Development, optimisation and validation of chromatographic and qNMR spectroscopic methods for the detection and quantification of controlled drugs, psychoactive substances and prescription-only medications.

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Development, optimisation and validation of chromatographic and qNMR spectroscopic methods for the detection and quantification of controlled drugs, psychoactive substances and prescription-only medications.

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Abstract

The supply chains for the illicit drugs market are unregulated and consistency between tablets and powders are not to be expected, typically varying in shape, colour and active API content. This thesis has an overarching focus on cocaine, which is known to be seen in a wide range of purities and can potentially contain a wide range of adulterants dangerous to health.

Three analytical techniques, attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and ion mobility spectrometry (IMS) have been assessed and outlined, providing an alternative to gas chromatography-electron ionisation-mass spectrometry (GC-EI-MS), which is hailed as the "gold-standard" for forensic analysis within current literature. This aims to showcase alternative techniques to GC-EI-MS and to increase accessibility for on-site harm reduction illicit sample testing.

Qualitative comparisons between GC-MS, bench-top NMR (BT-NMR) and FT-IR were made to assess their functionality for off-site harm reduction drug testing. GC-MS maintained the "gold-standard". FT-IR proved to be a useful tool due to its fast, non-destructive analysis but should be used with the use of spectral subtraction to allow multiple component analysis of binary and tertiary illicit drug samples. BT-NMR provides a useful alternative, fully identifying 53.3% of binary mixtures, consisting of illicit cocaine and ecstasy samples, with complete identification of cocaine binary mixtures achievable between 70 – 40 % (w/w) content, matching simulated sample analysis.

A ^{1}H qNMR method for the quantification of cocaine in either its freebase or hydrochloride salt forms, within illicit samples (n = 97) was outlined, in the presence of 7 common adulterants: caffeine, benzocaine, phenacetin, ketamine, aspirin, procaine and levamisole, using a low-field (60 MHz) benchtop instrument. Method uses spectral acquisition of ~4.5 minutes using cheap deuterated solvents D_2O and $CDCl_3$, with simple sample preparation. Sample analysis was compared with GC-EI-MS showing good correlation (R^2 = 0.9399). Method of analysis has also been adapted for no-D solvents allowing for cheaper and simpler analysis.

A commercial IMS instrument (IONSCAN 600, Smiths Detection™) was used for sample analysis, performing qualitative analysis on samples containing a high prevalence of “ecstasy” tablets, cocaine and other powders seized from a festival setting. IMS identified the illicit component in 98.2% of samples analysed. Levamisole was the only active adulterant identified within binary mixtures, producing a full identification in 34.0% of samples (n = 18) and no identifications of tertiary mixtures, due to IMS inactive adulterants and low content % (w/w). Positional isomeric discrimination was assessed, showcasing good separation within typically singularly substituted isomers generally following in the order of ortho > meta/para.

The characterisation and quantification for the new psychoactive substance 1-(1,3-benzodioxyl-5-yl)-2-(propylamino)butan-1-one (bk-PBDB, putylone), which has not been previously identified within the UK, was outlined. A reference standard was synthesised and common 1D and 2D NMR assignments are outlined, as well as proposed GC-EI-MS fragmentation patterns. This expands on the previous literature which only showed GC-EI-MS and allows other groups or organisations to identify and quantify as necessary.

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Abbreviations

APCI	Atmospheric Pressure Chemical Ionisation
API	Active Pharmaceutical Ingredient
ATR	Attenuated Total Reflectance
BrP	Bromophenidine
CD	Corona Discharge
$CDCl_3$	Deuterated chloroform
CH_3Cl	Chloroform
ClP	Chlorophenidine
CMC	Chloromethcathinone
CMPE	Cyclopentyl methyl ether
COSY	Correlation Spectroscopy
CP	Control Parameters
DFDP	Difluorodiphenidine
DFEP	Difluoroephenidine
DMP	Dimethylphenidine
DMPEA	3,4-dimethyoxyphenethylamine
DMS	Dimethyl sulphide
DMSO-d_6	Deuterated dimethyl sulfoxide
DMXP	Dimethoxyphenidine
EI	Electron Impact
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EP	Ephenidine
ESI	Electrospray Ionisation
FB	Freebase
FDP	Fluorodiphenidine
FEP	Fluoroephenidine
FF	Fluorofentanyl
FL	(Fluorophenyl)-*N*-methyl-2-phenylethanmine
FMC	Fluoromethcathinone
FMP	Floromethylphenidine
FP	Fluorophenidine
FT-IR	Fourier Transform – Infrared Spectroscopy
FWHM	Full Width at Half Maximum

GC	Gas Chromatography
GC-FID	Gas Chromatography – Flame Ionisation Detector
GC-MS	Gas Chromatography – Mass Spectrometry
GC-TMS	Gas Chromatography – Toroidal Ion Trap Mass Spectrometry
GHB	Gamma-hydroxybutyrate
GI-XRD	Grazing Incidence – X-Ray Diffraction
GMP	Greater Manchester Police
HCl	Hydrochloride
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC-HRMS	High Performance Liquid Chromatography – High Resolution Mass Spectrometry
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IP	Iodophenidine
IR	Infrared Spectroscopy
LC-MS/MS	Liquid Chromatography – Tandem Mass Spectrometry
LC-qTOF	Liquid Chromatography – Quadrupole Time of Flight Mass Spectrometry
LoD	Limit of Detection
LoQ	Limit of Quantification
LSD	Lysergic acid diethylamide
MA	Maleic acid
MANDRAKE	MANchester Drug Analysis and Knowledge Exchange
MC	Methcathinone
MDDP	Methylenedioxyphenidine
MDMA	3,4-methylenedioxymethamphetamine
MDP	Methyldiphenidine
MDPHP	3,4-methylenedioxy-α-pyrrolidinohexiophenone
MeCN-d_3	Deuterated acetonitrile
MeOH	Methanol
MMC	Methylmethcathinone
MMU	Manchester Metropolitan University
MS	Mass Spectrometry
MTBSTFA	N-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide

