix, sample preparation and compounds used.

Several issues of selectivity have been identified, the first of which was one of the isotope peaks of cocaine-d_3 ($M+H^+ = 307.1732$, with the relevant isotope being $M+H^+ = 308.1764$) which is close enough to zolpidem ($M+H^+ = 308.1757$). Both signals are still separated by their respective retention times with 13.1 min and 14.4 min respectively, as shown in **Figure 2.15** and identifiable by the relevant mass spectra as shown in **Figure 2.16**.

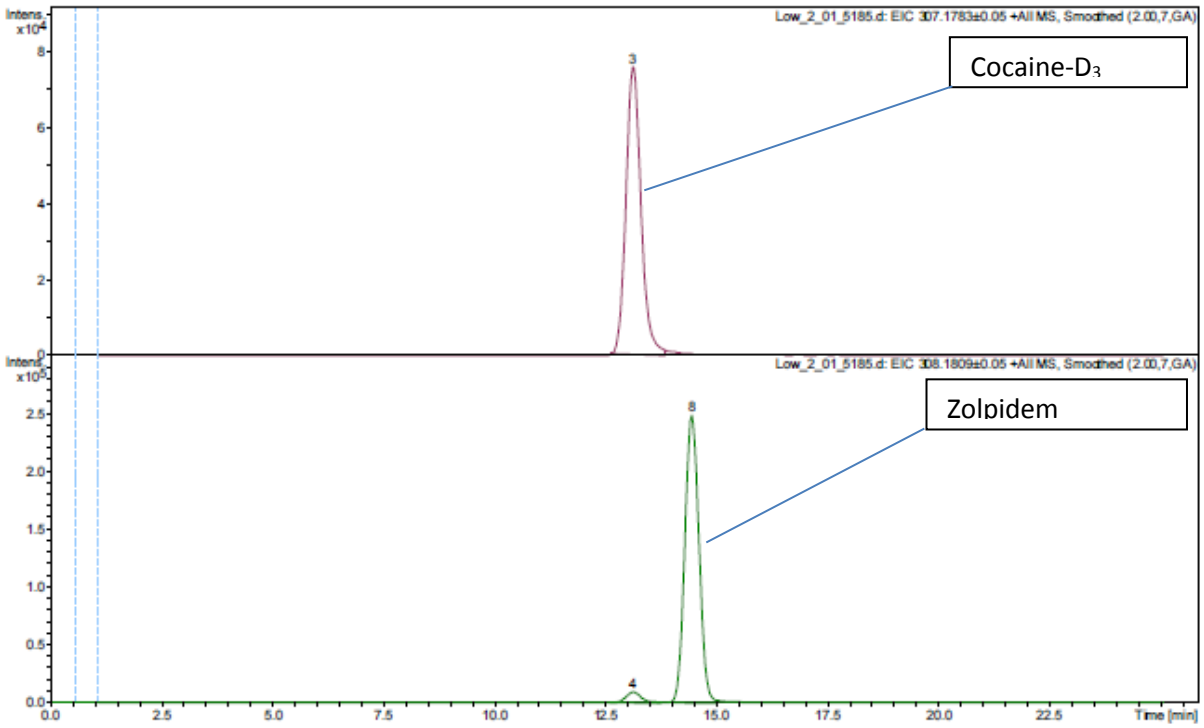


Figure 2.15 Chromatogram of Cocaine-D_3 and Zolpidem

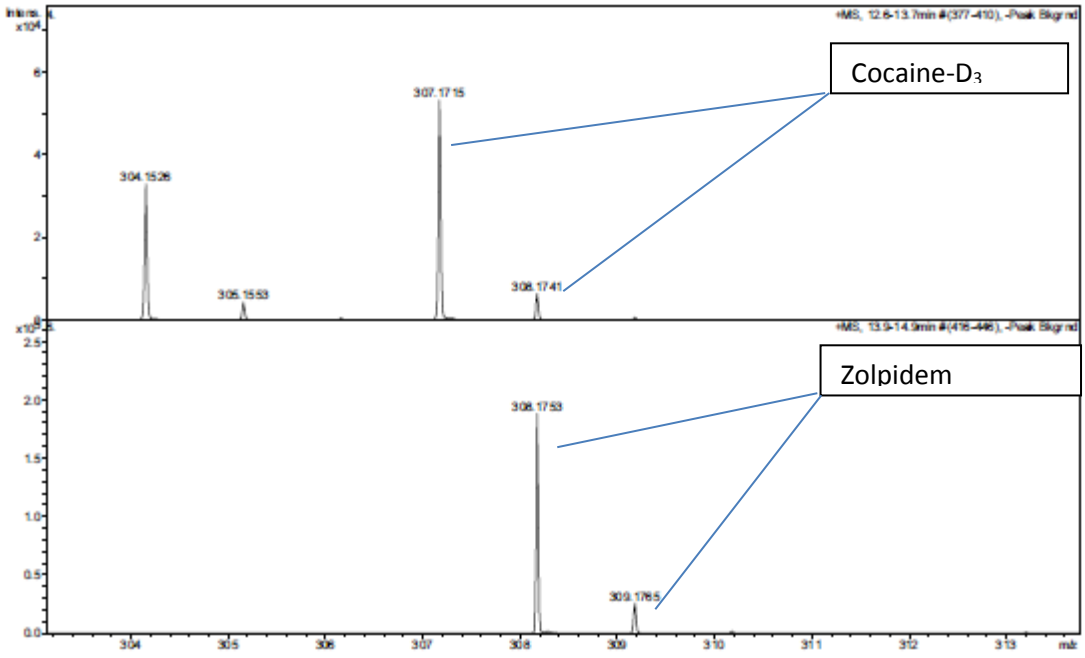


Figure 2.16 Mass spectra of Cocaine-D_3 and Zolpidem

The next identified selectivity issue is one of the isotope peaks of zolpidem-d_6 ($M+H^+$ = 314.2134, with the relevant isotope being $M+H^+$ = 315.2165) which is close to THC ($M+H^+$ = 315.2319). Both signals are still separated by their respective retention times,

with 14.4 min and 21 min respectively as shown in **Figure 2.17** and identifiable by the relevant mass spectra as shown in **Figure 2.18**.

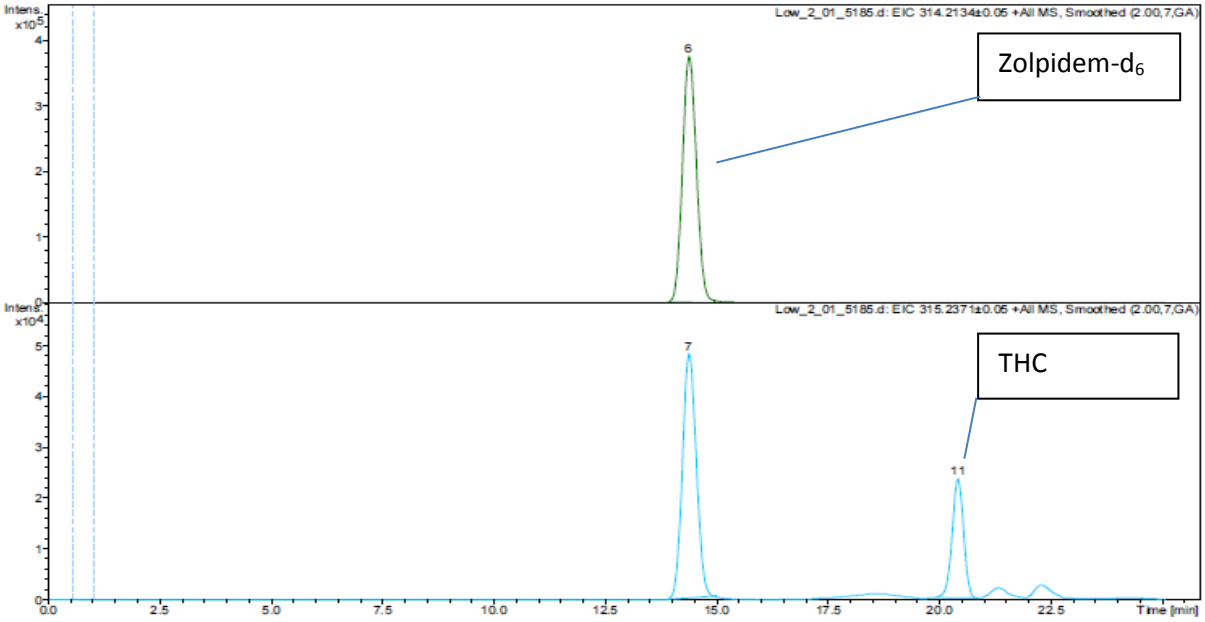


Figure 2.17 Chromatogram of Zolpidem-D_6 and THC

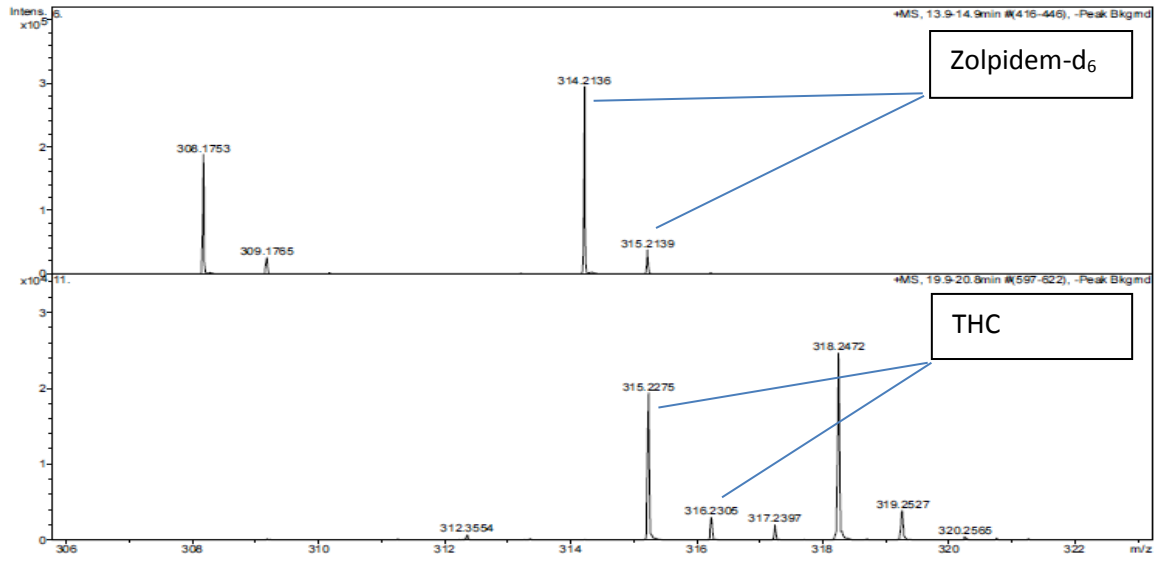


Figure 2.18 Mass spectra of Zolpidem-D_6 and THC

Two direct issues in regard to selectivity have been identified in the internal standards used. More precisely, the first isotope peaks of cocaine-d_3 and zolpidem-d_6 are close enough to

the signals of zolpidem and THC respectively to show up in their respective trace chromatograms. Naturally, the isotope peaks show up at the retention time of the compound they originate from and thus are easily distinguished from the compounds they interfere with as their retention times differ significantly. Furthermore, TargetAnalysis compares the isotope pattern of identified compounds with their theoretically determined isotope pattern as one parameter to establish a positive match. Consequently, isotope peaks, while appearing in the trace chromatograms, are not recognised as individual compound peaks of that compounds extracted ion chromatogram.

2.6.3.2 Exact Mass Library

Compounds are screened and identified in their respective extracted ion chromatogram (EIC) according to their exact masses and retention time. An additional score sigma (σ) is generated which compares the isotope pattern of the obtained mass spectra against the theoretical isotope pattern of the compound according to its chemical formula. These mass spectra are obtained by the software by creating extracted ion chromatograms for all compounds in the database and the recognition of relevant mass at the predetermined retention time.

- *Intensity ≥ 1000*

  Since the extracted ion chromatograms of compounds acquired with high resolution mass spectrometry are usually almost free of noise, the determination of signal-to-noise ratio (3:1) was deemed not feasible. While most compounds can reliably be acquired at as low an intensity of 500, a can go considerably lower. To avoid the reporting of false positive results the limit of identification was arbitrarily set to an intensity of 1000.

- Peak Shape

  While a slight broadening of peaks, depending on the analyte, can be expected all peaks should follow a Gaussian shape as much as possible. While this is a subjective

criterion that requires experience with the method, the peak shapes vary between analysed compounds and should not be filtered by a program.

- mSigma Score

  The mSigma (σ) score given by TargetAnalysis is the result of comparing the theoretically calculated isotope pattern to the identified isotope pattern. This is, however, not a completely reliable indicator. While a low mSigma score signals a positive match of the isotope pattern and is a very strong indicator of a positive result, a high mSigma score does not necessarily signal a negative result. Significant interference, background noise or co eluting compounds in the same mass range as the isotope pattern can cause a high mSigma score even in positive results

- Retention Time and Mass Error

  As in every chromatographic method, the retention time and mass match of the potential results are the main factors in determining positive or negative results. A retention time window of ±0.2 minutes was determined to be a good match and the widest detection range set to ±0.5 minutes to ensure detection in the case of a shift in retention time. The acceptable mass error was set to 5 mDa.

The identification and overall confidence in the results could be greatly increased by adding fragmentation of the desired analytes to the methods. However, several limitations prevented the establishment of a fragmentation library to support the exact mass recognition.

Firstly, while the software allows for MRM (Multiple Reaction Monitoring), the list for scanned precursor ions is limited to 50 entries, which severely limits this particular approach as the method can be easily expanded but already includes almost twice as many analytes as the software allows for MRM.

Secondly, setting a threshold to cause the fragmentation of any compound that exceed a minimum signal strength is inherently problematic in any method that screens a wide range of different compounds as the signal strength of many compounds can vary significantly even if they are present at the same concentration. Consequently, a threshold set too low would potentially trigger fragmentation for compounds that are part of the matrix, which

would clutter the chromatogram with undesired data, while a threshold set too high would potentially miss analytes of interest with an inherently low signal strength.

Lastly, half of every second of data acquisition is used by an untargeted fragmentation sweep, which fragments all ions present during that time by covering a wide range of fragmentation energies. While this results in the fragmentation of the desired analytes, it also results in a significant noise of lower mass ions – as not only the desired analytes are fragmented, but inherent matrix compounds as well – which made the recognition of qualitative ions impossible.

2.6.3.3 Matrix Effects

While all determined matrix effects showed an enhancing effect, the highest matrix effects were determined for THC with up to 53% enhancement. However, THC also showed a %CV of 19% which makes the results highly variable depending on the individual urine sample.

Matrix effects for morphine, amphetamine MDEA, cocaine, zolpidem and diazepam were determined between 10 and 23% enhancement with %CV's varying from 4-14%. The guidelines suggest that the average ion suppression or enhancement should not exceed ±25% and the %CV should not exceed 15%. These limitations are only exceeded in the case of THC which has already been determined as a compound with limited visibility when analysed in the context of this method.

2.7 Conclusion

The method established in this chapter consists of a LLE with MTBE and Tris and a mobile phase with the buffer concentration of 0.02 % formic acid and 0.4 µM ammonium acetate. Furthermore, the most suitable pH of the aqueous mobile phase has been established to be pH 5, while the preferred organic solvent was methanol.

Furthermore, while the proposed method shows a notable variance in peak area depending on the individual sample the results were also reproducible and the use of isotope labelled standards ensured that results from different samples can be compared. The wide range of proposed drug classes encompassed in this method makes individual analyte optimisation impractical. However, the method proved to be viable to screen for all representative compounds, with limited usability for THC analysis.

3 Case Samples

All urine samples were analysed using the extraction method detailed in **Section 2.4.2.1** and the data processed according to the methodology detailed in **Section 2.5.6**.

For the purpose of discussion, traditionally abused drugs and typical prescription drugs are combined under the nomination of drugs of abuse. Furthermore, the term Novel Psychoactive Substances (NPS) is expanded to entail substances not included in regular screenings methods as well as newer compounds that are not regularly screened for, such as etizolam.

Ethical approval for samples collected from Hospital A&E admissions was sought and granted from NHS GG&C Ethics and they deemed it as a service evaluation. Consent was waived for the study as this was considered a service development study as urine samples are sent for a toxicology screen as a standard of care and testing was carried out on samples already being obtained.

With regards to the Scottish Prison Service study, ethical approval was granted from the West of Scotland Research Ethics Service. A copy of the letter of approval can be found in the appendix.

3.1 Hospital A&E Admission Samples (Urine)

Glasgow Royal Infirmary is a large inner city emergency department, with ~86,000 attendances per year. During the time between 1 May 2014 and 29 July 2014, urine samples and data were collected by the treating clinicians, from patients who attended the department, according to their medical history or clinical suspicion on the ingestion of NPS. Patients younger than 16 were excluded from the study.

No case history was available in five cases, the remaining 75 were made up of 54 men (aged 17 – 55 years) and 21 women (aged 16 – 47 years). The source of referral was by ambulance (37), self-referral (14) and by the police (24).

3.1.1 Results

A wide range of drugs were detected as shown in **Table 3.1** with their respective prescription status.

Table 3.1 Frequency of detected drugs and their prescription status[48]

Drug	Not prescribed (%)	Prescribed (%)
Diazepam	24 (30)	9 (11.25)
MDMA	18 (22.5)	0
Cocaine	16 (20)	0
Amitriptyline	14 (17.5)	0
6-MAM	9 (11.25)	0
Etizolam	7 (8.75)	0
Amphetamine/MDA	6 (7.5)	0
Mirtazapine	6 (7.5)	3 (3.75)
Methadone	1 (1.25)	13 (16.25)
MDAI	4 (5)	0
Gabapentin	3 (3.75)	0
Methoxetamine	2 (2.5)	0
TFMPP	2 (2.5)	0
PMA/PMMA	2 (2.5)	0
Methedrone	1 (1.25)	0
Butylone	1 (1.25)	0
Ketamine	1 (1.25)	0
Buprenorphine	1 (1.25)	1 (1.25)

The majority of detected drugs were not prescription drugs, with the exception of methadone which was prescribed in 13 of the detected 14 cases. Diazepam, MDMA and cocaine were detected most frequently, while NPS such as methoxetamine, Butylone, MDAI and methedrone were detected, but only infrequently.

Figure 3.1 and **Figure 3.2** show the distribution of cases where no drugs, only NPS or only traditionally abused drugs were detected and the frequency of poly drug use respectively.