MXP	Methoxyphenidine
NEP	*N*-ethyl-pentylone
NMR	Nuclear Magnetic Resonance Spectroscopy
NPS	New Psychoactive Substances
PLS	Partial Least Squares
PoM	Prescription Only Medication
PTFE	Polytetrafluoroethylene
PULCON	Pulse Length-based Concentration Determination
QA/QC	Quality Assurance / Quality Control
QC	Quality Control
Qual-LoD	Qualitative Limit of Detection
qNMR	Quantitative Nuclear Magnetic Resonance Spectroscopy
RD	Relaxation Delay
RSD	Relative Standard Deviation
SIM	Selective Ion Monitoring
SINO	Signal-to-Noise Ratio
SOP	Standard Operating Procedure
SPoC	Single Point of Contact
SWGDRUG	Scientific Working Group for Analysis of Seized Drugs
Te-FEP	Tetrafluoroephenidine
TFMXP	Trifluoromethoxyphenidine
THC	Tetrahydrocannabinol
THCA-A	Tetrahydrocannabinolic acid
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
TMSP	Trimethylsilyl propionic acid
TP	(2-methylbibenzyl-α-yl)Piperidine
Tri-FEP	Trifluroephenidine
UNODC	United Nations Office on Drugs and Crime
USP	United States Pharmacopeia
XRD	X-Ray Diffraction
α-PVP	α-pyrrolidinovalerophenone

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1. Chapter 1 – Introduction

1.1. Introduction to Illicit Drug Testing

Drugs of abuse are typically taken by individuals for a number of reasons including (but not limited to): relaxation, to enhance socialisation or simply to 'become intoxicated'.[1] Unlike with other forms of legalised outlets, such as alcohol, tobacco or legalised substances that are generally heavily regulated,[2] the illicit drugs market is not, which poses a danger to users due to potential adulteration and a variety of purity levels.[3] In many countries, legislation is employed to restrict the possession, production and supply of substances which the potential to cause harm and thus, safe guard society.[4] In the United Kingdom, the key legislation employed for this purpose is the Misuse of Drugs Act 1971;[5] the Misuse of Drugs Regulations 2001 and the Psychoactive Substances Act 2016.[6, 7] Though this legislation is grounded in reducing criminality, these substances are still taken by users despite their controlled nature. To combat this risk, harm reduction testing is employed to treat drug intoxication and to inform healthcare professionals of potentially dangerous substances.[8] The analysis of biological fluids such as blood, saliva and urine are also good indicators of illicit drug prevalence but typically require tedious and lengthy analysis.[9] Similarly, self-reporting of drug use data relies on an accurate account from individuals which can be further skewed due to potentially mis-sold substances.[10] Alternatively, bulk sample analysis can be performed on the seized substances using a number of analytical methods as showcased in section 1.1 - Overview of Techniques. Similarly, a number of groups who perform bulk sample on-site/off-site harm reduction testing have been showcased in section 3.1 - Introduction to On-site / Off-site Testing Techniques.

1.1. Overview of Techniques

1.1.1. General Overview of All Techniques

A general overview of the typical analytical techniques used for illicit drug analysis has been provided in Table 1. This was adapted from a review written by Harper *et al.*[8]

Table 1 - Summary of the analytical techniques used in typical illicit drug analysis. Unless stated otherwise, information adapted from Harper et al.[8]

Method	Discrimination	Substances	Identify	Quantify	Sample Destruction	Portable	Typical Cost	Ease of use	Analysis time
GC-MS	5/5	Virtually any (volatiles)	✓	✓	Yes	No	£60k+	Intermediate - Advanced	Minutes
FT-IR	5/5	Virtually any	✓	Specialist	No	Yes	£10k+	Basic	Seconds
Raman	5/5	Virtually any	✓	Specialist	No	Yes	£10k+[11]	Basic	Seconds
NMR	5/5	Virtually any	✓	✓	No	Benchtop possible	£50k+ (Benchtop)	Intermediate - Advanced	Minutes
X-ray Diffraction	4/5	Crystalline solids	✓	✓	No	No	£41k - £205k	Advanced - Expert	Minutes - Hours
IMS	3/5	Small volatiles	✓	-	Yes	Yes	£18k+	Basic	Seconds
Ultraviolet Spectroscopy	3/5	Common drugs of abuse	✓	✓	No	Yes	£2.5k - £8.2k	Basic - Intermediate	Minute
Colour Tests	2/5	Previously characterised drugs of abuse	✓	-	Yes	Yes	£1.60 – £4.10 per test	Basic – Intermediate	Seconds - Minutes

The focus of this thesis is limited to only four techniques Fourier Transform – Infrared Spectroscopy (FT-IR), Gas Chromatography – Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR) and Ion Mobility Spectroscopy (IMS). These techniques were chosen for the reasons outlined within their respective introductory sections (sections 1.1.2, 1.1.3, 1.1.4 and 1.1.5). Research on illicit drug screening using GC-MS, FT-IR, NMR and IMS were selected to fill a specific gap within the literature, which shall be outlined within the remainder of this thesis.

There are a number of techniques which have potential to be used for illicit drug screening but were not outlined within this thesis. X-Ray Diffraction (XRD) (referring to specifically powder XRD not Grazing Incidence – X-Ray Diffraction (GI-XRD)) is an expensive to purchase technique that requires an expert level operator to use.[12] The instrument is limited to crystalline solid samples only, with typically long run times (10+ mins) compared to quicker techniques such as IMS and FT-IR (seconds).[13] Commercial databases for illicit drugs are not widely available, with even less availability for adulterants, proving mixture analysis difficult.[13] Diffractograms can even change depending on sample preparation and crystal structure.[13] The disadvantages and expertise required to perform large scale routine illicit drug analysis using XRD would not be attractive for small laboratories or point-of-care services.

Raman spectroscopy is a technique which would be suitable for bulk sample illicit drug analysis. It's ease of use, non-destructive, rapid analysis has all of the same benefits as FT-IR.[12] Handheld Raman spectroscopy units are useful for point-of-care services due to their portability. Similar studies on either FT-Raman or handheld-Raman devices could be included within the work showcased in this thesis. However, suitable assess to a FT-Raman instrument or a handheld Raman instrument is not possible.

Ultraviolet (UV) spectroscopy is an easy to use and portable technique.[12] Disadvantages of UV spectroscopy centre around the inherent lack of specificity making it not a confirmatory technique. Compounds without chromophores e.g. GBH cannot be analysed without complex sample preparation, UV spectral data can change dependant on pH and can be similar for similar classes of compounds and mixture analysis is complex.[12, 14]

Colour tests are one of the most widely used analytical methods used by laboratories who perform illicit drug analysis.[15] Colour tests are purchased in the form of an inexpensive 'kit' in which the analyst administers a series of reagents, to a small portion (less an 1 mg) of suspected illicit substance.[15] The identification of the substance is presented to the analyst, typically in a series of colour changes, in which the analyst has to deduce the identity of the compound by comparing to colour charts.[15] These colour tests are not a confirmatory technique and typically have a large number of interferences.[15] Due to colour tests not being a confirmatory technique, they are not included within work outlined in this thesis.

1.1.2. Fourier Transform – Infrared Spectroscopy

FT-IR is often employed as a complimentary technique for the analysis of illicit drug samples.[16-18] The infrared (IR) activity of a molecule is caused as the bonds absorb IR energy producing a change in dipole moment. The change in the intensity of IR light is measured by the instrumentation and is outputted as spectral data, outlining a "fingerprint" of bond data for each component within the sample. This data is then matched to a spectral library containing an illicit drug database. This technique is useful for routine illicit drug analysis as virtually all pharmaceutical compounds absorb IR radiation. IR spectrometers are cheap to purchase, require minimal maintenance, no sample preparation, provides non-destructive analysis, identifies between free-base or salt-form derivatives of drugs and is relatively portable.[12] However, since the IR spectrum only supplies information based on the bonds present within the molecules, this technique struggles to identify unknowns that are not within the library spectra due to the high amount of bond data provided and the spectral overlap that occurs. Other drawbacks of the instrumentation have been evaluated by Goncalves *et al*.,[19] showcasing 8% of samples tested (n = 11) did not match qualitative analysis provided by HPLC-HRMS (High Performance Liquid Chromatography – High Resolution Mass Spectroscopy) and has showcased that FT-IR analysis is limited to powder and tablet type matrixes, being unsuitable for blotters, cannabis and mixed/low content substances due to insensitivity.[20]

Quantification has been shown to be possible with IR for a variety of illicit drug compounds, typically using chemometric analysis but this is mainly for specialist applications.[21-23] Mass drug testing will be typically performed by comparing each

spectrum with a library. SWGDRUG (Scientific Working Group for the Analysis of Seized Drugs) produce and distribute their own illicit drug spectral library for FT-IR analysis,[20] the use of which has been showcased within various illicit drug analysis.[24-26] The main issue with complex samples such as cocaine, is that a spectral library will most likely not contain a wide variety of cocaine spectral data mixed with common adulterants / diluents as binary and tertiary mixtures, so another method must be used to analyse complex mixtures, such as spectral subtraction.[27]

1.1.3. Gas Chromatography – Mass Spectrometry

Another technique used for off-site testing is GC-MS. GC-MS is hailed as the 'gold-standard' for illicit drugs analysis allowing chromatographic separation of volatile compounds to then be analysed *via* mass spectrometry, producing 'fingerprint' spectral data specific to each compound (in the case of hard ionisation sources such as EI (electron ionisation)).[8] This allows each compound to be compared to a MS (mass spectrometry) spectral library for identification based on their fragmentation pattern as well as quantification of compounds based on the response of the instrument against reference material.[12] However, this spectral data cannot differentiate between the free-base and salt-form of a compound and can typically struggle to resolve between near identical structural isomers without the use of a specialist column.[28] Other sources of ionisation can be used e.g. soft ionisation sources such as ESI (Electrospray Ionisation) / APCI (Atmospheric Pressure Chemical Ionisation) typically allow for minimal fragmentation of analytes to allow the detection of the molecular ion fragment weight.[8]

GC-MS specifically has a disadvantage for non-volatile compounds as these compounds will not be separable using GC (Gas Chromatography). This can be an issue for illicit drug samples, as diluents such as various sugars, which have been used in cocaine samples since the 1970's and used as tablet fillers, are not GC active and require derivatisation.[29, 30]

GC-MS has proven useful for on-site laboratory analysis but has a significant disadvantage for off-site analysis. The instrumentation can be costly dependant on the detector, not portable as they are typically heavy and fragile, require a constant gas supply, require dilution during sample preparation and should be operated by a trained individual. However, portable GC units are available and methods have been developed

within the literature for its use. Fiorentin *et al.*,[31] adapted the validated screening method for use on a portable GC-TMS (Gas Chromatography – Toroidal ion trap Mass Spectrometry). GC-TMS is a portable unit with a toroidal mass spectrometer containing a battery-operated self-contained unit, light enough to carry by hand and contains helium cylinders to operate the GC. The main issue with this unit is the low resolution of the mass spectrometer, with studies reporting a minimum mass resolution (Δm) of 0.32.[32] Δm is the minimum difference that two MS peaks need in order to be baseline separated by the instrumentation with a common quadrupole mass spectrometer obtaining a mass resolution of Δm = 0.1.[33] Due to this low-resolution, quantification could not occur using GC-TMS but is perfectly suited for drugs screening using this method**.** Similar comparisons were made with LC-MS/MS (liquid chromatography tandem-mass spectroscopy) during the study and found an average accuracy of 90.1% with cocaine and four adulterants.

1.1.4. Nuclear Magnetic Resonance Spectroscopy

NMR is an efficient analytical technique for structural elucidation of molecules which has been applied to the identification of illicit drugs in a number of methods.[34, 35] NMR has the benefit of also being a quantitative technique and there is a large number of applications for this technique to quantify illicit drugs.[36-39]

Typically, within laboratory settings, analysis by NMR is performed using high field instrumentation, which are costly, require the frequent use of liquid gases for cooling and are not portable.[40] Benchtop NMR spectrometers use a permanent magnet so there is no need for gases, reducing the operating cost of the instrument which allows the instrumentation to be portable and cheaper to purchase and maintain.[40]