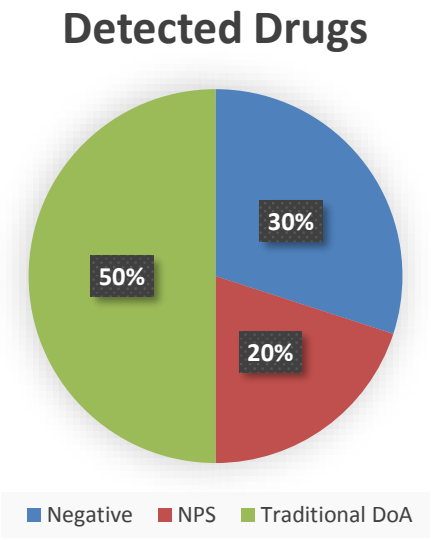


Figure 3.1 Distribution of detected drugs for A&E admissions

As shown in **Figure 3.1**, the majority of samples tested positive for drugs (50%) contained only traditionally abused drugs, while NPS were only detected in 16 (20%) of cases. 24 samples tested negative for all drugs.

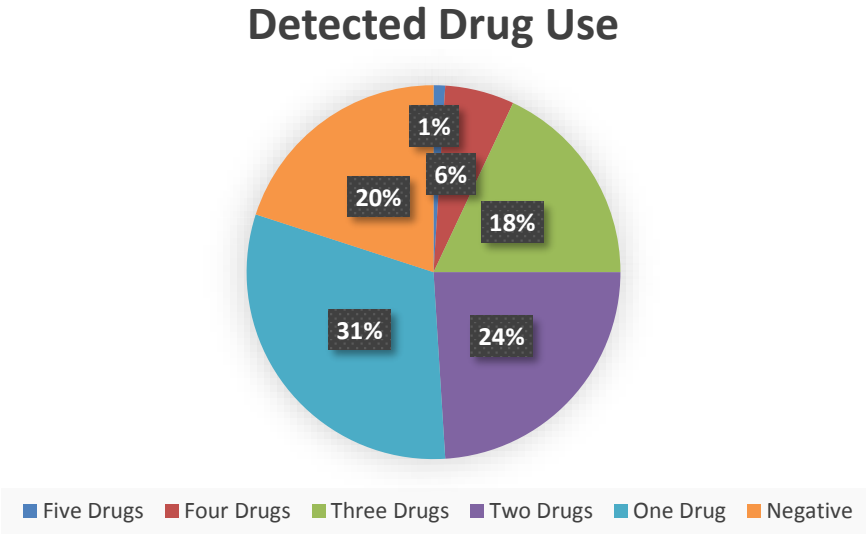


Figure 3.2 Frequency of poly drug use in A&E admissions[48]

The majority of drug use was determined to be single drug use with 31%, while 49% (39) samples showed more than one drug present.

Table 3.2 Drugs detected versus patient reports[48]

Reported ingestion	Toxicology results
No history	Dihydrocodeine*, citalopram*, methoxetamine,
Cocaine	Diazepam* etizolam, cocaine, methadone*, amitriptyline
No History	Lignocaine*, mirtazapine, lamotrigine*, amitriptyline, methadone*. MDAI, MDA
NPS	PMA, PMMA, cocaine
No History	Diazepam, codeine*, etizolam citalopram*
Cocaine	Cocaine, MDA, diazepam, MDAI, carbamazepine*
Cocaine	TFMPP, cocaine
No history	Etizolam, codeine*, 6-MAM, carbamazepine*, methoxetamine
Diazepam, gabapentin	Etizolam, gabapentin, methadone*, MDMA
Heroin, amitriptyline	Lidocaine*, 6-MAM, codeine*, butylone
Diazepam	Amitriptyline, methadone*, 6-MAM, etizolam
Diazepam	Gabapentin, carbamazepine*, etizolam, methedrone. MDMA
Cocaine	Quetiapine, MDAI, cocaine, codeine*
No history	MDAI, diazepam, fluoxetine*, dihydrocodeine
Ecstasy	TFMPP, MDMA
No History	Etizolam, methadone*, amitriptyline, diazepam*

*** indicates drugs known to be prescribed to that individual**

Table 3.2 highlights the disparity between patient reports and toxicological findings, however it is unknown if this is intentional or if the patients were unaware of their consumption.

3.1.2 Discussion

Of particular interest were the 24 samples which tested negative despite the specific patient selection for this study which targets cases with a history of drug of abuse or self-reported drug consumption. The negative results may be due to the concentration being too low as the analyte may not have been passed into the urine by the time of collection or the urine present concentration was below the limits of detection. Another possibility is that the analyte was not in the library and thus not detected, which could be remedied by processing the obtained high resolution data again with an expanded library – a distinct advantage of the data obtained by time-of-flight instrument.

The positive results as shown in **Table 3.2** show diazepam, MDMA, cocaine and amitriptyline as the mainly abused substances, which, with the exception of amitriptyline, corresponds to drugs listed in *Drug-related deaths in Scotland in 2014 (© Crown copyright. Data supplied by National Records of Scotland)* as drugs regularly involved in drug-related deaths. The abuse of amitriptyline is known in patients on methadone therapy [49], however only one patient reported the consumption of amitriptyline.

Mirtazapine and etizolam have both been detected in significant quantities, even though none of the patients reported intentional consumption and the abuse of mirtazapine is not referenced in the literature. In the case of etizolam, only one person had a history of benzodiazepines consumption and etizolam was detected in combination with other illicit drugs present in all samples. Furthermore, etizolam and MDAI, which was detected in 4 samples, are not currently regulated by the Misuse of Drugs Act in the UK which may indicate a tendency towards unregulated compounds for the purpose of abuse.

3.2 Prison Prevalence Samples (Urine)

Urine samples were provided by the Scottish Prison Service in the context of their 2013 prevalence study and were collected over the period of one month, which yielded a total of 904 samples. Prisons included in the data acquired for this work were Perth, Corton Vale, Low Moss, Barlinnie, Addiewell, Edinburgh and Polmont as they were located in the central belt of Scotland, chosen for logistical reasons, and Greenock as they expressed a high interest

in our study. The Scottish Prison Service expressed interest in the study as they were concerned that their own regular drug tests would not recognise the full spectrum of abused substances, especially NPS as they are not part of their own testing regime

Table 3.3 shows the distribution between admission and liberation as well as the total number of all samples analysed for this work.

Table 3.3 Distribution of provided admission and liberation samples by individual prisons

Prison	***Total***	***Admission***	***Liberation***
Perth	187	123	64
Corton Vale	101	90	11
Low Moss	118	100	18
Barlinnie	172	106	66
Addiewell	63	33	30
Edinburgh	85	25	60
Greenock	27	11	16
Polmont	151	102	49
Total	904	590	314

3.2.1 Results

Figure 3.3 shows the overall positive results for all admission samples analysed (n = 590) and **Figure 3.4** shows the overall positive results for all liberation samples analysed (n = 314). Results were split between drugs of abuse and novel psychoactive substances as defined previously. Furthermore, potential metabolites are regarded as the precursor drug in the discussion even though the metabolites are drugs in their own right.

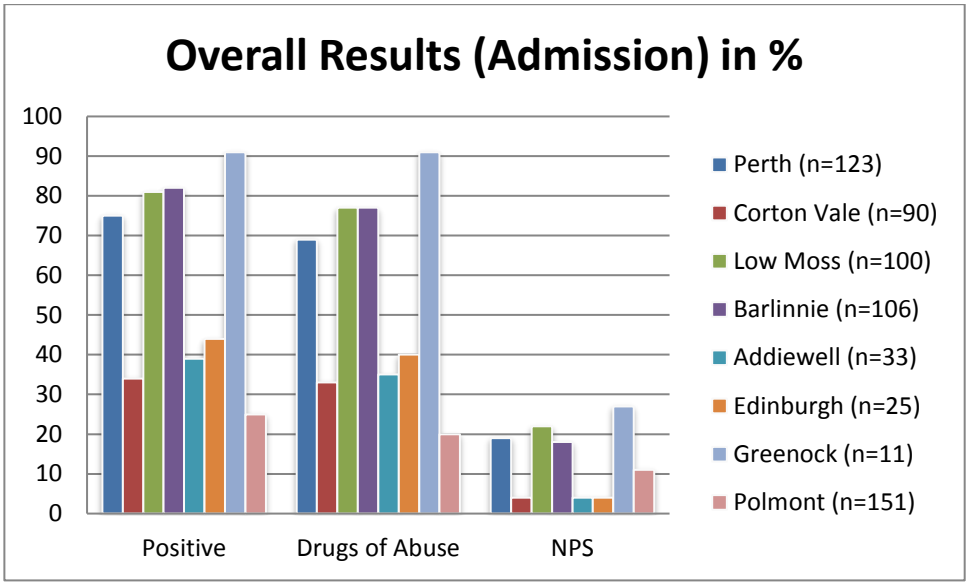


Figure 3.3 Overall positive results for all prisons for admission (n = 590)

Most of the positive admission samples are drugs of abuse, while NPS are significantly less frequent. Especially Perth, Low Moss, Barlinnie and Greenock have a very high percentage of positive results. In the case of Greenock however, this may be due to the small sample size (n = 11).

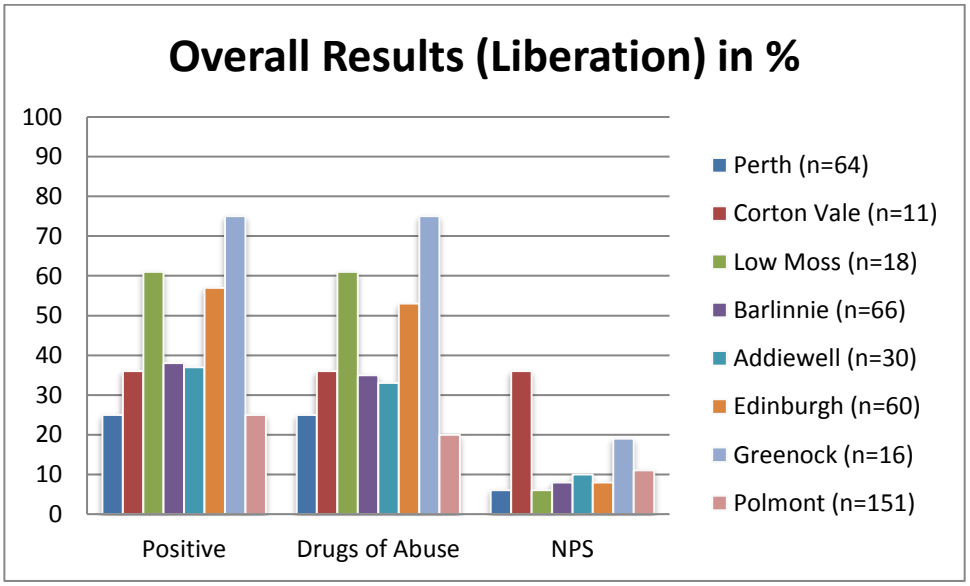


Figure 3.4 Overall positive results for all prisons for liberation (n=314)

Liberation shows again a more significant occurrence for drugs of abuse, with reduced NPS frequency, the difference, however, is not as pronounced as with admission samples. Low Moss and Greenock show, similar to admission, the highest abundancy of positive results

together with Edinburgh. Again, the low sample volume of Greenock (n = 16) might play a factor in the respective percentage.

3.2.2 Results for Individual Prisons

The results for the individual prisons are presented in the following and are displayed as overall results, split into drugs of abuse and novel psychoactive substances as discussed at the beginning of this chapter. Diagrams of the complete data for each prison are presented in the appendix. The following highlighted drugs were common to all prisons for both admission and liberation and included mirtazapine, amitriptyline, diazepam, methadone, cocaine and DHC in drugs of abuse and benzedrone MDAI, JWH-250, etizolam, butylone and methoxetamine in the NPS group. Other drugs were only found in isolated cases amongst the 904 samples.

3.2.2.1 Perth

Figure 3.5 shows the positive results from the samples received from Perth prison and shows the distribution between drugs of abuse and novel psychoactive substances.

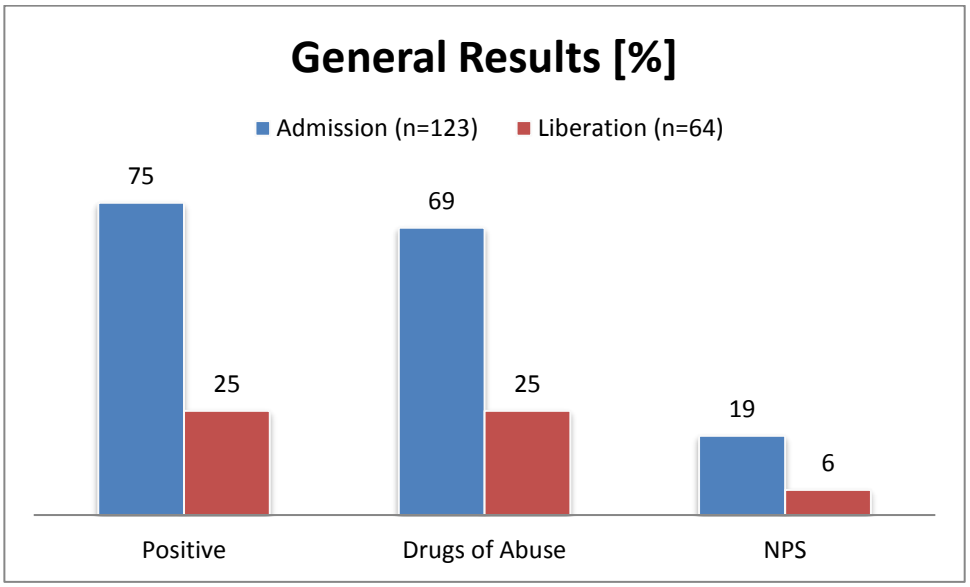


Figure 3.5 General positive results for Perth prison in percent

While the abundance of positive results is relatively high in admission samples for drugs of abuse with still significant abundance for NPS, liberation samples showed significant reduction in these numbers. However, 25% prevent of liberation samples have been tested positive for drugs of abuse.

Figure 3.6 and **Figure 3.7** show the distribution of the overall most prevalent drugs of abuse and novel psychoactive substances, respectively, in Perth prison.

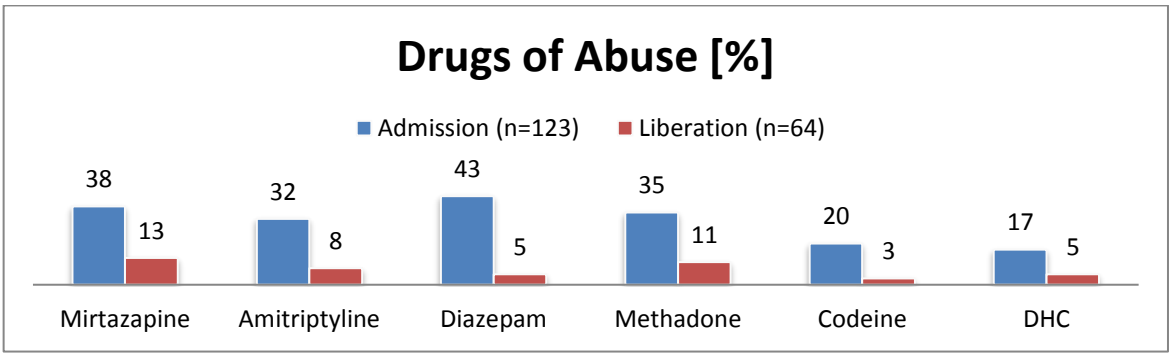


Figure 3.6 Most overall prevalent drugs of abuse in samples provided by Perth prison

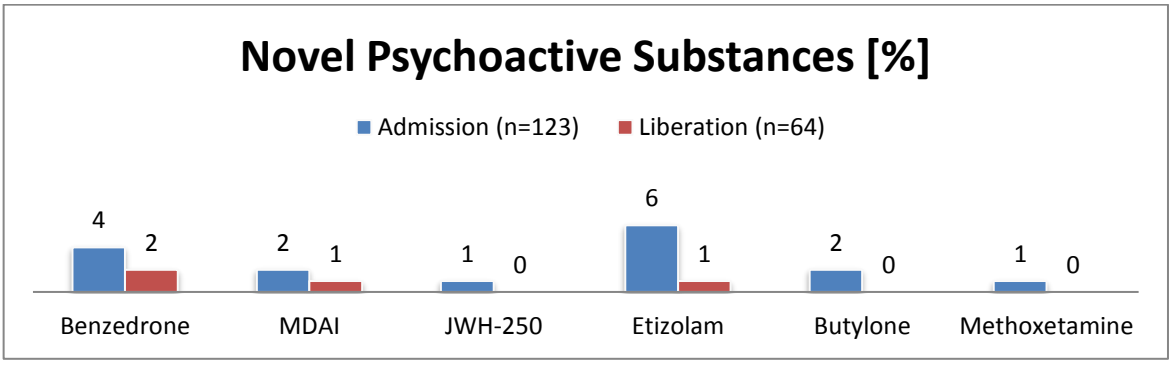


Figure 3.7 Most overall prevalent novel psychoactive substances in samples provided by Perth prison

Figure 3.6 and **Figure 3.7** show a significantly reduced number of positive samples for liberation when compared to admission for drugs of abuse as well as NPS. The strongest reduction is apparent in diazepam, which went from 43% to only 5%, and etizolam which went from 6% to 1%. However, it should be noted that the sample volume for liberation (n = 64) is just over half that for admission (n = 123).

3.2.2.2 Corton Vale

Figure 3.8 shows the positive results from the samples received from Corton Vale prison and shows the distribution between drugs of abuse and novel psychoactive substances.

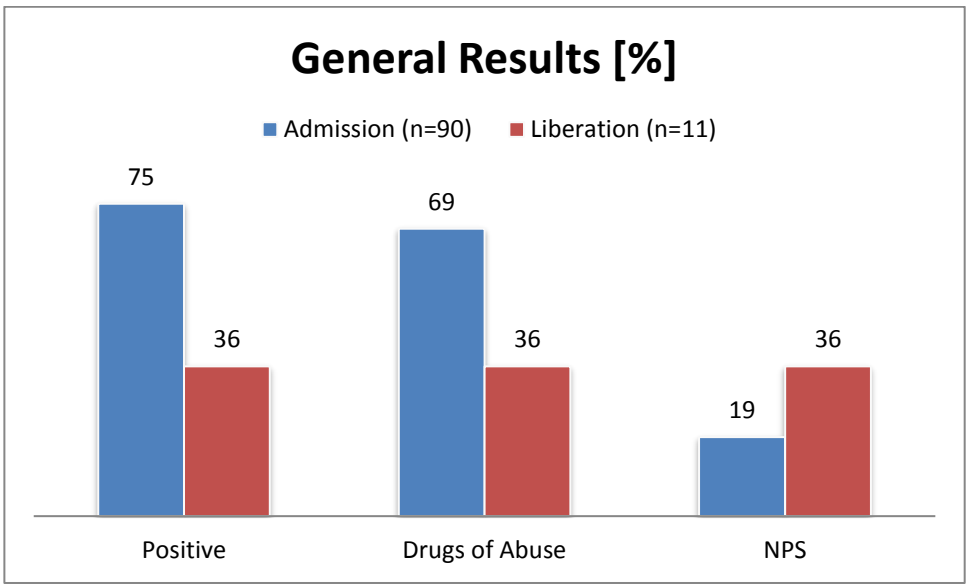


Figure 3.8 General positive results for Corton Vale prison in percent

Corton Vale shows a similar reduction in overall positive results and drugs of abuse from admission to liberation as previously discussed for Perth. However, while the percentage of NPS detected in liberation samples (36%) is significantly higher than in admission samples (19%) it should be noted that the sample volume for liberation was very limited (n = 11) and this result may simply be an artefact of that.