The detection of illicit substances using NMR spectroscopy has been outlined by Antonides *et al.,*[41] and aims to bridge the gap between rapid and simple off-site testing with the improved analytical performance of on-site analysis. This system uses a low field benchtop NMR system to analyse illicit drug samples and compares the spectral data acquired to a database using partial least squares analysis (PLS). PLS is a form of statistical regression analysis which compares two different matrixes of data. This can outline to the user the closest matching spectra with a score of spectral similarity, allowing the identification of compounds to occur. A varying number of scans can be used during analysis, to provide a greater sensitivity for the detection of compounds

samples with very little material. Analysis time is roughly three minutes for bulk samples, with less concentrated samples requiring 30 minutes. For herbal samples which need extraction, the analysis time is roughly fifteen minutes with *circa* five minutes of sample preparation. The longer the analytical run time, the more scans that are recorded during analysis. The greater number of scans improve the signal-to-noise ratio (SINO) of the spectra which may allow smaller peaks to be identified, allowing more chance of a positive identification to occur. If the sample analysed did not match any of the 302 compounds in the reference spectral library to a sufficient match score (> 83.8%) then the class of the compound will be reported to the user, allowing a partial identification for novel compounds. Providing a match score differs from most of the current literature which either compares the two spectral data by eye or uses another analytical technique to identify the samples first. In this study performed by Antonides *et al.*,[41] 432 seized drug samples were analysed using the NMR system and compared to GC-MS analysis. Out of the samples which produced an analytical signal from the GC-MS, 93% matched the result of the NMR system and 6% showed partial agreement, showing reliability within the qualitative analysis from the instrument. The overall sample preparation is simple; 5-10 mg of bulk sample is dissolved in deuterated solvent and filtered into an NMR tube. Herbal samples required grinding, sonication for ten minutes then filtering directly into an NMR tube for analysis. Due to the easy to use software and simple sample preparation, this technique can be employed by users that are typically untrained chemists, allowing on-site analysis to be more accessible.[42]

Further work carried out by Hussain *et al.,*[42] analysed 20 seized illicit tablets, 15 of which were quantitatively analysed using the 60 MHz low field qNMR (quantitative NMR) drugs detector described previously by Antonides *et al*.[41] Two methods were employed, a manual approach using simple linear regression and an automated approach which used PLS analysis to first identify the compound and to select the integral used for linear regression. Both approaches used deuterated solvents which were absent of internal standards. The results from both methods were compared with results obtained *via* GC-MS analysis, showing good agreement between the three methods, with the average content of MDMA (methylenedioxymethamphetamine) in tablets reported as 42.6%, 45.9% and 44.0% (w/w) for the manual, automated and GC-MS methods respectively. The LoD and LoQ reported were 5.5 mg/mL and 16.5 mg/mL respectively, which is more than adequate for routine “Ecstasy” tablet analysis

considering the average seized tablet reported during this study was 193.0 mg/tablet and is typically between 84-160 mg/tablet across Europe.[43]

To the writer's knowledge, benchtop-NMR analysis of illicit cocaine samples has not been previously studied and there is a clear gap to create a method for analysis using a more robust and potentially field deployable alternative to high-field NMR. A full assessment of the current literature of qNMR analysis is performed in section 4.1.2 - qNMR Literature Examples. Cocaine specifically is the choice of focus due to the wide range of purities and potentially harmful adulterants typically identified.[3, 44]

1.1.5. Ion Mobility Spectroscopy

Ion mobility spectroscopy is a technique used to separate ions in an inert gas, based on their collision cross-section, decreased mass and charge.[14] This technique is used for a wide range of applications: explosives,[45] chemical warfare agents,[46] environmental,[47] biomedical and clinical analysis.[48] Pharmaceutical analysis also includes quality assurance / quality control (QA / QC) testing, since the technique has a low LoD (ng). Contaminants will be detected during sample analysis or from swabs which are used to check if instrumentation is clean.[49] Typically, IMS instrumentation in laboratories are custom built, with increased resolving power over commercial instrumentation.[49]

Figure 1 shows the main components of an IMS instrument. The swab is first placed within the inlet chamber which is heated to 260 ^{o}C, vaporising the adhered components. The gaseous atoms are then transferred to the ionisation chamber by an inert gas, then ionised using corona discharge (CD). This method allows both positive and negatively charged ions to be detected by the technique. The shutter acts as a barrier between the ionisation region and the drift tube. Once sufficient ionisation has occurred, the shutter opens and the ions are transferred to the drift tube by gas flow. The size and width of the peaks can be increased by increasing the time in which the shutter is open, effectively allowing a greater number of ions into the drift tube, across a longer time period.[50] In the drift tube, the separation of ions occurs, with the gas flowing effectively towards the ions as an electrical field carries the charged ions towards the Faraday plate. Gaseous ions in an electrical field will accelerate until they collides with a neutral ion, each gaseous ion then accelerates with a unique ion velocity, resulting in a characteristic drift time for each ion.[51] Once the ion reaches the Faraday plate, a small electrical current is generated, amplified and then recorded by

the software. The time from when the shutter is opened to when an ion reaches the plate is labelled as the drift time for the component.[51]

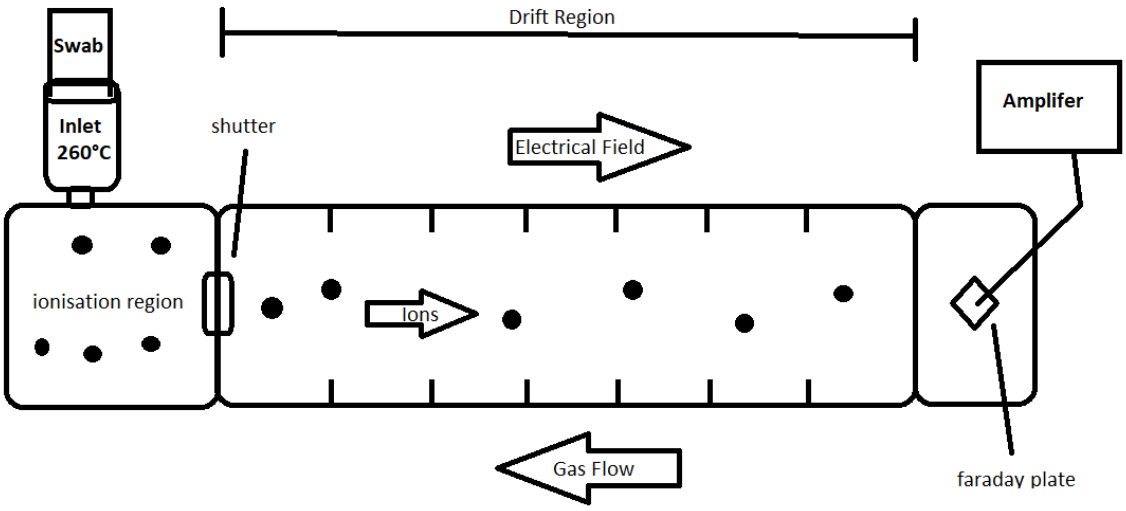


Figure 1 - The principal components of IMS

Typical advantages of IMS are its low running costs, minimal sample preparation, rapid analysis time, very high sensitivity and the inherent ease of use, making it suitable for non-trained analysts.[14] However, analysis can suffer from a number of potential issues: matrix effects within samples, changes in environmental conditions (temperature, pressure and humidity), detector saturation, poor mixture analysis and low resolving power resulting in low selectivity and potential false positives.[14, 52] To account for the small changes in temperature, pressure and humidity in a normal working environment, K_0 values are used (opposed to drift times), which are the ratios between the positive calibrant nicotinamide and the analyte peak, to account for potential changes in conditions.

Overall, the separation and identification of analytes using IMS is based on the analytes mobility when traveling through the drift tube. Analyte mobility (K) is defined by Equation 1.

$$K = \frac{3}{16}\sqrt{\frac{2\pi}{\mu k_B T}} \times \frac{ze}{N\Omega}$$

Equation 1 - Analyte mobility (K) - μ = reduced mass of ion-gas pair - k_b = Boltzmann constant - ze = analytes charge - T = Gas Temperature - Ω = collision cross section - N = gas number density

For analysis performed on the same instrument with the same physical conditions (temperature, pressure etc.), the terms which show the difference in analyte mobility

are reduced mass and the collision cross section of the respective analyte. Thus, separation is based on mass and overall shape of the molecule.[50, 53]

A comprehensive overview of the current literature that performs illicit drug analysis using IMS, has been overview in a later chapter, section 5.1.1 - Literature Examples.

1.2. Cocaine

Cocaine (Figure 2) was first isolated in 1855 by Gaedcke *et al*.[54] It was extracted from the leaves of the coca plant after Spanish colonists noticed indigenous south Americans chewing these leaves to enhance endurance.[55] Throughout the late 1800's further research was conducted on this new compound for its uses both medically and recreationally. Koller *et al*.,[56] demonstrated its uses for optometry as a pupil dilatant and various companies started incorporating the compound into wine / cola drinks.[55] From this point cocaine was being produced in a number of forms including powders, cigarettes and injectable liquids and was being sold as a 'cure all' stimulant.[55] It wasn't until the early 1900's, when the negative effects of cocaine use were discovered, that the use of cocaine was restricted to medicinal use only (1922).[55]

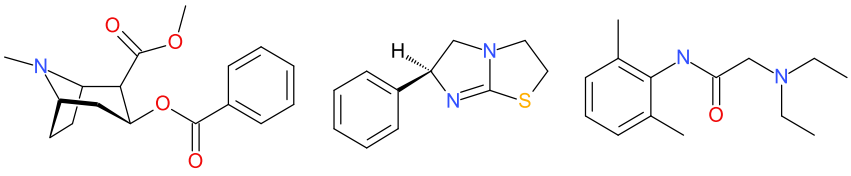


Figure 2 - Structures of cocaine, levamisole and lidocaine

Due to the complex alkaloid structure of cocaine, it is extracted from the leaves of the *Erythroxylum Coca* plant which grow easily in countries with hot climates such as Peru and Colombia,[55] opposed to being synthesised. However, the synthesis has been accomplished by a number of chemists from commercially available and simple precursors.[57, 58] The leaves are mixed with kerosene and sodium bicarbonate in order to extract the cocaine hydrochloride as a paste, which is then dried and transferred to clandestine laboratories for further clean-up.[55]

Cocaine induces an effect on the user by blocking dopamine transporters within the brain. Since dopamine is no longer recycled within the synapses, a build-up occurs over activating the dopamine receptors and producing feelings of europhia.[59, 60] The speed

and the severity of the effects are mainly influenced by the route of administration. The free-base form of cocaine, often named as 'crack', is often smoked and almost instantly has an effect on the user due to the efficient transfer across the alveoli into the blood within the lungs, lasting around 30 - 45 minutes.[60] The hydrochloride salt form is either taken intranasally giving the user a reduced effect peaking between 25 - 50 minutes but lasting longer overall, or can be administered intravenously, with a very similar duration and peak to smoking.

Typical doses of cocaine include 20 - 100 mg intranasal route, 10 - 50 mg intravenous route and 50 - 200 mg when smoked, with heavy users of cocaine typically requiring a higher dose as a tolerance to the drug is built up.[39] This is a concern as users may opt to take a higher dose of cocaine in order to obtain a consistent effect, which may put themselves at risk of an overdose, which can potentially cause life threatening complications such as seizures or fatal cardiac arrythmia.[39] Cocaine is normally heavily adulterated due to the suppliers desire to maximise profits, with purity of typical cocaine samples reported on average between 52 – 83% within European seizures.[61]. Other smaller scale studies have identified a wider range of sample purities e.g. 1 – 95% with an average of 60%.[62] Due to inconsistent purity levels it may cause the user to overdose, if one batch is in higher purity than what the user normally takes. Purity levels within this context refer to the percentage of the desired form of cocaine (base/salt) within the total sample. This is either reduced through intended adulteration, from the inclusion of other active pharmaceutical compounds or from other diluents e.g. carbonates / borates.[63] Another way in which purity is reduced is during the initial production of the cocaine, either from the inclusion of the other alternative form of cocaine, or from the inclusion of residual solvents.[64] Quantitative analysis of seized samples containing cocaine should be performed in order to alert the public to any batches which are of a higher purity, thus making them more cautious and hopefully prevent fatalities.