Figure 3.9 and **Figure 3.10** show the distribution of the overall most prevalent drugs of abuse and novel psychoactive substances, respectively, in Corton Vale prison.

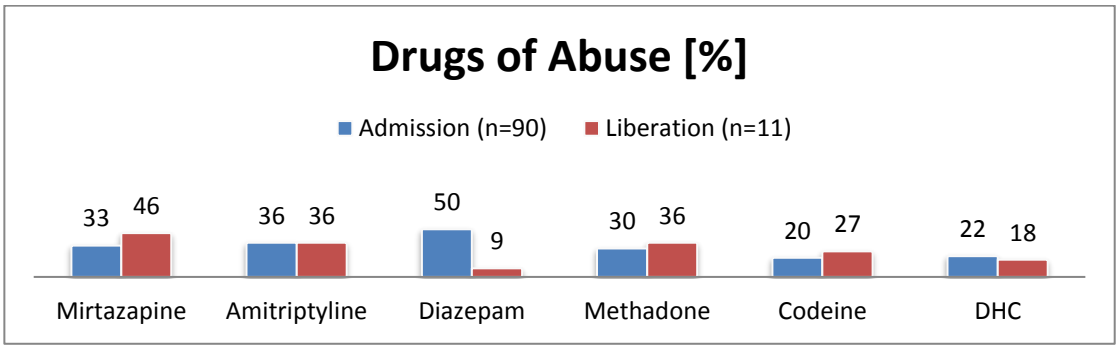


Figure 3.9 Most overall prevalent drugs of abuse in samples provided by Corton Vale prison

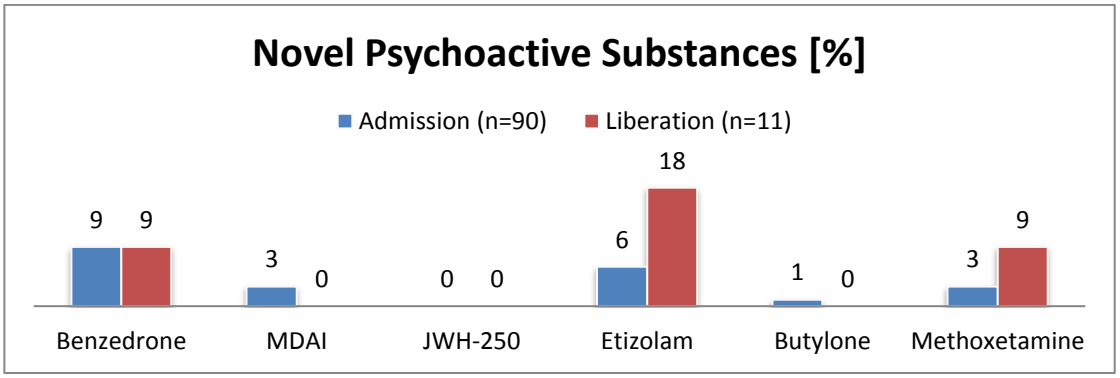


Figure 3.10 Most overall prevalent novel psychoactive substances in samples provided by Corton Vale prison

While the overall prevalence of drugs of abuse has decreased from admission to liberation samples, as seen in **Figure 3.8**, the percentage of the five most abundant drugs of abuse has actually hardly changed or even increased with the exception of diazepam which went from 50% admission to 9% liberation. Again, the limited sample volume of liberation samples (n=11) may entirely be responsible for this occurrence.

3.2.2.3 Low Moss

Figure 3.11 shows the positive results from the samples received from Low Moss prison and shows the distribution between drugs of abuse and novel psychoactive substances.

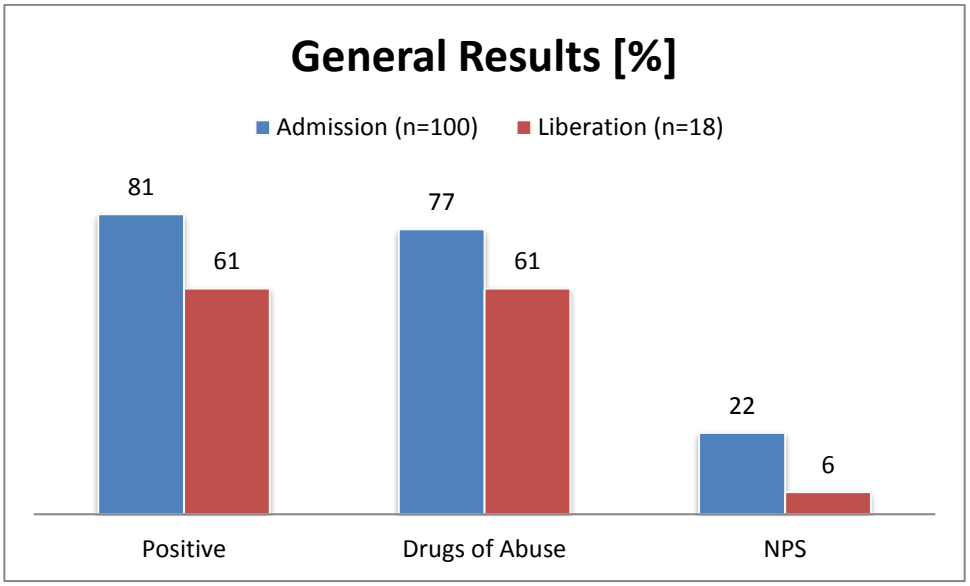


Figure 3.11 General positive results for Low Moss prison in percent

The reduction of samples positive for drugs of abuse from admission to liberation is comparatively minor, however when the difference in sample numbers for admission (n = 100) and liberation (n = 18) is taken into account the difference is substantially bigger. Especially the reduction for NPS positive samples from admission to liberation is even more pronounced when the different sample volumes are taken into consideration.

Figure 3.12 and **Figure 3.13** show the distribution of the overall most prevalent drugs of abuse and novel psychoactive substances, respectively, in Low Moss prison.

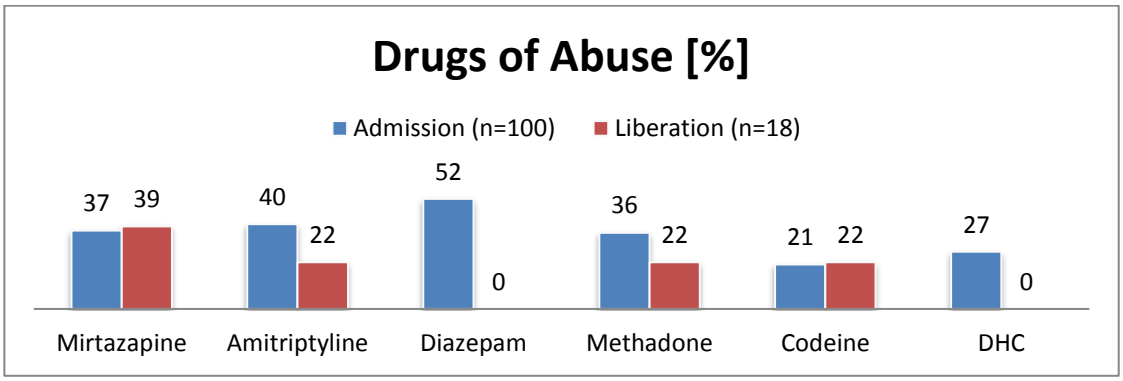


Figure 3.12 Most overall prevalent drugs of abuse in samples provided by Low Moss prison

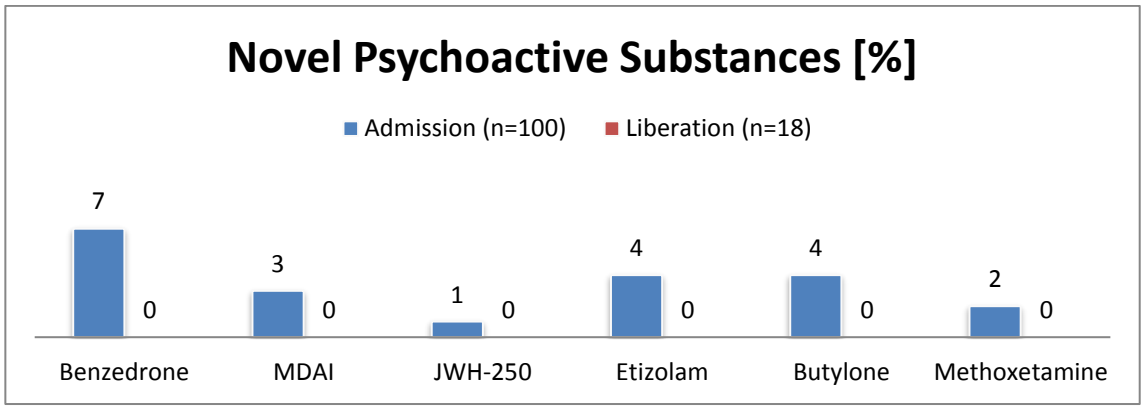


Figure 3.13 Most overall prevalent novel psychoactive substances in samples provided by Low Moss prison

The complete lack of diazepam or DHC positives in liberation samples is a remarkable difference as they showed a high prevalence in admission samples with 52% and 27% respectively. Most remarkable in Low Moss is the complete absence of positive NPS samples in regard to the most prevalent NPS encountered in this study. Consequently, the detected NPS in Low Moss liberation samples (APB) has been very rarely identified in the course of this study.

3.2.2.4 Barlinnie

Figure 3.14 shows the positive results from the samples received from Barlinnie prison and shows the distribution between drugs of abuse and novel psychoactive substances.

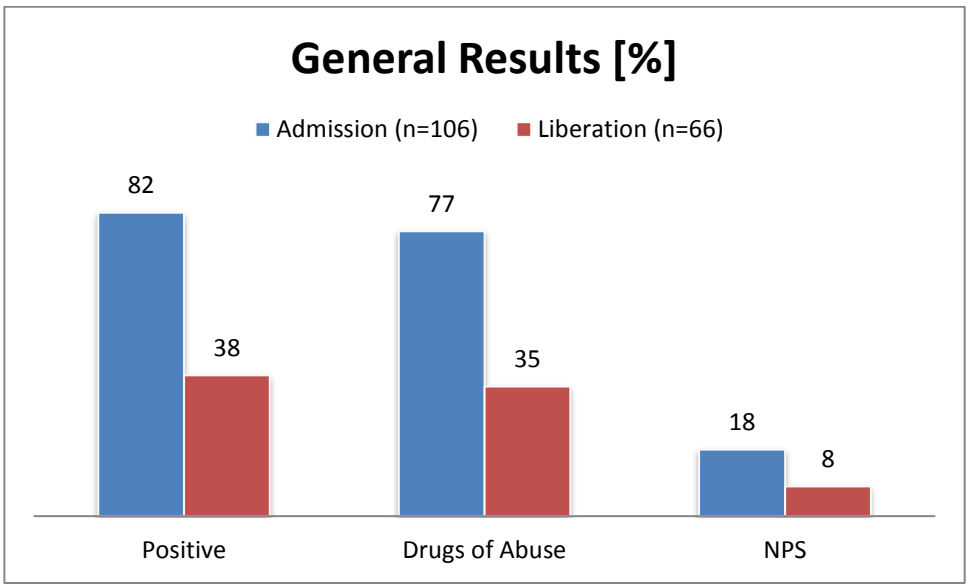


Figure 3.14 General positive results for Barlinnie prison in percent

The results from Barlinnie prison were show an expected decline in positive liberation samples in drugs of abuse as well as NPS. While the sample volume for liberation samples (n = 66) is lower than for admission samples (n = 106) the difference is not as extreme as in other populations.

Figure 3.15 and **Figure 3.16** show the distribution of the overall most prevalent drugs of abuse and novel psychoactive substances, respectively, in Barlinnie prison.

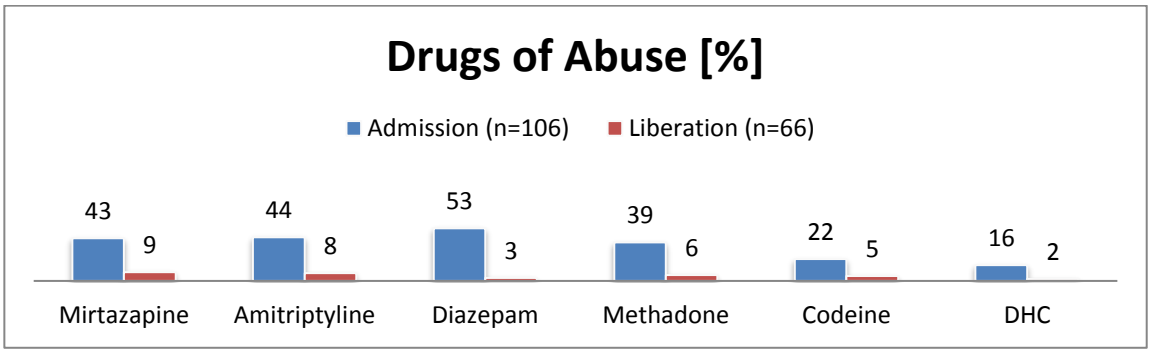


Figure 3.15 Most overall prevalent drugs of abuse in samples provided by Barlinnie prison

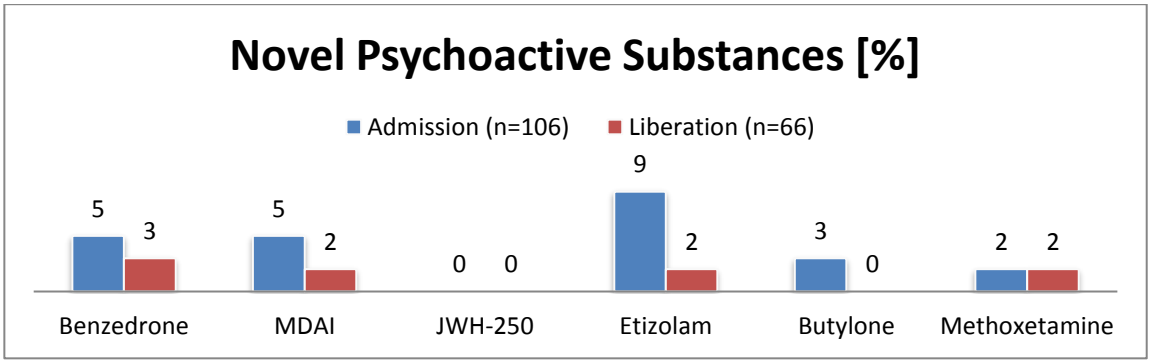


Figure 3.16 Most overall prevalent novel psychoactive substances in samples provided by Barlinnie prison

The overall results are even more pronounced when drugs of abuse and NPS are displayed separately, as the abundance of all drugs has been significantly reduced.

3.2.2.5 Addiewell

Figure 3.17 shows the positive results from the samples received from Addiewell prison and shows the distribution between drugs of abuse and novel psychoactive substances.

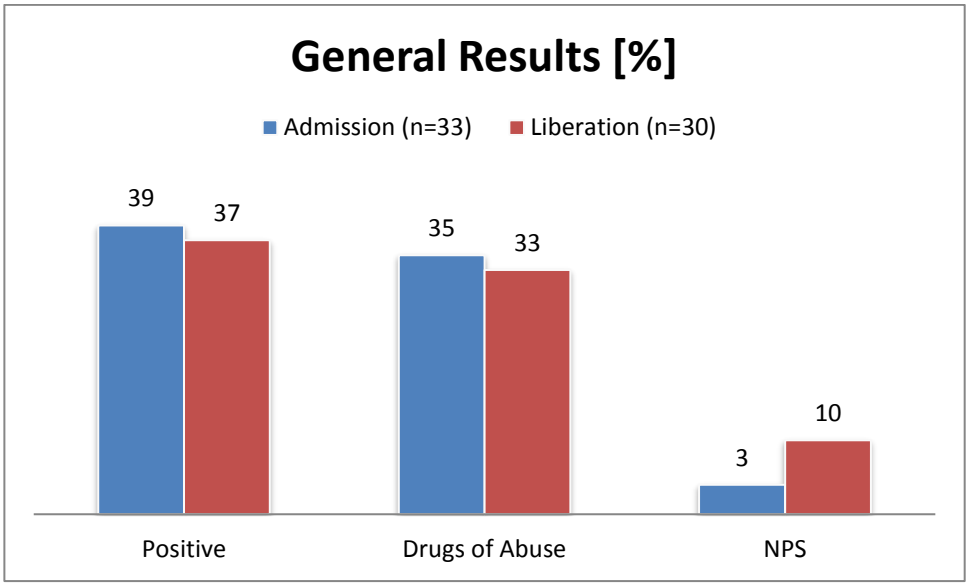


Figure 3.17 General positive results for Addiewell prison in percent

The sample pools of admission samples and liberation samples for Addiewell are almost identical with 33 and 30 respectively. However, this makes the almost non-existent reduction in positive samples from admission to liberation even more significant. Notable as well is the increase in positive NPS samples in liberation when compared to admission, even though the actual number of positive cases only changed from 1 to 3.

Figure 3.18 and **Figure 3.19** show the distribution of the overall most prevalent drugs of abuse and novel psychoactive substances, respectively, in Addiewell prison.