Cocaine toxicity affects every organ within the body; however, cardiovascular system experiences the greatest effects.[65] In the case of an overdose, urgent treatment is required for tachycardia, dysrhythmia, hypertension and other heart related issues.[65] With cocaine being the second most used drug within Europe, only behind cannabis, where 2.5 million 15 – 34 year olds used cocaine across 2022, there is a large number

of users who may experience adverse effects or even death.[61] Within Spain, cocaine was involved within 52% of drug related deaths in 2021,[61] and similarly, in England there was 857 deaths from cocaine use reported in 2022, 2.0% higher than the previous year and seven times greater than 2011.[66] This further showcases the need for harm reduction programmes to monitor and assess the purity of cocaine samples, regionally and across events. The LD_{50} reported for cocaine is to be 95.1 mg/kg (mice)[67] with the average human weight in England reported to be 85.4 kg for men and 72.1 kg for women,[68] the calculated lethal dose is between 6.86 – 8.12 grams, which is notably larger than the described averages doses (20 - 100 mg intranasal, 10 - 50 mg intravenous and 50 - 200 mg smoked).[39] This is overshadowed by the number of potential interactions with drugs, other substances and potential adulteration present within samples, on patients with previous history of conditions which causes lethality of cocaine.[69, 70]

1.3. Cocaine Adulteration

Adulterants within cocaine samples are also a concern as competitive interactions between cocaine and other substances may increase risk of medical attention being needed. Levamisole (Figure 2), is the most common adulterant found in cocaine samples that were analysed from Spain.[44] It is typically used because it is cheap and appears identical to cocaine but is twice as dense and mimics nasal numbness giving a false sense of high potency.[44, 71] Although levamisole typically only gives the user mildly adverse effects, there has been cases of toxicity from the use of cocaine adulterated with levamisole, causing 79% of all cases of methemoglobinemia within the USA, a serious condition which can reduce the amount of oxygen within the blood.[72] Similarly lidocaine (Figure 2), which is a known cocaine adulterant, has been found to be the cause of ventricular arrhythmia which led to the subsequent death of a 23 year old, after administration of the cocaine / lidocaine mixture.[73]

Identifying the common adulterants within cocaine samples can alert the public to the dangers of specific batches or inform medical staff to help administer the correct treatment. A selection of the most commonly used adulterants of cocaine are shown in Figure 3, derived from an eight year study from illicit cocaine seizures in Spain, (2007 - 2014) performed by Núñez et al. consisting of 8644 samples in total.[44]

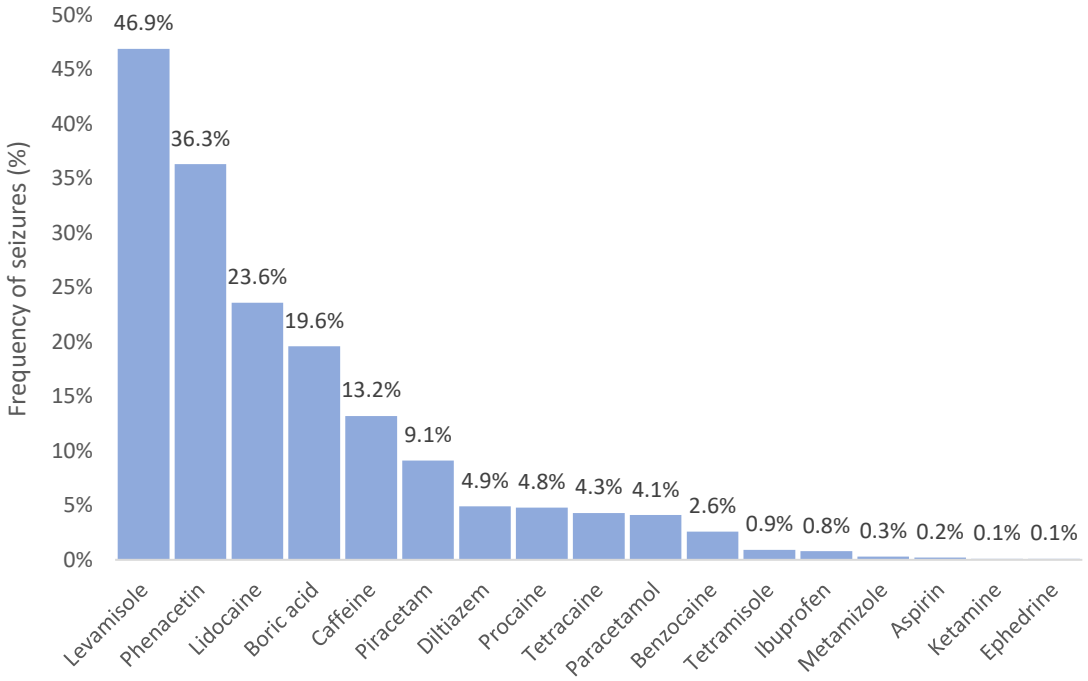


Figure 3 - Most prevalent cocaine adulterants seized from across Spain (n = 8644, 2007 - 2014).[44]

Another study performed by Broséus *et al.* analysed 6586 cocaine samples seized across a nine-year period in western Switzerland.[74] The prevalence of adulterants for this study is shown in Figure 4.

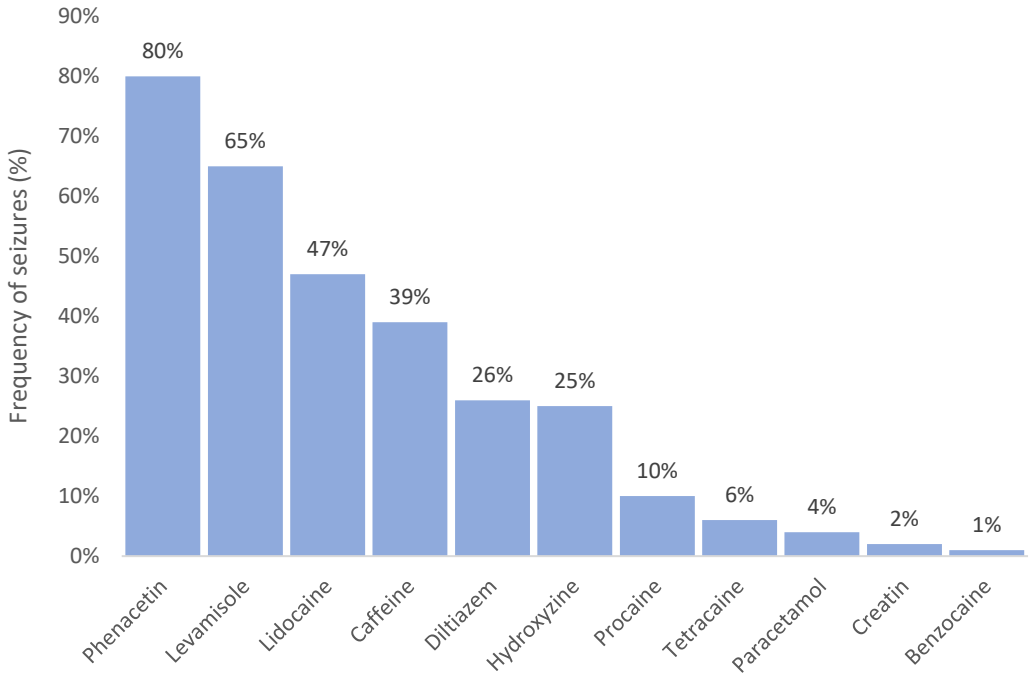


Figure 4 - Most prevalent cocaine adulterants seized from across western Switzerland (n = 6586 , 2006-2014) – Study does not report at higher significant figures.[74]