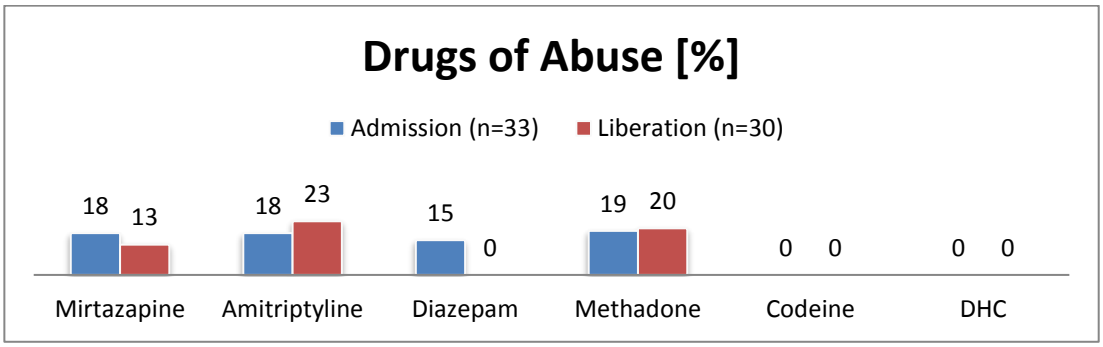


Figure 3.18 Most overall prevalent drugs of abuse in samples provided by Addiewell prison

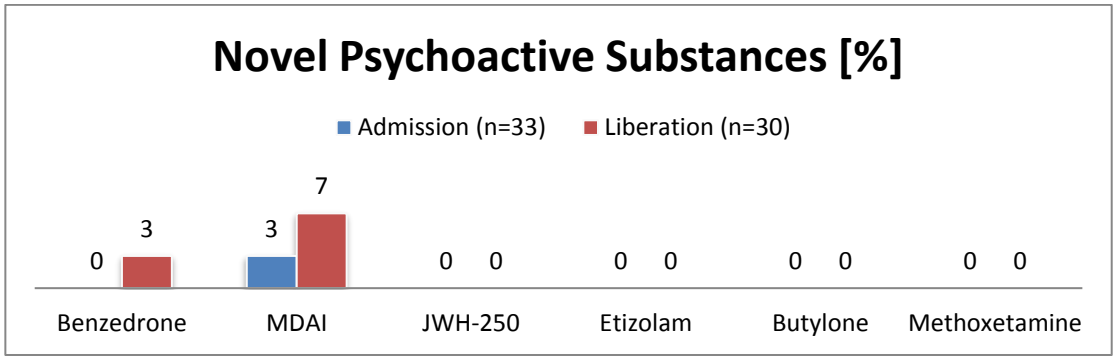


Figure 3.19 Most overall prevalent novel psychoactive substances in samples provided by Addiewell prison

The decline in positive results for drugs of abuse are, despite the significant reduction of diazepam from 15% to 0%, explained by less often encountered drugs such as tramadol and quetiapine. Most significant, however, is the increased amount of positive NPS in liberation samples which is due to an increased presence of benzedrone and MDAI.

3.2.2.6 Edinburgh

Figure 3.20 shows the positive results from the samples received from Edinburgh prison and shows the distribution between drugs of abuse and novel psychoactive substances.

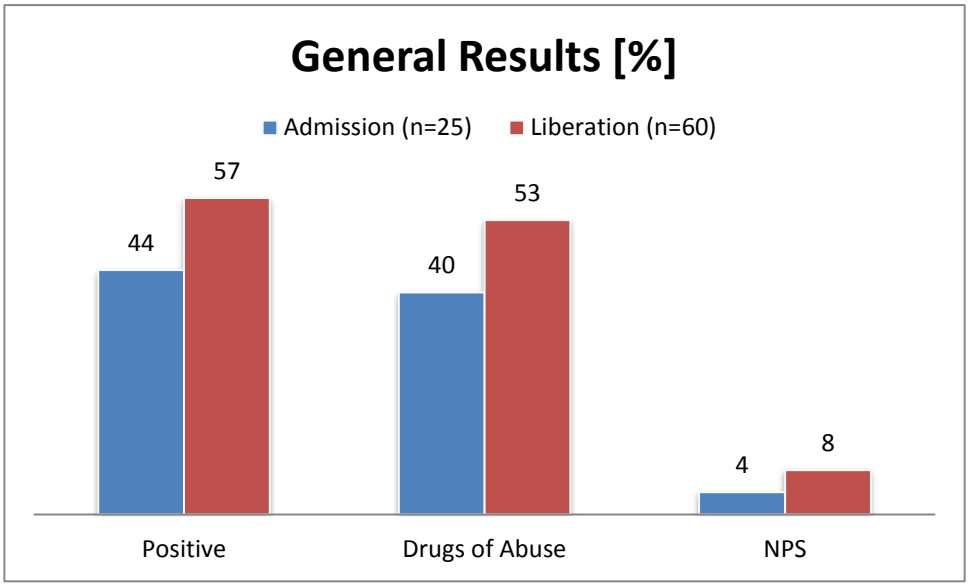


Figure 3.20 General positive results for Edinburgh prison in percent

Drugs of abuse and NPS show more positive results in liberation samples with 53% and 8% respectively, than in admission samples, with 40% and 4% respectively. However, as the sample volumes for both admission as well as liberation are comparatively small with 25 and 60 respectively, even a very few positive samples may have a huge impact on the percentage values.

Figure 3.21 and **Figure 3.22** show the distribution of the overall most prevalent drugs of abuse and novel psychoactive substances, respectively, in Edinburgh prison.

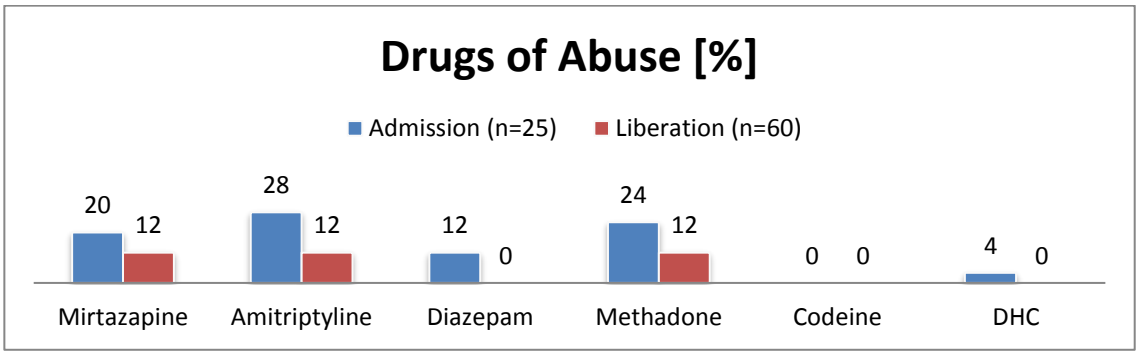


Figure 3.21 Most overall prevalent drugs of abuse in samples provided by Edinburgh prison

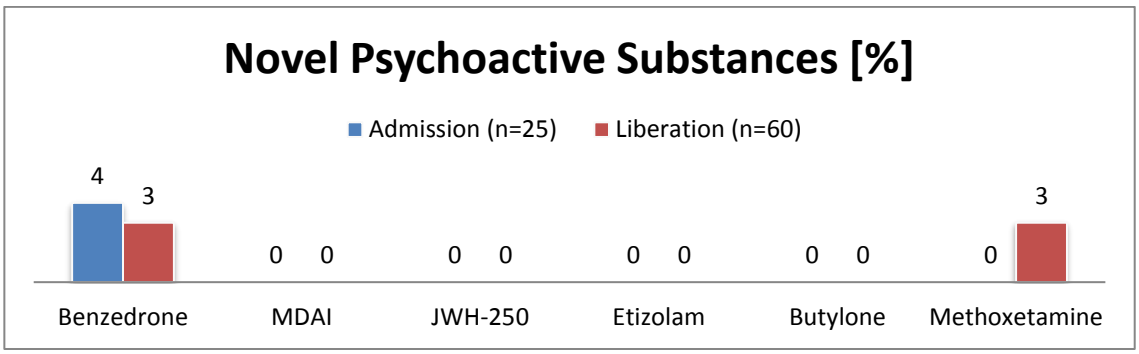


Figure 3.22 Most overall prevalent novel psychoactive substances in samples provided by Edinburgh prison

The minor increase in NPS in positive liberation samples can be explained by an almost unchanged amount of benzedrone positive samples with a new population of methoxetamine positive samples. The increase of drugs of abuse is not due to the most prevalent drugs shown in **Figure 3.21**, but to less often encountered drugs, such as tramadol and trazadone in this case.

3.2.2.7 Greenock

Figure 3.23 shows the positive results from the samples received from Greenock prison and shows the distribution between drugs of abuse and novel psychoactive substances.

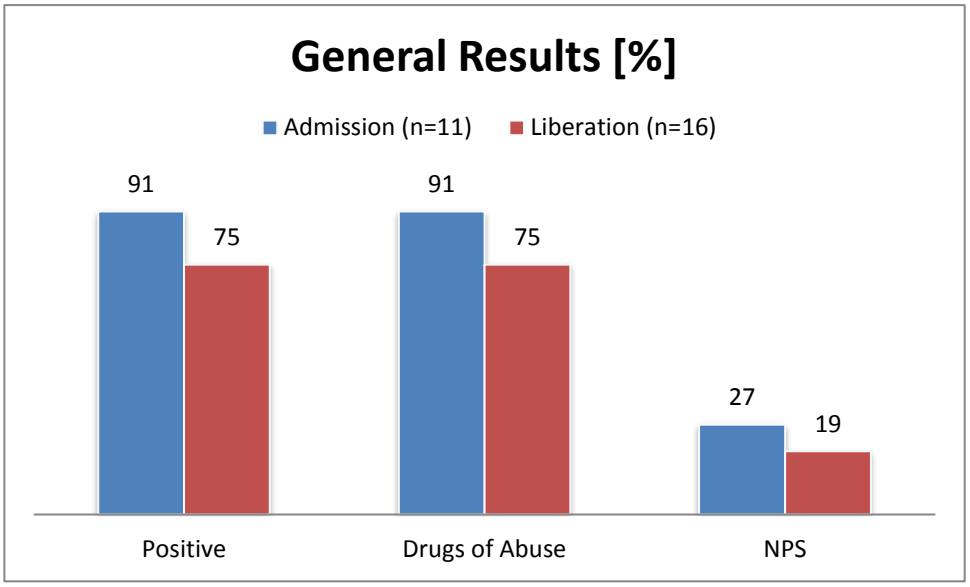


Figure 3.23 General positive results for Greenock prison in percent

While the percentage of positive results in admission as well as liberation are comparatively high, the sample number of both groups was extremely limited with 11 and 16 respectively. Consequently, even a single positive sample has a huge impact on the percentages which should be taken into consideration. As the sample numbers are so low it cannot be ensured that the results are statistically relevant.

Figure 3.24 and **Figure 3.25** show the distribution of the overall most prevalent drugs of abuse and novel psychoactive substances, respectively, in Greenock prison.

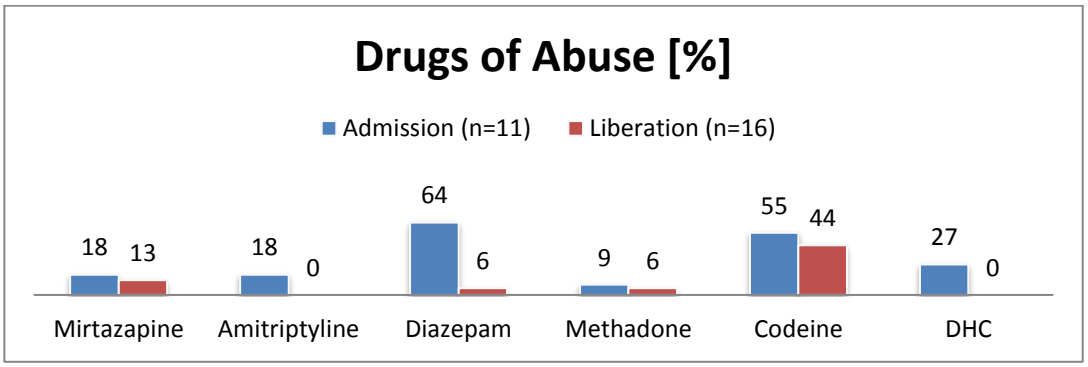


Figure 3.24 Most overall prevalent drugs of abuse in samples provided by Greenock prison

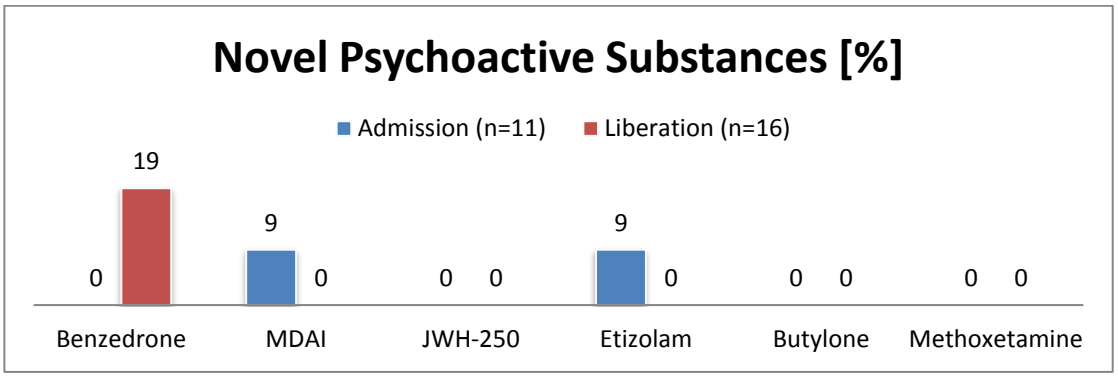


Figure 3.25 Most overall prevalent novel psychoactive substances in samples provided by Greenock prison

Similar to the overall results, the percentages are heavily influence by minute changes in positive sample amounts and consequently, it can only be said that admission and liberation samples show minor differences in abundancies. Interesting, however, is while MDAI and etizolam are only present in admission samples, benzedrone was only detected in liberation samples.

3.2.2.8 Polmont

Figure 3.26 shows the positive results from the samples received from Polmont prison and shows the distribution between drugs of abuse and novel psychoactive substances. No data concerning admission and liberation was available for Polmont.

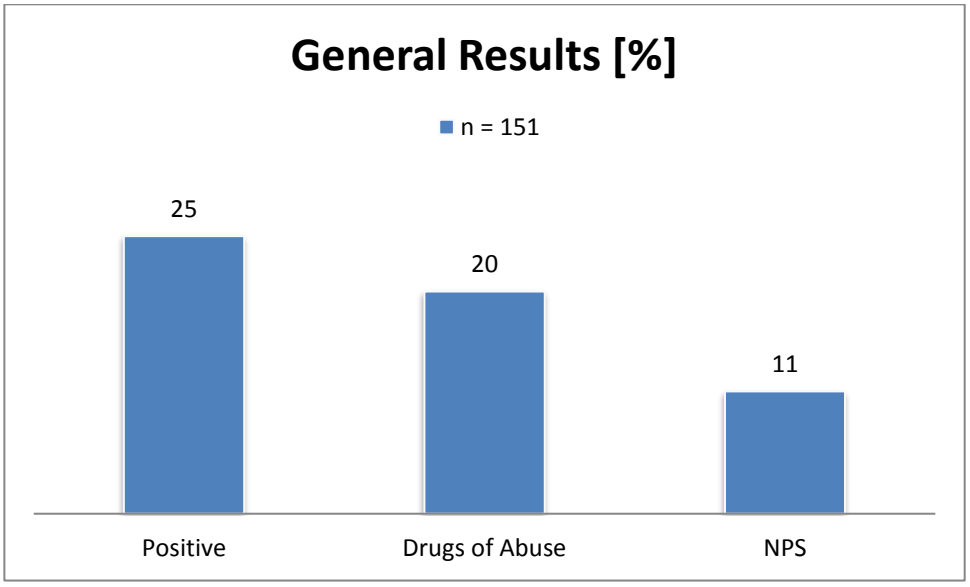


Figure 3.26 General positive results for Polmont prison in percent

Even though a significant number of samples was received from Polmont the overall prevalence of drugs of abuse and NPS is comparatively low with only 20% and 11% respectively.

Figure 3.27 and **Figure 3.28** show the distribution of the overall most prevalent drugs of abuse and novel psychoactive substances, respectively, in Polmont prison.

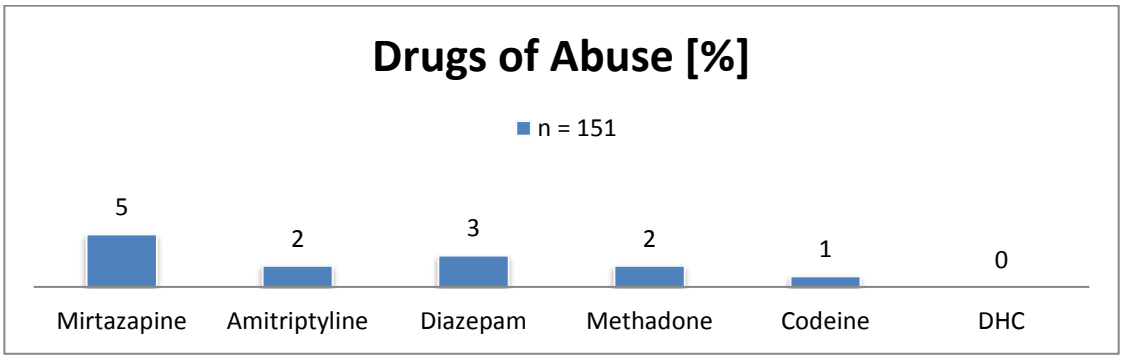


Figure 3.27 Most overall prevalent drugs of abuse in samples provided by Polmont prison

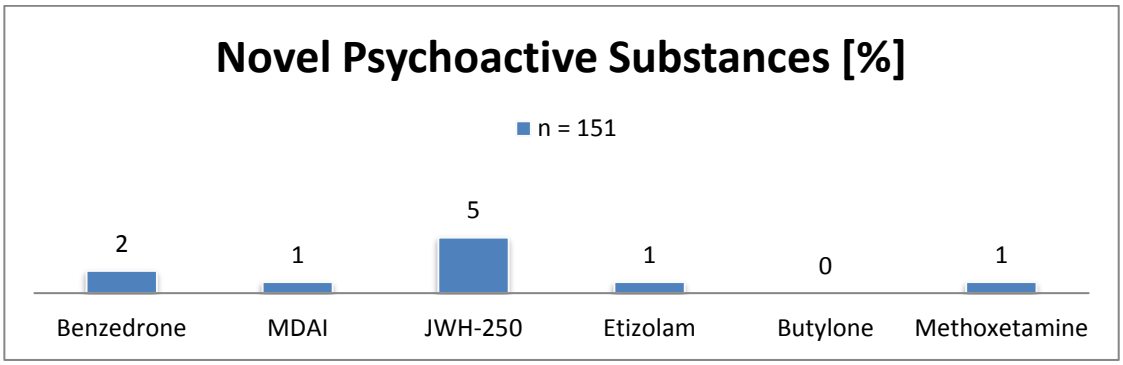


Figure 3.28 Most overall prevalent novel psychoactive substances in samples provided by Polmont prison

Most significant is the high prevalence of JWH-250 hydroxypentyl metabolite, shortened to JWH-250 in the diagram.

3.3 Discussion

3.3.1 Prison Prevalence Study

The overall results for each prison shown in **Figure 3.3** for admission samples and **Figure 3.4** for liberation samples show that the vast majority of detected drugs in admission samples are classical drugs of abuse and prescription medication, which were combined under the nominator Drugs of Abuse, while novel psychoactive substances (NPS) make up only a fraction of positive results. Liberation samples show the same tendency towards drugs of abuse, albeit not as pronounced as in admission samples. While this can be partly be attributed to the fact that significantly fewer liberation samples have been received than admission samples and thus the results (shown in percentages) may appear greater at first glance - the alternative to present the data in total numbers would present a similar problem in that the numbers might appear less significant than they are due to the limited amount of liberation samples available. Another reason for this difference in admission and liberation samples is most likely the measures in place to prevent inmates from access to drugs, which is considerably easier to accomplish for more common and better known drugs of abuse than for unknown NPS.