By comparing these two large-scale studies it is observed that the three highest prevalent cocaine adulterants correlate between Spain and Switzerland, with the most prevalent compound shifting from levamisole to phenacetin. The Spanish study identified 17 different adulterants opposed to 11 identified across Switzerland. Despite the differences, the wide range of potential adulterants showcased within these studies shows cocaine adulteration as dynamic and potentially dangerous for the end users due to the added risk of complications for adulterant toxicities.[74]

Focusing on the region of Manchester, Antonides *et al.* analysed a wide range of illicit samples seized and tested at a live festival event.[41] Out of the 85 cocaine samples seized, 22 were adulterated, the details of which are shown in Figure 5.

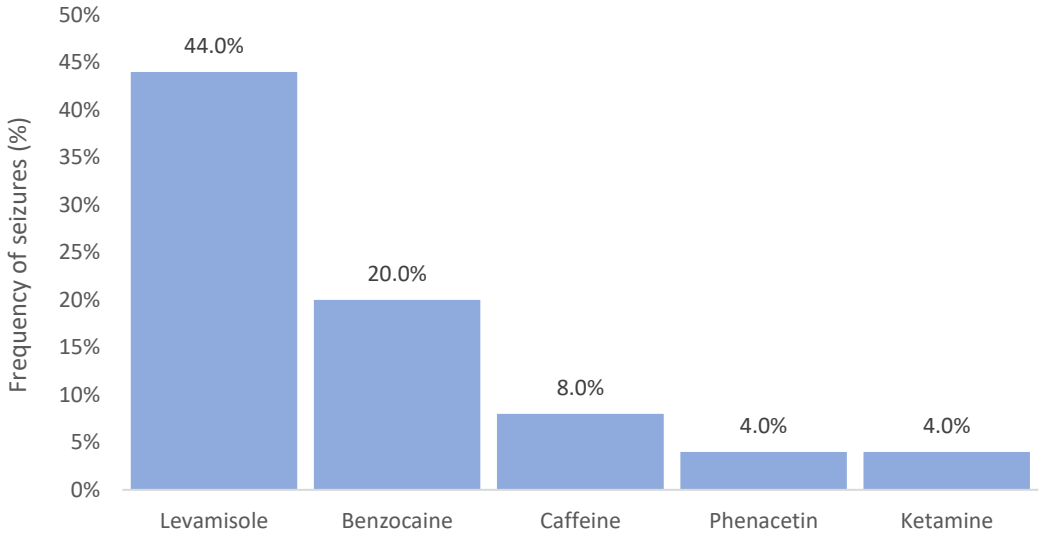


Figure 5 - Adulteration prevalence identified for cocaine samples tested at a live Manchester based festival in August 2018 (n = 22).[41]

Only a small range of adulterants were identified. However, this is due to the significantly smaller sample range between this study and the two wide range studies presented. The unusually high prevalence of ketamine has been noted in full sample prevalence (17.0%) and is not assumed unusual within the study.

Similar results are showcased within the Greater Manchester Trends reports, which are based on qualitative interviews backed up by qualitative and quantitative analysis of illicit samples, which reports on illicit drug trends within the area of Greater Manchester.[75, 76] In 2021, phenacetin was identified as the main cocaine adulterant and in 2022 only benzocaine, caffeine and levamisole were identified as cocaine adulterants.[75, 76]

1.4. Aims and Objectives

The main aims and objectives of this thesis are to compare and adapt analytical techniques which are frequently used for illicit drug bulk sample analysis. This may indirectly promote harm-reduction by providing more robust, alternative methods of analysis.

A direct comparison between GC-MS, BT-NMR and FT-IR to outline strengths and weaknesses for each technique, allowing analysts to make better informed decisions on their choice of technique for bulk sample testing.

Production of a BT-NMR method to quantify cocaine within adulterated samples, to provide comparable data to GC-MS. Allowing analysts a lower cost and portable alternative for on-site illicit drug analysis.

Adaptation of a commercial trace analysis IMS instrument for bulk sample illicit drug analysis. This allows another easy to use, cheap, portable technique to be used as a field deployable analytical technique.

Showcase characterisation and synthesis data for an illicit cathinone, “putylone” to enable other analysts to identify during illicit drug screening, allowing alerts and reports to welfare staff to be made.

2. Chapter 2 – Experimental

2.1. General Information

2.1.1. Seized Samples

All samples were provided by Greater Manchester Police (GMP), *via* the MANchester DRug Analysis and Knowledge Exchange (MANDRAKE) partnership in accordance with Manchester Metropolitan University's Home Office licence, allowing the possession, supply and production of controlled drugs (Schedules 1 – 5) under the Misuse of Drugs Act (1971) and Misuse of Drugs Regulations (2001). All operations (receipt, possession, analysis and destruction) were undertaken in accordance with agreed Standard Operating Procedures (SOPs) and the Memorandum of Understanding (on file in Manchester Metropolitan University's (MMU) Legal Department) between the institution and GMP, using a Single Point of Contact (SPoC), to ensure a traceable chain-of-custody and legal compliance with Home Office licence requirements.

2.1.2. Standard Equipment

Weighing out of materials was performed using a Mettler Toledo AB104-5 analytical balance calibrated using internal weights before every set of measurements. Sample dilutions were performed using an SGE Analytical Science eVol XR Electronic Syringe, coupled with a 100 µL and 1 mL syringe, both calibrated as per manufactures guidelines. All sonication was performed on a Grant XUAB3 ultrasonic bath (Grant Instruments, Cambridge, UK).