For each prison, the five most prevalent drugs of abuse and five most prevalent NPS, determined by the prevalence over all samples, have been compared for admission and liberation separately.

Perth and Barlinnie prison, shown in **Figures 3.5 - 3.7** and **Figures 3.14 - 3.16**, follow the same pattern as discussed for the overall results for all prisons in **Figures 3.3** and **Figure 3.4** for admission and liberation respectively. Drugs of abuse have been detected significantly more often than NPS with considerably higher rates in admission samples than in liberation samples, even when taking the number difference between admission (Perth: n = 123; Barlinnie: n = 106) and liberation (Perth: n = 64; Barlinnie: n = 66) into account.

Corton Vale prison (**Figures 3.8 - 3.10**) shows a deviation from the overall trend in that while admission samples have again a higher prevalence of positive drug of abuse results, liberation samples show a higher percentage of NPS (36%) detected than drugs of abuse (19%). However, due to the limited sample number for liberation with only n = 11, compared

to admission samples with n = 90, this is most likely not a true instance of increased NPS usage in prison. It is worth mentioning though that the liberation samples showed the same percentage of positive results (36%) for drugs of abuse and NPS but again the statistical relevance of this result is not clear due to the limited sample pool available.

The results from Addiewell prison (**Figures 3.17 - 3.19**) show an almost identical percentage of drugs of abuse detected in admission (35%) and liberation (33%) which is of particular interest as both sample pools have almost the same number of samples with n = 33 and n = 30 respectively. This may indicate the same quantity of usage of drugs of abuse inside the prison as outside and a potential drug prevention behaviour in Addiewell prison. Additionally, while NPS are considerably rarer detected in admission as well as in liberation, fewer positive results have been found in admission samples than in liberation samples with 4% and 10% respectively.

Low Moss prison, as shown in **Figures 3.11 - 3.13**, shows results comparable to those discussed previously for Addiewell prison with a very high number of positive results for drugs of abuse even in liberation samples (61%) when compared to the number of positive results in admission samples (77%). The number of results for NPS are significantly lower in admission (22%) as well as in liberation (6%). While this may indicate a relative high use of drugs of abuse in the prison, only a small number of liberation samples (n = 18), compared to significantly higher number of admission samples (n = 100), was available for analysis which, consequently, makes a definite conclusion difficult.

Greenock prison is of special interest as they explicitly asked to be part of this study as they voiced concerns over drug use in their facility. It was not part of the originally planned seven prisons selected as it is a smaller prison with an upper limit of nearly 300 in custody; compared to i.e. Barlinnie with an average of 1305 in custody. A high percentage (**Figure 3.23**) of samples tested positive for drugs of abuse and NPS in admission samples, with 91% and 75% respectively, as well as liberation samples, with 27% and 19% respectively. While the number of samples received were comparably small for admission (n = 11) and liberation (n = 16), the fact that admission as well as liberation show similar percentages for drugs of abuse as for NPS might indicate similar drug use inside the facility as well as outside.

Edinburgh prison (**Figures 3.20 - 3.22**) was the only instance where liberation samples tested positive for a higher prevalence of drugs of abuse as well as NPS in liberation samples, with 53% and 8% respectively, than in admission samples, with 40% and 4% respectively. While the number of samples available for admission (n = 25) and liberation (n = 60) might influence the percentages to some degree, the results indicate significant drug use inside the facility in respect to drugs of abuse. While results for NPS showed more positive in liberation than in admission samples as well, their percentages are low enough (8% and 4% respectively) to fall behind the more significant numbers for drugs of abuse.

Polmont prison has been evaluated differently due to the fact that even though samples for admission (n = 102) and liberation (n = 49) were available for the study, the process of freezing and thawing those samples made a significant amount of the used labels illegible. As it was not clear whether these now unspecified samples were original admission or liberation samples, all samples (n = 151) were processed without making the distinction between admission and liberation to avoid unintentional bias in case more samples got removed from one group than the other. As a consequence, the results as shown in **Figures 3.26 - 3.28** present an expected pattern of more positive results for drugs of abuse (20%) than NPS (11%). However, given the total sample size (n = 151) the number of positive samples for drugs of abuse and NPS is comparatively low. Additionally, Polmont showed the only instance of significant amounts of JWH-250 hydroxypentyl metabolite, shortened to JWH-250 in the diagrams, of all samples screened. This finding is significant in that Polmont is a holding facility for young male offenders and thus represents a segment potentially more prone to turn to NPS in their drug use.

Comparison of the results with the data obtained by the prevalence study conducted by the SPS (Appendix), from which these samples were obtained, showed some differences in both the amount of positive results obtained and the drugs identified by the dip tests employed by the SPS.

A reason for the higher number of overall positive results in the SPS prevalence study is a higher number of samples tested, as not all were passed on for this study, that the developed method shows a comparatively low sensitivity for cannabis which was identified in considerable amounts by the drug dip tests. Especially samples collected at admission

showed a high number of cannabis positive results, while only Polmont showed any significant number in liberation samples.

Further impact on the number of obtained positive identifications from this study had the degradation observed in the obtained samples. Initially, the consistent cooling during storage until pick up was not guaranteed and while they were frozen upon arrival, pick up did not occur daily. Consequently, considerable degradation of sample matrix was observed and it follows that potential drug concentrations may have suffered similar degradation before they could be tested.

3.4 Conclusion

The application of high performance liquid chromatography coupled with time-of-flight mass spectrometry proved to be essential in analysing the samples from A&E admissions as well as from the Scottish Prison Service as its high resolution allowed the analysis of samples containing a wide variety of compounds from a single extract. This is demonstrated in **Figure 3.29** and **Figure 3.30** in which 8 and 9 compounds were identified respectively, many of which eluted simultaneously or in close proximity.

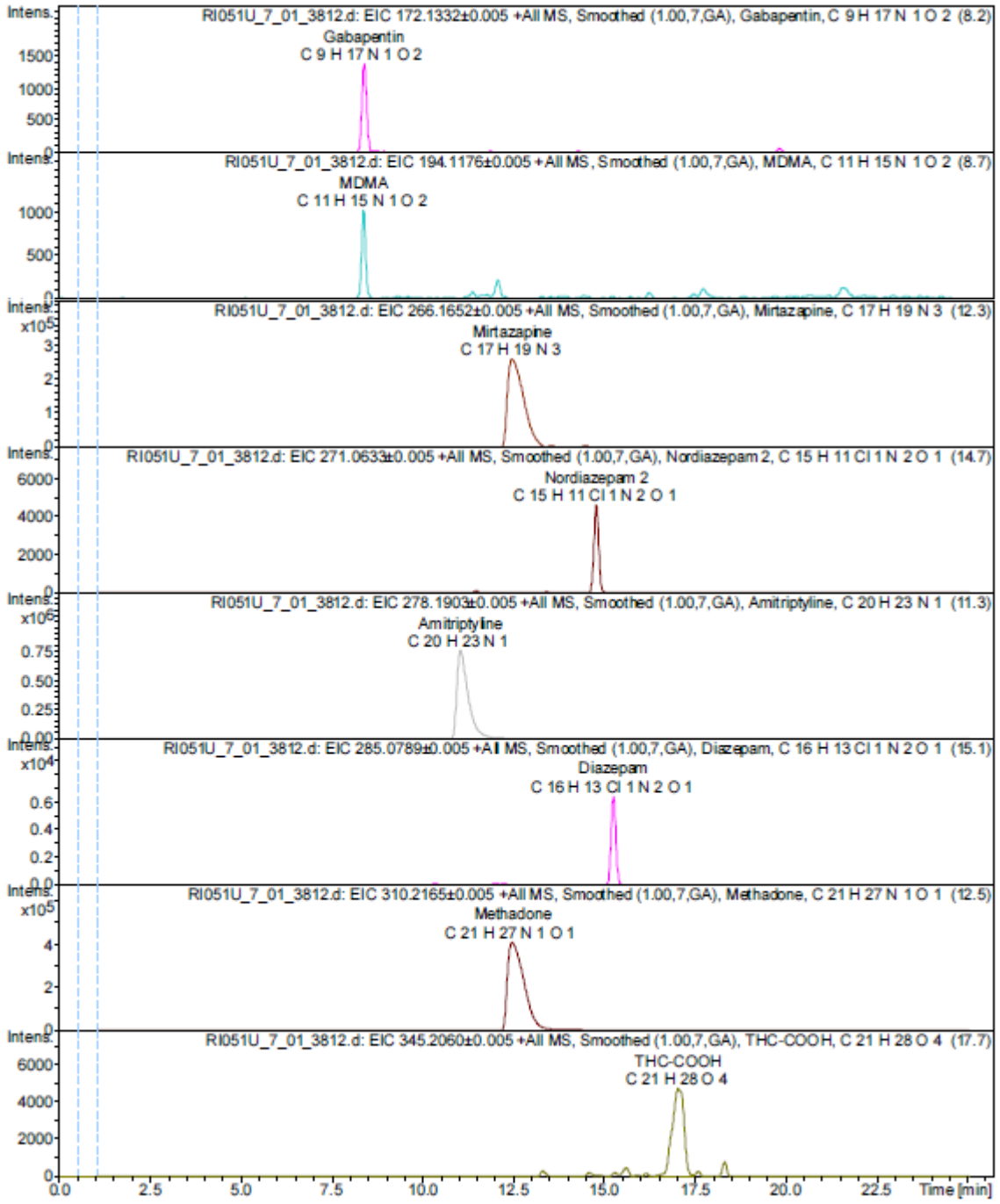


Figure 3.29 A&E admission sample (#51) containing multiple compounds

Furthermore, gabapentin was added to the library after the sample shown in **Figure 3.29** had already been processed but the comprehensive data obtained by the time-of-flight instrument allowed for a simple reprocessing of the obtained data with the expanded library.

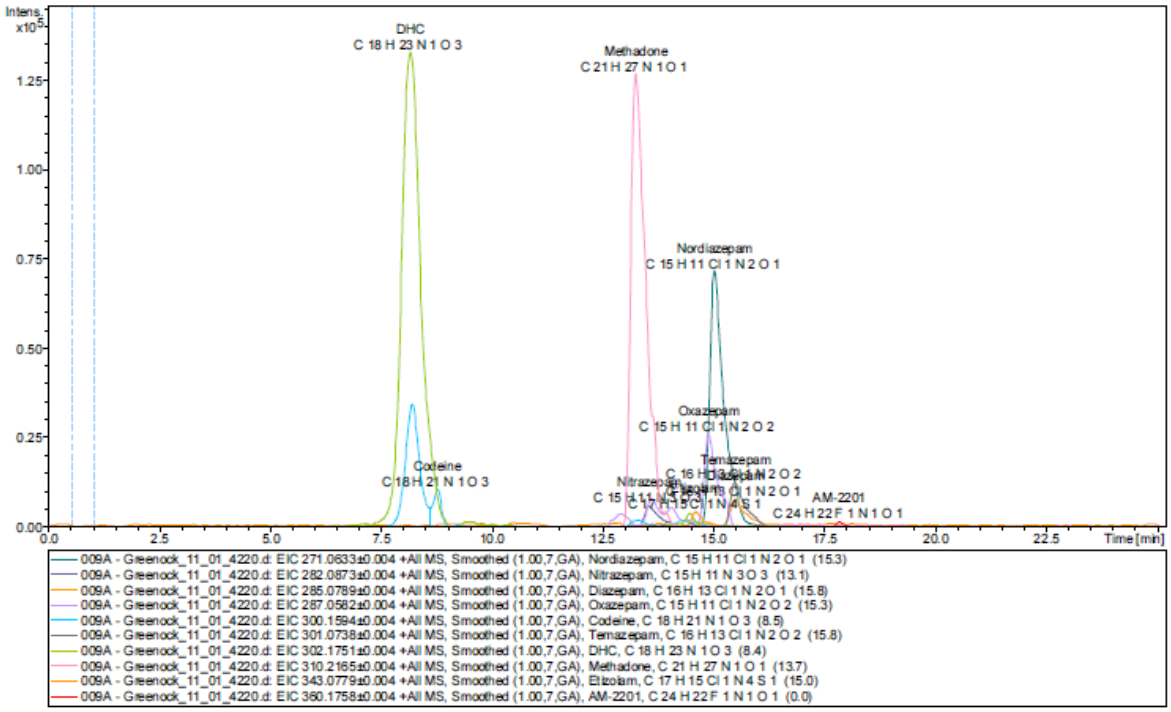


Figure 3.30 Sample received from the Scottish Prison Service (Greenock #9 Admission)

The variety in chromatograms obtained by the established method is shown in **Figure 3.30** which features compounds with very strong responses, such as DHC, methadone and nordiazepam, and compounds with significantly lower responses such as nitrazepam, temazepam and etizolam. The signal marked AM-2201 was later determined to be an unknown compound with the same chemical formula as AM-2201, as its isotope pattern is recognised by the software but AM-2201 has been shown to elute earlier – as discussed previously.

The utility of extracted ion chromatograms using exact mass obtained from the time-of-flight instrument is immensely powerful in analyte rich samples such as shown in **Figure 3.31** as the generated chromatograms are mostly free of any background noise.

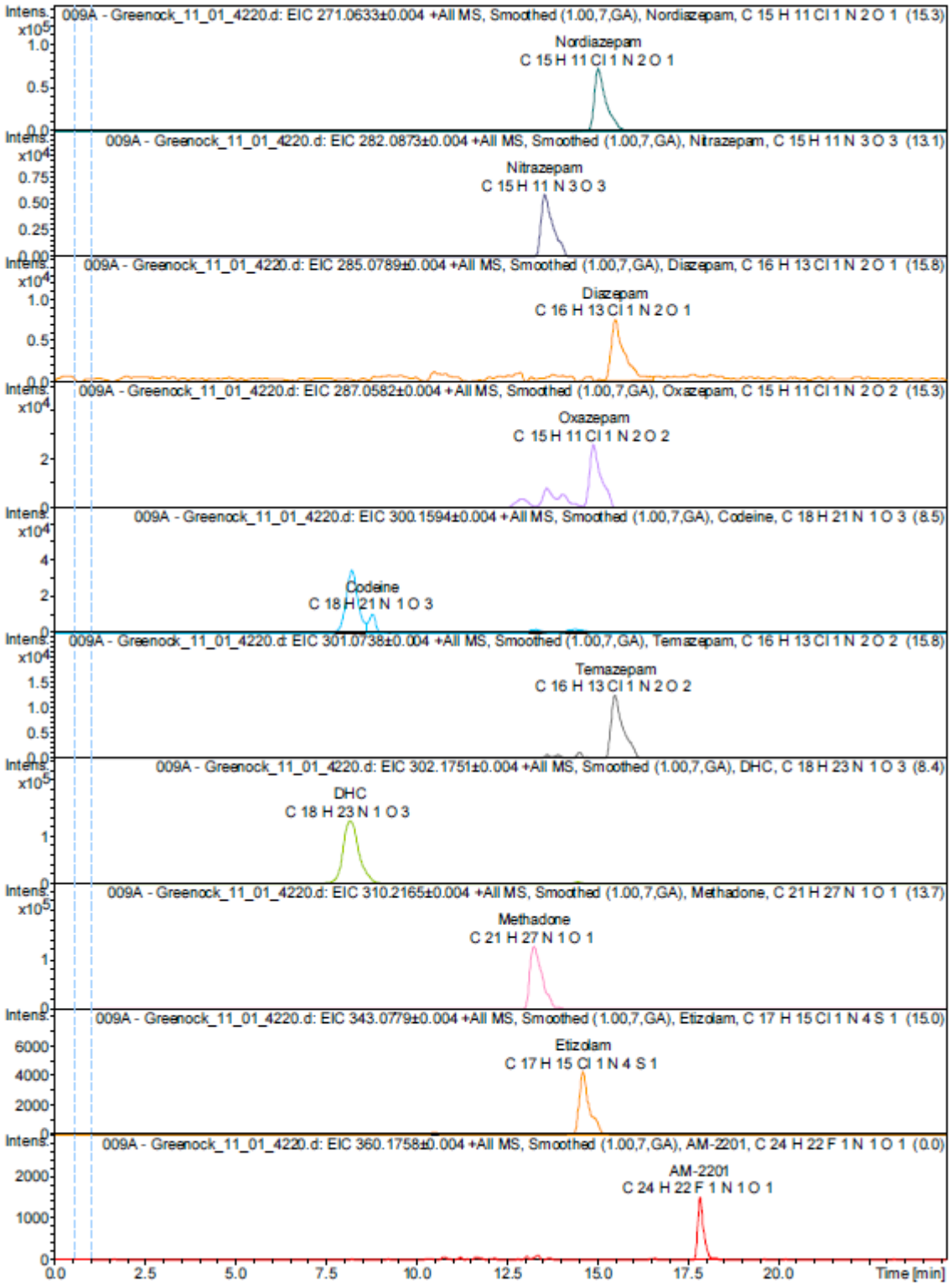


Figure 3.31 Sample received from the Scottish Prison Service (Greenock #9 Admission)

Only the extracted ion chromatograms of diazepam and oxazepam show slight increase in background noise which does not impede the analysis of these compounds.

Interpretation of the obtained results and matching the positively identified drugs with corresponding drug use can be extremely difficult for various reasons. Metabolic rates vary between individual users and are mostly unknown for NPS which is further complicated by the fact the time between ingestion and samples acquisition is vague or unknown and may even give only limited information if the same or similar compounds are ingested on a regular basis.

4 Conclusion

Time-of-flight mass spectrometry proved to be a capable tool in the general qualitative screening of a wide range of analytes and invaluable in the capability to retroactively process data with an updated library.

The established limits of detection for the liquid-liquid extraction were determined to be between 5-20 ng/mL for a range of known analytes and considered a suitable range for qualitative screening in urine. Extractions with a stronger base agent (NaOH) proved to result in more background noise and interference, which resulted in significantly lower limits of detection. Furthermore, optimisation of mobile phase conditions showed that a lower buffer concentration and a higher pH created more favourable ionisation conditions for the compounds tested.

The true capabilities of time-of-flight mass spectrometry with the established method were shown when analysing the case samples received from the A&E Department of Glasgow Royal Infirmary, taken in cases of suspected drug abuse, and the Scottish Prison Service, taken in course of their own prevalence study. Established extracted ion chromatograms (EIC) by the data processing software TargetAnalysis were extremely effective in the identification of overlapping or co-eluting compounds. EIC are remarkably background free chromatograms, in most cases, which allows clear identification and quick recognition of interference. However, the mass spectra and corresponding isotope patterns are still subject to interference and especially compounds with nearly identical retention times and masses can make identification more complicated, but by no means impossible.

The method produced variable results for amphoteric compounds such as morphine and THC. The effect on morphine is significant peak broadening and tailing and a changeable shift in retention time window, while THC shows a good peak shape and solid retention time but the amount detected is highly variable between samples and injections. Furthermore, matrix effects remain highly dependent on specific drug investigated and while these effects have been taken into consideration for the representative compounds, specific matrix effects for other, even similar, compounds were only extrapolated.

Furthermore, the application of the developed method to samples received from A&E admissions and the Scottish Prison Service detected many NPS that would not have been detected otherwise. In fact, in A&E admissions etizolam was frequently detected in patients who reported the ingestion of other drugs or no ingestion of drugs at all. While this may have been intentional, this may also indicate that the drug was taken unwittingly and is sold as another drug for example. In the samples received from the Scottish Prison Service NPS have been detected in the admission pools of every prison and in most of the liberation pools. While the numbers themselves were low, this suggests a widespread use in and outside of the prison.

5 Further Work

While the established method has been shown to work for urine, an adaption to blood would open up many analytical advantages, as blood can indicate intoxication at the time the sample was taken, while urine only shows past exposure. The liquid-liquid extraction shown in this work, could not be adapted for blood, but one of the investigated SPE extractions might prove just as suitable for blood as for urine. Another potential matrix, oral fluid, has not been investigated but the method should be easily adaptable from urine to oral fluid on a technical basis.

The simplest approach, however, would be the expansion of the library and retesting already processed results to gain more information. This kind of application would also make a powerful tool for post mortem toxicology were samples are not available for an unlimited timeframe, if the method can be adapted to potentially heavily degraded matrixes. In fact, any application that has to make use with a very limited samples volume would profit greatly from this capability of reprocessing data already obtained.

Another target for the instrumentation are NPS and synthetic cannabinoids, two extremely rapidly changing fields. While considerable adaptation of the method might be necessary to focus on these fields, the previously mentioned capabilities should prove very powerful in detecting these compounds.

Furthermore, while extraction and mobile phase have been extensively investigated, a different chromatography column might offer advantages that could not be investigated in this work. While the scope of different potential analytes prohibits a too specialised column, there are many general chromatography columns available for reverse phase and normal phase chromatography.

The inclusion of fragmentation into the screening procedure has been determined to be not suitable as the instrument and software impose limitations on the amount of compounds that can be scheduled for fragmentation which was exceeded by the library early on. A general untargeted fragmentation approach might be considered, but preliminary tests showed almost complete fragmentation of the precursor ion and no recognisable fragmentation pattern. While more advanced instrumentation or software might be able to include

fragmentation into the screening procedure, it might be worthwhile to outsource any needed fragmentation to a more specialised instrument such as a LC-QQQ with far superior fragmentation capabilities.

6 Appendix

6.1 Exact Mass Library

Accurate Mass	RT	Formula	Name
258.9858	9.1	C9H10IN	5-IAI
135.1048	8.5	C9H13N	AMP
171.1259	8.2	C9H17NO2	Gabapentin
255.0079	9.8	C9H7Cl2N5	Lamotrigine
177.0790	10.2	C10H11NO2	MDAI
179.0946	8.3	C10H13NO2	MDA
149.1205	8.6	C10H15N	MAMP
165.1154	8.4	C10H15NO	PMA
199.1209	5	C10H17NO3	Ecgonine methyl ester
230.1031	10.7	C11H13F3N2	TFMPP
175.0997	10	C11H13NO	5-APB
175.0997	9.5	C11H13NO	6-APB
193.1103	8.7	C11H15NO2	MDMA
193.1103	8.2	C11H15NO2	Methedrone
179.1310	8.8	C11H17NO	PMMA
221.1052	8.5	C12H15NO3	Butylone
207.1259	9.3	C12H17NO2	MDEA
237.0920	9.3	C13H16ClNO	Ketamine
234.1732	9.4	C14H22N2O	Lignocaine / Lidocaine
266.1631	7.8	C14H22N2O3	Atenolol
347.9665	14.5	C15H10BrClN2O	Phenazepam
320.0120	13.6	C15H10Cl2N2O2	Lorazepam
315.0411	13.1	C15H10ClN3O3	Clonazepam
270.0560	14.7	C15H11ClN2O	Nordiazepam
286.0509	14.2	C15H11ClN2O2	Oxazepam
281.0801	13.1	C15H11N3O3	Nitrazepam
237.0790	9.1	C15H11NO2	Methoxetamine
236.0950	12.9	C15H12N2O	Carbamazepine
254.1055	12.3	C15H14N2O2	Licarbazepine
233.1780	10.1	C15H23NO	3-MeO-PCE
284.0717	15.1	C16H13ClN2O	Diazepam
300.0666	14	C16H13ClN2O2	Temazepam
299.0825	14.4	C16H14ClN3O	Chlordiazepoxide
274.1237	12.2	C16H19ClN2	Chlorpheniramine
289.1314	9.5	C16H19NO4	Benzoylecgonine
263.1885	9.1	C16H25NO2	Tramadol
342.0706	14.1	C17H15ClN4S	Etizolam
283.1209	11	C17H17NO3	3-4-MDBC
309.1340	12.9	C17H18F3NO	Fluoxetine
318.0958	14	C17H19ClN2S	Chlorpromazine

265.1579	12.3	C17H19N3	Mirtazapine
253.1467	12.1	C17H19NO	Benzedrone
285.1365	4.8	C17H19NO3	Morphine
284.1347	12.6	C17H20N2S	Promethazine
255.1623	11.2	C17H21NO	Diphenhydramine
303.1471	10.4	C17H21NO4	Cocaine
277.2042	10.2	C17H27NO2	Venlafaxine
267.1623	10	C18H21NO	Pipradol-3-Isomer
299.1522	8.5	C18H21NO3	Codeine
266.1783	17.8	C18H22N2	Desipramine
301.1678	8.4	C18H23NO3	DHC
317.1627	10.3	C18H23NO4	Cocaethylene
273.2093	10.4	C18H27NO	3-MeO-PCP
329.1427	12.3	C19H20FNO3	Paroxetine
307.1685	13	C19H21N3O	Zolpidem
327.1471	8.5	C19H21NO4	6-MAM
371.1513	12.8	C19H22ClN5O	Trazodone
314.1550	14	C19H23ClN2	Clomipramine
281.1780	8.7	C19H23NO	Naphyrone
324.1638	10.9	C20H21FN2O	Citalopram
277.1831	11.3	C20H23N	Amitriptyline
375.1401	11.4	C21H23ClFNO2	Haloperidol
383.1668	13.1	C21H25N3O2S	Quetiapine
309.2093	13.7	C21H27NO	Methadone
344.1988	17.7	C21H28O4	THC-COOH
314.2246	20	C21H30O2	THC
351.1835	15.2	C22H25NO3	JWH-250 Hydroxypentyl metabolite
414.1614	12.1	C22H26N2O4S	Diltiazem
339.2198	12.2	C22H29NO2	Propoxyphene
474.2049	14.1	C22H30N6O4S	Sildenafil
343.1572	15.8	C23H21NO2	JWH-073 4-Hydroxybutyl metabolite
410.2118	12.4	C23H27FN4O2	Risperidone
359.1685	16.7	C24H22FNO	AM-2201
375.1635	15.6	C24H22FNO2	AM-2201 4Hydroxypentyl metabolite
357.1729	11.4	C24H23NO2	JWH-018 4-Hydroxypentyl metabolite
357.1729	11.4	C24H23NO2	JWH-018 5-Hydroxypentyl metabolite
349.2406	13.1	C24H31NO	Dipipanone
413.2566	10.2	C25H35NO4	Norbuprenorphine
454.2832	11.8	C27H38N2O4	Verapamil
467.3036	15.6	C29H41NO4	Buprenorphine

6.2 Letter for Ethical Approval

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

West of Scotland REC 4
Ground Floor, Tennent Building
Western Infirmary
38 Church Street
Glasgow
G11 6NT
www.chsssr.org.uk

Mrs Shaza Deeb
PhD Student
University of Glasgow
Forensic Medicine and Science
University Place
Glasgow
G12 8QQ

Date 11 February 2013
Direct line 0141-211-1722
Fax 0141-211-1847
e-mail evelyn.jackson@ggc.scot.nhs.uk

Dear Mrs Deeb

Study title:	**Determination of New Antiepileptic Drugs in Human Samples (Urine and Head Hair) Using Chromatographic Techniques Coupled with Mass Spectrometry As Prospective Drugs of Misuse**
REC reference:	**12/WS/0312**
Amendment number:	**AM01**
Amendment date:	**08 February 2013**
IRAS project ID:	**104688**

Thank you for your letter of 8 February 2013, notifying the Committee of the following amendments:

- Falkirk Police Office added as an additional non-NHS site.
- Sponsor's contact changed to Dr Debra Stuart.

The Committee does not consider this to be a "substantial amendment", as defined in the Standard Operating Procedures for Research Ethics Committees. The amendment does not therefore require an ethical opinion from the Committee and may be implemented immediately, provided that it does not affect the approval for the research given by the R&D office for the relevant NHS care organisation.

Documents received

The documents received were as follows:

Document	Version	Date
Notification of a Minor Amendment	AM01	08 February 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

12/WS/0312: Please quote this number on all correspondence

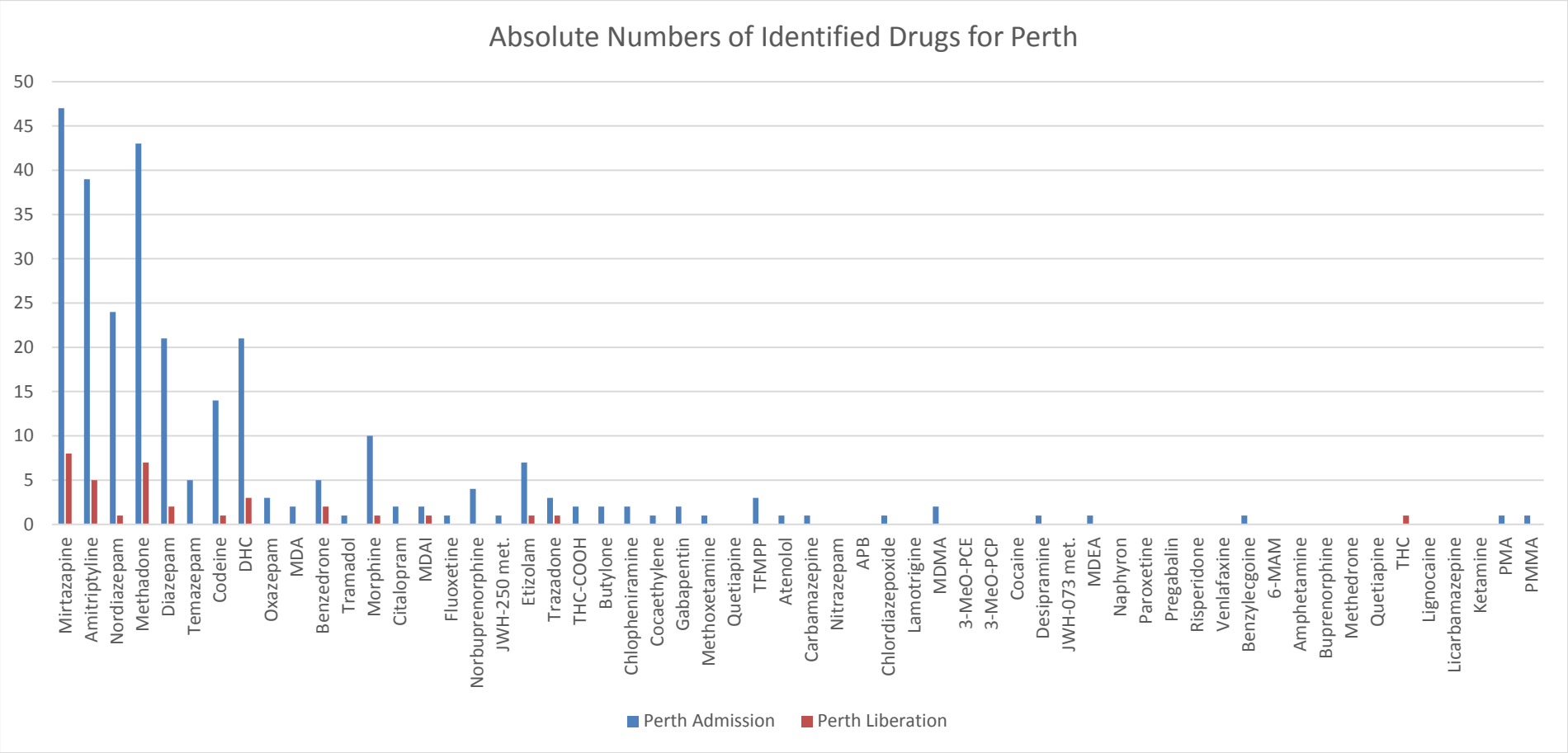
Yours sincerely

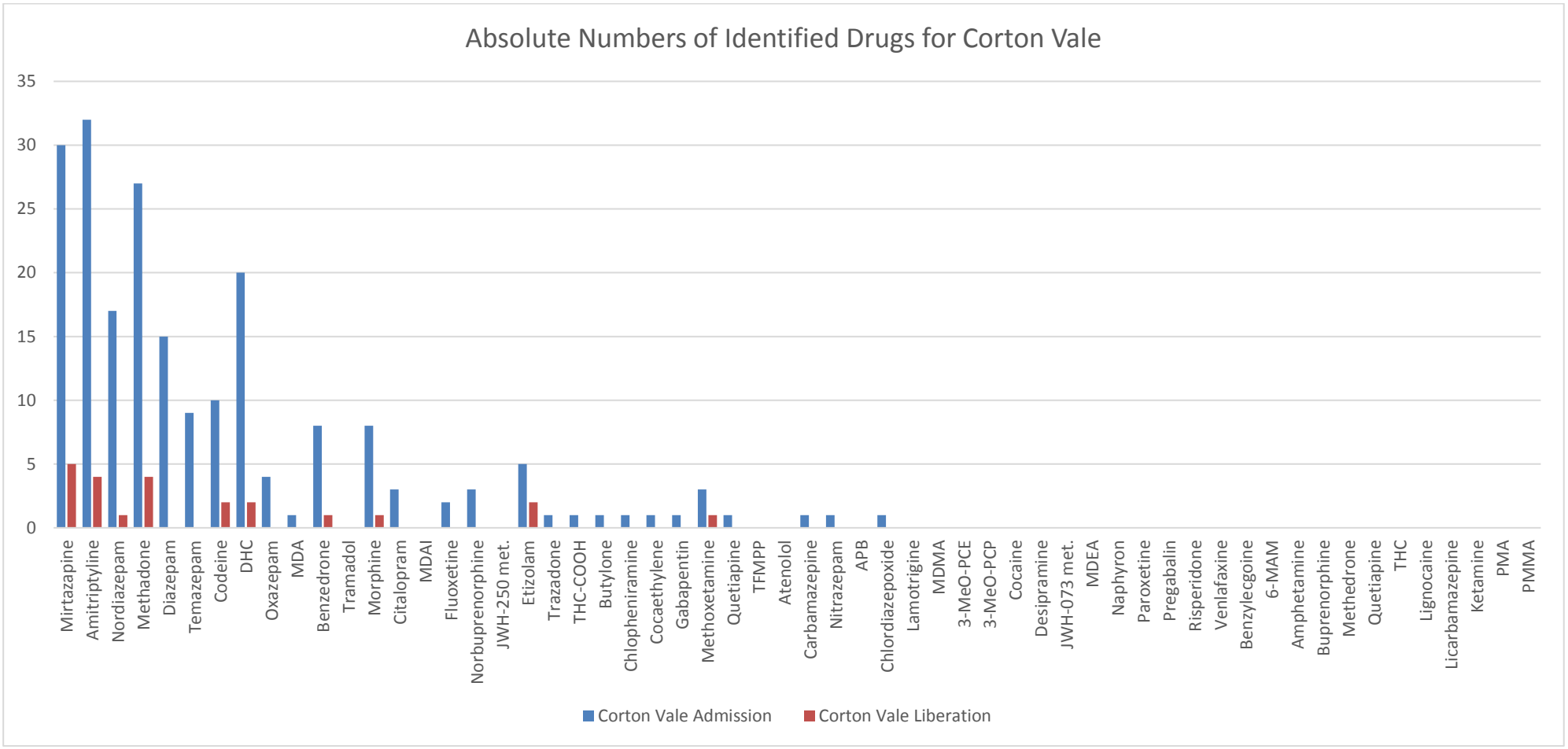
Evelyn Jackson

Ms Evelyn Jackson
Committee Co-ordinator

Copy to: *Dr Oliver Frenschock*
Dr D Stuart, University of Glasgow, Tennent Building

6.3 Comprehensive Prison Data

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Mirtazapine
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Codeine
DHC
Oxazepam
MDA
Benzedrone
Tramadol
Morphine
Citalopram
MDAI
Fluoxetine
Norbuprenorphine
JWH-250 met.
Etizolam
Trazadone
THC-COOH
Butylone
Chlopheniramine
Cocaethylene
Gabapentin
Methoxetamine
Quetiapine
TFMPP
Atenolol
Carbamazepine
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APB
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Lamotrigine
MDMA
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3-MeO-PCP
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Desipramine
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MDEA
Naphyron
Paroxetine
Pregabalin
Risperidone
Venlafaxine
Benzylecgoine
6-MAM
Amphetamine
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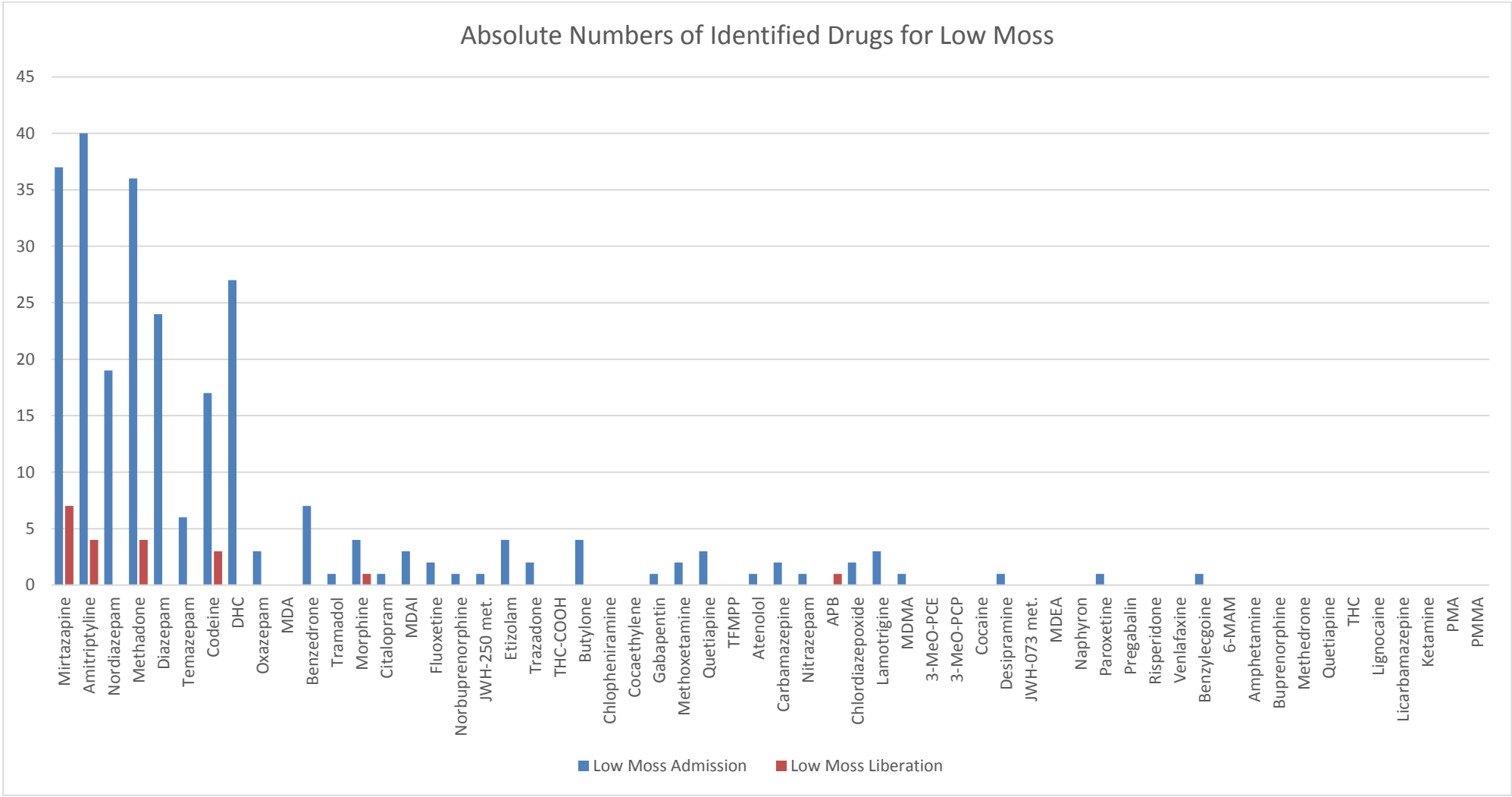
PMMA
Corton Vale Admission
Corton Vale Liberation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Chlopheniramine
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MDEA
Naphyron
Paroxetine
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Risperidone
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Benzylecgoine
6-MAM
Amphetamine
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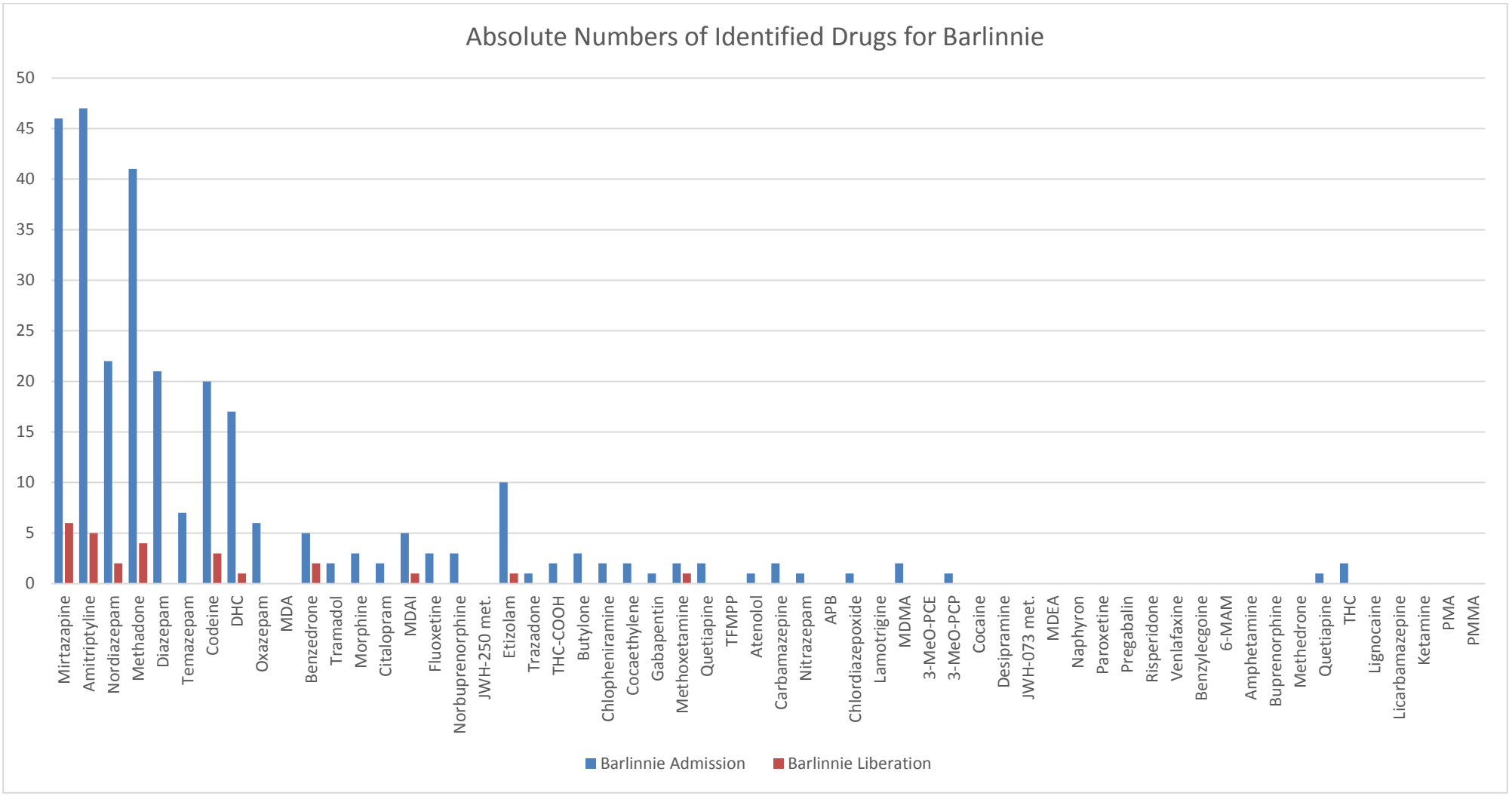
Low Moss Admission
Low Moss Liberation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DHC
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3-MeO-PCP
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Pregabalin
Risperidone
Venlafaxine
Benzylecgoine
6-MAM
Amphetamine
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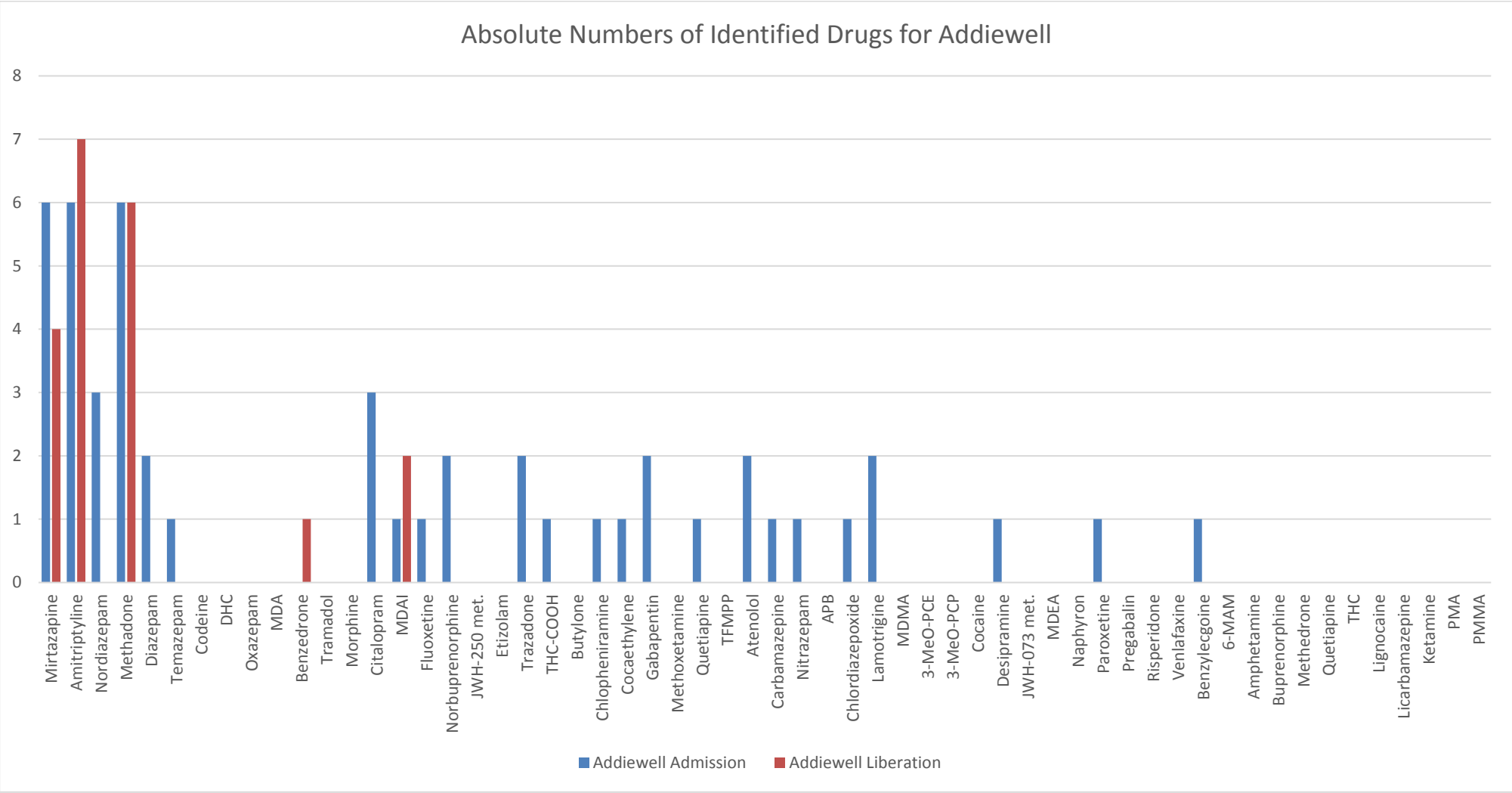
Barlinnie Admission
Barlinnie Liberation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Chlordiazepoxide
Lamotrigine
MDMA
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3-MeO-PCP
Cocaine
Desipramine
JWH-073 met.
MDEA
Naphyron
Paroxetine
Pregabalin
Risperidone
Venlafaxine
Benzylecgoine
6-MAM
Amphetamine
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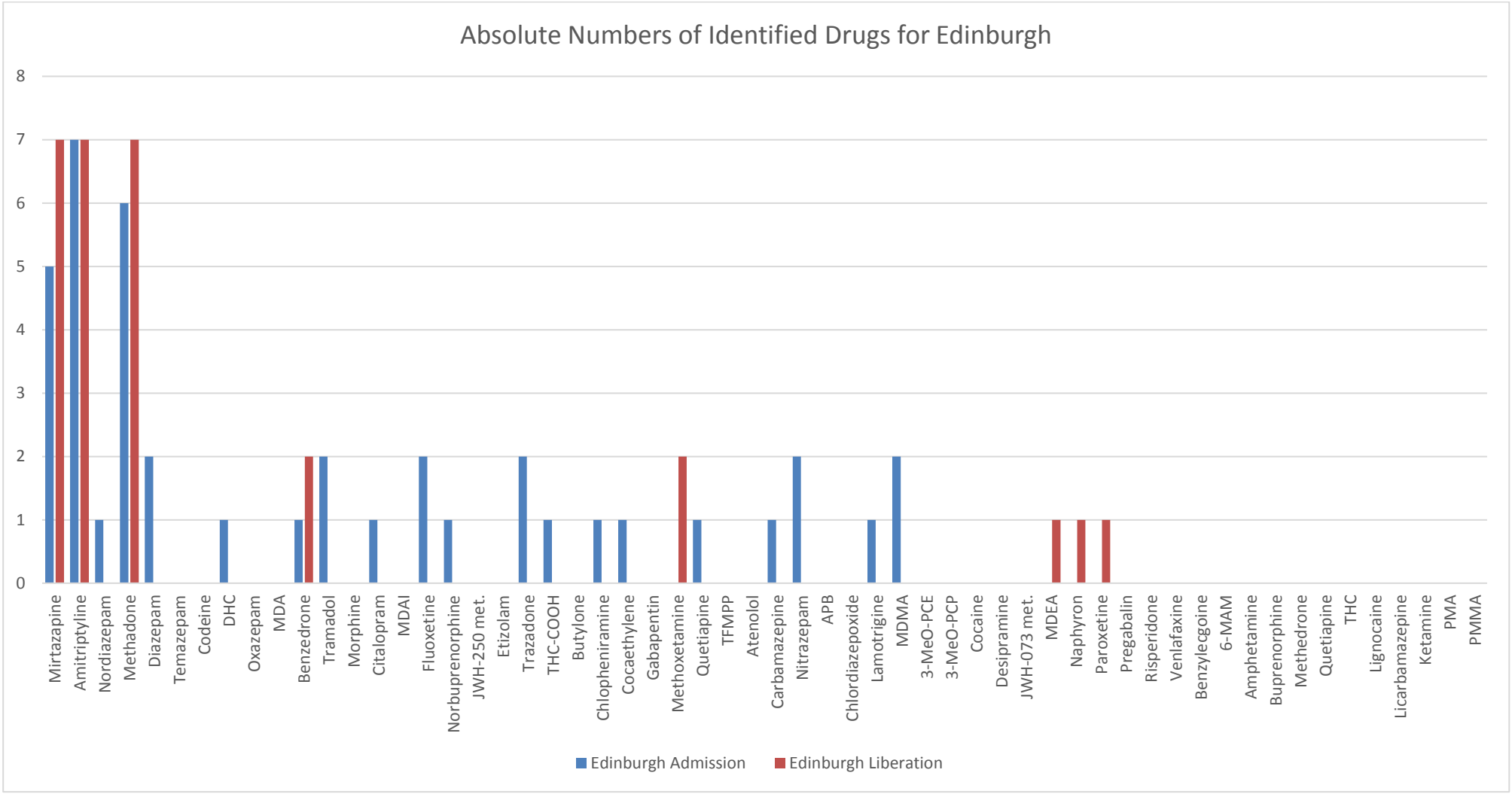
Addiewell Admission
Addiewell Liberation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DHC
Oxazepam
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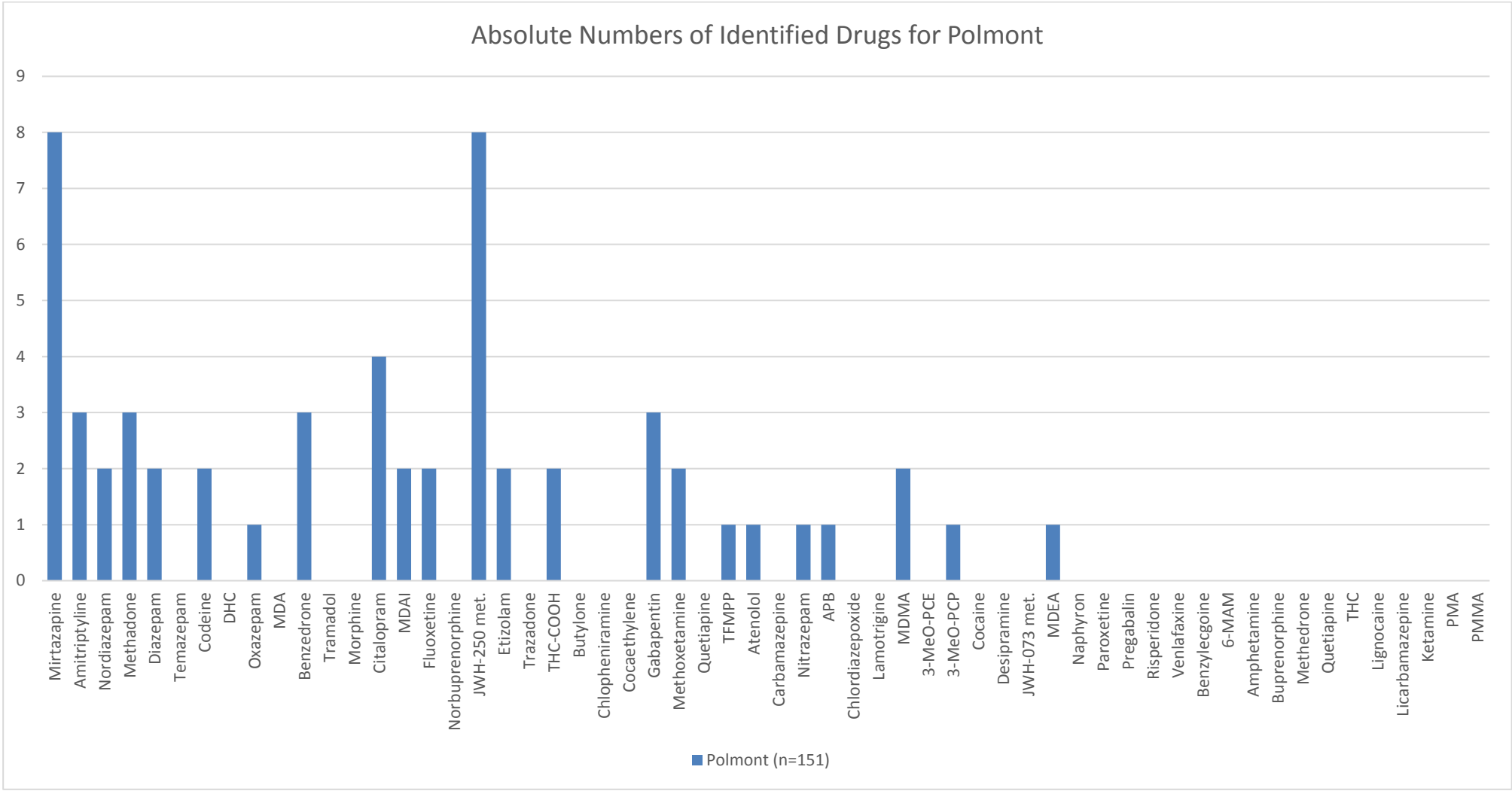
Etizolam
Trazadone
THC-COOH
Butylone
Chlopheniramine
Cocaethylene
Gabapentin
Methoxetamine
Quetiapine
TFMPP
Atenolol
Carbamazepine
Nitrazepam
APB
Chlordiazepoxide
Lamotrigine
MDMA
3-MeO-PCE
3-MeO-PCP
Cocaine
Desipramine
JWH-073 met.
MDEA
Naphyron
Paroxetine
Pregabalin
Risperidone
Venlafaxine
Benzylecgoine
6-MAM
Amphetamine
Buprenorphine
Methedrone
Quetiapine
THC
Lignocaine
Licarbamazepine
Ketamine
PMA
PMMA
Edinburgh Admission
Edinburgh Liberation


Absolute Numbers of Identified Drugs for Polmont
9
8
7
6
5
4
3
2
1
0
Mirtazapine
Amitriptyline
Nordiazepam
Methadone
Diazepam
Temazepam
Codeine
DHC
Oxazepam
MDA
Benzedrone
Tramadol
Morphine
Citalopram
MDAI
Fluoxetine
Norbuprenorphine
JWH-250 met.
Etizolam
Trazadone
THC-COOH
Butylone
Chlopheniramine
Cocaethylene
Gabapentin
Methoxetamine
Quetiapine
TFMPP
Atenolol
Carbamazepine
Nitrazepam
APB
Chlordiazepoxide
Lamotrigine
MDMA
3-MeO-PCE
3-MeO-PCP
Cocaine
Desipramine
JWH-073 met.
MDEA
Naphyron
Paroxetine
Pregabalin
Risperidone
Venlafaxine
Benzylecgoine
6-MAM
Amphetamine
Buprenorphine
Methedrone
Quetiapine
THC
Lignocaine
Licarbamazepine
Ketamine
PMA
PMMA
Polmont (n=151)

6.4 Publications

6.4.1 SODAS: Surveillance of Drugs of Abuse Study

SODAS: Surveillance of Drugs of Abuse Study

David J. Lowe[a], Hazel J. Torrance[b], Alastair J. Ireland[a], Felix Bloeck[b] and Richard Stevenson[a]

Objective Novel psychoactive substance (NPS) as a form of recreational drug use has become increasingly popular. There is a paucity of information with regard to the prevalence and clinical sequelae of these drugs. The aim of this study was to detect NPS in patients presenting to the emergency department with suspected toxicological ingestion.

Patients and methods The prospective study was performed in a large emergency department in the UK. During a 3-month period 80 patients were identified by clinicians as having potentially ingested a toxicological agent. Urine samples were analysed using liquid chromatography high-resolution mass spectrometry, and basic clinical data were gathered.

Results Eighty patients with a history of illicit or recreational drug consumption had urine screenings performed. Forty-nine per cent (39) of the patients undergoing a screen had more than one illicit substance detected. Twenty per cent (16) of the patients tested positive for at least one NPS.

Conclusion Almost half of the presented patients revealed ingestion of multiple substances, which correlated poorly with self-reporting of patients. Developing enhanced strategies to monitor evolving drug trends is crucial to the ability of clinicians to deliver care to this challenging group of patients. *European Journal of Emergency Medicine* 00:000–000

European Journal of Emergency Medicine 2015, 00:000–000

Keywords: forensic drug analysis, identification, legal highs, novel psychoactive substances, prevalence

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Introduction

Attendances in emergency departments (EDs) due to the sequela of the effects of drugs of abuse creates a significant burden [1–3]. Drug abuse became regarded as an epidemic problem with the rise of heroin in the 1960s [4]. Recreational drug use is more prevalent among young adults and is associated with significant short-term and long-term health implications [5,6]. Novel psychoactive substance (NPS) is a generic term used to describe substances that mimic the effects of traditional illicit drugs. These psychoactive substances are newly available and, although not prohibited, pose a public health threat comparable to that of traditional illicit substances [7].

There has been a steady rise in the number of synthetic and plant-based psychoactive substances, with an exponential growth in the production and distribution of such drugs [8–10]. The unknown safety profile, active ingredients and quantity increase the risk of overdose and serious clinical consequences [11,12]. There remains inconsistent sampling and reporting of attendances to the ED following ingestion of NPS and of the clinical manifestations [13]. Proliferation of NPS abuse has been facilitated by inconsistent legislation allowing uncontrolled access to substances. The ability to evade detection by standard toxicological screens allied with easy availability in shops and on the Internet has made these drugs increasingly attractive recreational substances [14]. Developing analytical profiling of agents and reference standards is an area of considerable ongoing work [15,16].

Surveillance of drug abuse patterns is crucial to developing strategies to direct both clinical-based and community-based interventions [17]. Creating a detailed understanding of current trends is challenging in the face of constantly evolving habits [18,19]. Although population surveys provide useful information, the illicit nature of drug abuse and the reliability of respondents pose problems [20,21].

The clinical challenge is to safely treat patients who have ingested unknown substances, which is achieved by responding to the toxidrome on presentation [22]. The paucity of analytical confirmation of hazardous substances prevents clinicians from effectively managing these patients [23]. Identification of these novel substances enables tracking of use, effects from ingestion of these novel drugs and also the changing patterns of abuse [24,25]. Coingestion of synergistic or antagonistic

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substances can lead to diagnostic and treatment challenges [26].

Before this surveillance study, urine samples were sent for analysis using a commercially available testing kit for traditional substances of abuse (Alere Inc., San Diego, California, USA). It was increasingly recognized that this screen did not encompass the range of substances that were self-reported or corresponded to the toxidrome of the patient [21]. Evolution of drug behaviours within the local population had extended beyond the testing capabilities of the ED and local laboratory provision.

Patients and methods

Patients and sampling

This was a single-center prospective observational study. Glasgow Royal Infirmary is a large inner city ED with ~86 000 attendances each year. During a 3-month period (1 May 2014 to 29 July 2014), data were collected of all patients who attended the department and who were identified by the treating clinicians based on their history or clinical suspicion as attending because of ingestion of NPS, for which a urine sample was collected. Patients were excluded if they were younger than 16 years of age.

Data were collected using a standardized proforma. Patient's data were anonymized and linked to presentation by Bruker, Billerica, Massachusetts, USA a unique code number. Urine samples were stored in additive-free containers in a laboratory refrigerator until testing within a week of collection. Data analysis was performed using Microsoft Excel (2011; Microsoft Corporation, Redmond, Washington, USA).

Urine samples were extracted using a simple liquid–liquid procedure with MTBE and Tris buffer and analysed using liquid chromatography high-resolution mass spectrometry. A Bruker MicrOTOF-Q (Bruker, Billerica, Massachusetts, USA) with an Agilent 1260 Infinity (Agilent, Santa Clara, USA) HPLC was used for analysis. Identification was achieved by matching retention time, mass (four decimal places) and isotope pattern.

Table 1 Range of drugs detected and frequency

Drugs	Not prescribed (%)	Prescribed (%)
Diazepam	24 (30)	9 (11.25)
Ecstasy (MDMA)	18 (22.5)	0
Cocaine	16 (20)	0
Amitriptyline	14 (17.5)	0
Heroin (as 6-MAM)	9 (11.25)	0
Etizolam	7 (8.75)	0
Amphetamine/MDA	6 (7.5)	0
Mirtazapine	6 (7.5)	3 (3.75)
Methadone	1 (1.25)	13 (16.25)
MDAI	4 (5)	0
Gabapentin	3 (3.75)	0
Methoxetamine	2 (2.5)	0
TFMPP	2 (2.5)	0
PMA/PMMA	2 (2.5)	
Cathinone (methedrone, butylone)	2 (2.5)	0
Ketamine	1 (1.25)	0
Buprenorphine	1 (1.25)	1 (1.25)

6-MAM, 6-monoacetyl-morphine; MDA, 3,4-methylenedioxyamphetamine; MDAI, methylenedioxyaminoindane; MDMA, 3,4-methylenedioxymethamphetamine; PMA, paramethoxyamphetamine; PMMA, methyl-MA, 4-methoxy-N-methylamphetamine; TFMPP, 3-trifluoromethylphenylpiperazine.

Ethics

Ethics was sought and granted from NHS GG&C Ethics as a service evaluation. Consent was waived for the study as this was considered a service development study as urine samples are sent for a toxicology screen as a standard of care.

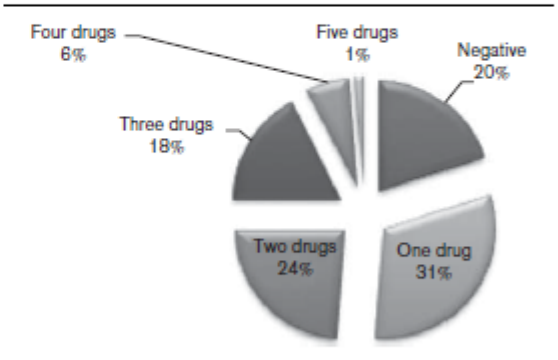
Results

Eighty patients with suspected ingestion of recreational drugs presenting at the ED had urine screenings performed. For the purposes of the study, an NPS was defined as a drug acting on the central nervous system, out-with those traditionally recognized as recreational drugs. The additional NPS tested for were: methoxetamine, etizolam, methlyenedioxyaminoindane (MDAI), piperazines [including 3-trifluoromethylphenylpiperazine (TFMPP)], paramethoxyamphetamine (PMA) and any cathinones. Case histories were not available for five patients; hence, demographic data are not available.

The range of drugs detected and respective frequencies are shown in Table 1. There was a male predominance; 54 men (aged 17–55 years) compared with 21 women (aged 16–47 years). The source of referral for patients was ambulance [49% (37)], self-referral [19% (14)] and police [32% (24)]. Thirty-six per cent (27) of the patients required admission, with the remaining 64% (48) discharged directly from the ED. Twenty per cent (16) of the patients tested positive for at least one NPS.

The total number of nonprescribed drugs detected in patients is reported in Fig. 1. Forty-nine per cent (39/80) of the patients undergoing a screen had more than one illicit substance detected.

Fig. 1



Number of drugs.

Table 2 Results of self-reported novel psychoactive substance ingestion

Reported NPS ingestions	Toxicology result
'Green apple' (tablet)	Diazepam, cocaine, lidocaine
'MCAT' (mephedrone)	PMA, PMMA, cocaine
'MCAT' (mephedrone)	Diazepam
'MCAT' (mephedrone)	None detected
'MCAT' (mephedrone)	None detected

NPS, novel psychoactive substance; PMA, paramethoxyamphetamine; PMMA, methyl-MA, 4-methoxy-*N*-methylamphetamine.

Table 3 Results for positive novel psychoactive substance ingestion and patient report

Reported ingestions	Toxicology result
No history	Dihydrocodeine[a], citalopram[a], **methoxetamine**
Cocaine	Diazepam[a], **etizolam**, cocaine, methadone[a], amitriptyline, mirtazapine[a]
No history	Lidocaine[a], mirtazapine, lamotrigine[a], amitriptyline, methadone[a], **MDAI**, MDA
NPS	**PMA**, **PMMA**, cocaine
No history	Diazepam, codeine[a], **etizolam**, citalopram[a]
Cocaine	Cocaine, MDA, diazepam, **MDAI**, carbamazepine[a]
Cocaine	**TFMPP**, cocaine
No history	**Etizolam**, codeine[a], 6-MAM, carbamazepine[a], **methoxetamine**
Diazepam, gabapentin	**Etizolam**, gabapentin, methadone[a], MDMA
Heroin, amitriptyline	Lidocaine[a], 6-MAM, codeine[a], **butylone**
Diazepam	Amitriptyline, methadone[a], 6-MAM, **etizolam**
Diazepam	Gabapentin, carbamazepine[a], **etizolam**, methedrone, MDMA
Cocaine	Quetiapine, **MDAI**, cocaine, codeine[a]
No history	**MDAI**, diazepam, fluoxetine[a], dihydrocodeine
Ecstasy	**TFMPP**, MDMA
No history	**Etizolam**, methadone[a], amitriptyline, diazepam[a]

Text in bold indicates NPS agent.
MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymphetamine; MDAI, methylenedioxyaminoindane; NPS, novel psychoactive substance; PMA, paramethoxyamphetamine; PMMA, methyl-MA, 4-methoxy-*N*-methylamphetamine; TFMPP, 3-trifluoromethylphenylpiperazine.
[a]Indicated prescribed drugs.

Only five patients reported consumption of an NPS; the results of their screenings are shown in Table 2.

Table 3 lists the patients who tested positive for NPS agents and their reported ingestions. Eleven patients were unable to give a history of ingestion because of their medical condition on arrival.

Discussion

The study reports the urine screening results of those patients who reported drug consumption for recreational purposes, or who presented with a clinical toxidrome suggestive of acute drug intoxication. The main objective was the detection of NPS in patients presenting to EDs; in our study 16 patients had NPS agents detected, of which only five patients reported consumption of these drugs. It is unclear whether there was deliberate misreporting by patients or whether patients were not aware that they had ingested an NPS.

A selection of NPS agents were detected, predominantly the ecstasy MDMA 'mimics', such as PMA/PMMA, MDAI, and TFMPP; only one patient gave a history of ecstasy consumption and tested positive for one of these compounds. From the NPS detected, only MDAI and etizolam are not currently regulated by the Misuse of Drugs Act in the UK. Only five patients reported taking an NPS (referred to as a 'legal high' in their own terms); one patient tested positive for PMA, whereas the rest were negative for NPS; however, this may have been a synthetic cannabinoid agent that had not been tested for in this study.

Of interest was the detection of amitriptyline and mirtazapine in our patient population; all the amitriptyline detected was present in patients also testing positive for methadone, diazepam, and other illicit substances. Only one patient reported the ingestion of amitriptyline, none reported the use of mirtazapine; abuse of amitriptyline by patients on methadone substitution therapy has been recognized since 1978 [1]; however, the nonprescription use of mirtazapine has not been reported in the literature.

Etizolam, a thienodiazepine, is not currently regulated in the UK; as with amitriptyline, it was only detected in patients who tested positive for other illicit drugs. No patients reported the intentional consumption of etizolam and only two patients reported a history of consumption of benzodiazepines out of the seven who tested positive for the drug.

Limitations

Our study had several limitations. First, it was performed at a single institution and limited to patients whom individual clinicians had identified as having ingested a toxicological agent. Retrospective review of triage notes did not reveal any clearly missed patients but relied upon individual clinician's identification and subsequent inclusion in the study. Synthetic cannabinoids were not included in this study but will be included in future studies. Because of the lack of reference standards, some novel agents may not have been identified. There is the potential for degradation of metabolites and no quantitative work was performed on the analytes. In addition, 24 patients who were enrolled in the study had negative samples for which there are a number of explanations: the patient had not ingested the substance; we did not test for the analyte; an error had occurred during storage/sampling; or the patient had been incorrectly enrolled.

Future

The purpose of this study was to evaluate the current toxicology screening against the potential range of substances ingested presenting in a large city ED. Sharing of the findings within the ED and other agencies raised the awareness of these varied and potentially hazardous substances. Development of point of care testing to enable rapid identification during presentation would aid treatment and risk stratification. Incorporating testing for NPS in postmortems and development of new standards for testing may facilitate greater recognition of the

contribution of these substances in forensic cases and inform future drug surveillance and regulation strategies.

Conclusion

Only a small percentage of samples tested positive for NPS. Most samples were positive for more commonly encountered drugs of abuse. It is important to understand the range of drugs that are affecting our local population. The extent of polyingestion has significant implications for management of these patients within the ED. The poor correlation between reporting and detection emphasizes the need for clinicians to have a high degree of suspicion and treat the presenting toxicological syndrome. This may, of course, represent a lack of knowledge by patients about the substances they are ingesting. The combination of both illicit drugs, newer drugs of variable legal status and those previously unconsidered drugs represents a substantial challenge to the treating physician. By identifying the individual drugs and trends that are prevalent, we can direct resources into understanding their effects and implications on this challenging group of patients.

Acknowledgements

The authors thank the emergency department staff and laboratory staff of Glasgow Royal Infirmary for their invaluable efforts.

Conflicts of interest

There are no conflicts of interest.

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6.4.2 Poster Abstract: TIAFT 2014 Argentinia

Utilising Time-of-Flight Mass Spectrometry to Profile the Drug Use as Identified from A&E Admissions and Prisoner Populations in Scotland

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Aims: The aim of this study was to evaluate the suitability of high resolution mass spectrometry (HRMS), as a general screening method including 80 drugs (benzodiazepines, opiates, stimulants, antihistamines, antidepressants, antipsychotic, antiepileptic, hypnotics and synthetic cathinones) for the assessment of the prevalence of drugs in hospital admissions and prisoner populations in Scotland.

Method: A liquid-liquid extraction (MTBE/TRIS) for urine in combination with a Full Scan (ESI+) LC HRMS method on a Phenomenex Fusion-RP column was developed. Limits of detection were found to be 4 40 ng/mL, with most drugs at 20 ng/mL or lower, while recovery ranged between 20% (THC) and 97% (Diazepam). The impact of matrix effects was evaluated and ranged between 23% ion enhancement and 19% ion suppression, depending on the compound, most prominently THC. Compounds were identified by accurate mass, retention time, isotopic pattern and signal-to-noise ratio.

Results: 75% of urine samples (n=90) from hospital admissions were positive for drugs included in the library. The prevalent drugs found were “classical” drugs of abuse including MDMA, Diazepam, Cocaine and more unusually PMA, PMMA and Etizolam. Also identified were prescription drugs such as Lignocaine and Amitriptyline. 904 urine samples from Scottish prison facilities were analysed with 37% positive results overall and with 21% of liberation samples (n=265) and 44% of admission samples (n=639) tested positive. The most commonly identified drugs were prescription drugs including Methadone, Amitriptyline and Benzodiazepines (Diazepam, Temazepam, etc.).

Conclusion: HRMS allowed for the parallel identification of coeluting and isobaric compounds without further chromatographic separation. The results show that mainly "classical" drugs of abuse continue to be abused and NPS make up a minority. Further work

is ongoing to include synthetic cannabinoids and NPS as required in future analysis of these samples.

Keywords: time-of-flight, general screening, drugs of abuse, NPS, hospital admissions, prison

*Presenting author

6.4.3 Oral Presentation: UKIAFT 2014 Leicester

Utilising Time-of-Flight Mass Spectrometry to Assess the Prevalence of Drugs in A&E Admissions and Prisoner Populations in Scotland

Felix Bloeck*, Hazel Torrance

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A comprehensive and general toxicological screen for both common and newly emerging drugs is a recognised first step in a toxicological sample work up. However, this proves to be an analytical challenge, as an extremely wide range of drugs are available, and potentially abused, consisting of vastly different chemical properties. Screening techniques are designed to target ranges of drugs or drug families at the cost of excluding more exotic or rare substances that might still be abused. While these techniques are constantly evolving to include newer and rarer substances, there can be a considerable delay between recognition of an abused substance and commercially available screening techniques.

The aims of this study are to evaluate the suitability of high resolution mass spectrometry (Bruker micrOTOF-Q) as a general screening method and the consequent assessment of the prevalence of drugs in A&E admissions and prisoner populations in Scotland. The application of this technology offers several advantages, most notable the ability to analyse a multitude of drugs simultaneously and library independence.

For this purpose, a general and unspecific liquid-liquid extraction method for urine in combination with liquid chromatography-high resolution mass spectrometry was developed and applied to samples received from a local A&E service and Scottish prison facilities. The screening method includes many commonly prescribed drugs, commonly abused drugs, synthetic cannabinoids and a range of novel psychoactive substances (NPS).

Keywords: time-of-flight, general screening, drugs of abuse, synthetic cannabinoids, NPS

*Presenting author

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