2.1.3. Solvents

All solvents were analytical grade quality purchased and used without further purification. Methanol (MeOH) (>99.9%), chloroform (CH_3Cl) (>99.8%), cyclohexane (>99.8%), diethyl ether (>99.0%), acetone (>95.0%) and dichloromethane (>99.8%) was obtained from Fisher Scientific (Loughborough, UK). Deuterium oxide (99.9% D), $CDCl_3$ (99.9% D), dimethyl sulfoxide-d_6 (DMSO-d_6) 99.96% D with 0.03% v/v TMS) and benzene (>99.0%) were obtained from Sigma-Aldrich (Gillingham, UK).

2.1.4. Consumables

Sample filtering was achieved using HENKE-JECK 1 mL syringe (Henke Sass Wolf, Tüttlingen, Germany), to pass solution through a 0.45 µm 13 mm PTFE

(polytetrafluoroethylene) syringe filter (Fisherbrand, Loughborough, UK). For GC-MS analysis, prepared solutions were stored in a 1.5 mL (32 x 11.6 mm) amber glass, short thread vial secured (Agilent Technologies, Cheadle, UK) with a 9 mm blue cap (Agilent Technologies, Cheadle, UK). Samples for benchtop NMR analysis were prepared using 5 mm ColorSpec® NMR tubes (Sigma-Aldrich, MO, USA) rated for 200 MHz spectrometer usage. Samples for high-field NMR analysis were prepared in DEU-500 Boro 5.1 NMR Tube (Asynt, Cambridgeshire, UK) rated for 500 MHz spectrometer usage.

2.1.5. Analytical Reagents

Cocaine hydrochloride, ketamine hydrochloride, phenacetin (>98.0%), procaine (>97.0%), benzocaine, tetramisole hydrochloride (>97.0%), caffeine (>97.0%), lactose monohydrate (>98.0%), diphenhydramine (>99.0%), boric acid (>98.5%), aspirin (>98.0%) and paracetamol (>99.5%) were obtained from Sigma-Aldrich (Gillingham, UK). Methyl stearate (99.0%) was obtained from Alfa Aesar (Lancashire, UK). Cocaine freebase was purified from previously seized samples. TMSP (trimethylsilylpropanoic acid) (98.0%+ D) and TMS (Tetramethylsilane) (99.9%+ D) was obtained from Thermo Scientific (Cambridge, UK). 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one hydrochloride and 1-(1,3-benzodioxol-5-yl)-2-(ethylamino)pentan-1-one hydrochloride were obtained from LGC Standards (Teddington, UK).

2.1.6. Synthesis Reagents

3’,4’-(methylenedioxy)butyrophenone (99.5%+), bromine (>99.99%), hydrobromic acid (48.0% aq.), Hydrogen chloride solution, (3M in cyclopentyl methyl ether (CMPE) and N-propylamine (>99.0%) was obtained from Sigma-Aldrich (Gillingham, UK).

2.2. Instrumentation Utilised

2.2.1. FT-IR Analysis

Between 1- 2 mg of sample was clamped onto a FT-IR diamond for analysis. All infrared analysis was performed on a Nicolet iS5 (Thermo Scientific, Cambridge, UK) utilising OMNIC 8.2.388 Spectral acquisition was achieved using 16 scans with 2 cm^{-1} resolution across 4000 – 650 cm^{-1}, reacquiring background spectra every 2 hours. Spectral data was matched against two spectral libraries, SWGDRUG 3.9 (August 2019, VA, USA)[77] and an in-house library.

Spectral subtraction was achieved through the OMNIC 8.2.388 software for the Nicolet iS5. Each binary mixture was first library searched to give one component. Reference spectra for the identified component was then subtracted using a subtraction factor calculated by eye. The resulting spectra was then re-analysed against the libraries to identify the second component. The process was repeated to confirm the presence of the original component minus the second component interference within the spectral data.

2.2.2. NMR Instrumental Methods

Qualitative ^{1}H NMR analysis was performed solely on a Pulsar benchtop NMR spectrometer (Oxford Instruments, Abingdon, UK) utilising a custom version of Spinflow 2.3.0, operating at a frequency of 59.3 MHz. Before each analysis, the instrument was shimmed automatically ensuring at most 0.5 Hz full width at half maximum (FWHM) of a 12% TMS in CH_3Cl reference standard.

Quantitative ^{1}H NMR analysis was performed on both a Pulsar benchtop NMR spectrometer mentioned previously and a JEOL 500 High field NMR (JEOL U.K., Hertfordshire, UK), utilising Delta (Version 5.0, JEOL UK, Hertfordshire, UK) operating at a frequency of 500.15 MHz. The JEOL 500 was shimmed to each individual sample using AUTOLOCK set to the respective solvent peak.

"Drugs detector" qualitative analysis: Spectral acquisition for bulk powders were collected using 16 scans, relaxation delay of 5 seconds, 32k data points across a filter width of 5000. For herbal and samples which contained very little material, more scans were acquired to improve SINO (64 and 128 scans respectively). After the sample was inserted, an automated process would commence, engaging the lock onto the DMSO-d_6 frequency before spectral acquisition. Following acquisition, the data was processed automatically using an MestReNova script (Version 9.1, Mestrelab Research, Santiago de Compostela, Spain) consisting of, apodization along T_1, phase and baseline correction before zero filling to 128k and phase correction by magnitude to make comparable between samples of varying concentration. The spectra were then saved as .csv files and then processed through Matlab (The Mathworks Inc, Cambridge, UK) developed in house, which removes the solvent shifts in the spectra and uses a minimum distance classifier to compare the multivariate differences between reference and sample spectral data. The match score was then determined by one

minus the Pearson correlation score, to which the threshold is set at 0.838 for a reliable reading. This value has been set empirically but typically values outputting a match score more than 0.7 are of "strong correlation", so 0.838 was chosen to include only results with a high degree of reliability.[41]

qNMR spectral acquisition: All samples, unless stated otherwise, were held within the probe of the instrument for 5 minutes before acquisition to ensure samples become acclimated with the temperature of the probe. Triplicate analyses were performed using 8 scans, relaxation delay of 45 seconds, 32768 data points with a width of 5000 Hz. The samples analysed using the JEOL 500 spectrometer, were not analysed with spinning enabled, to ensure consistency between instrumentation.

2.2.3. GC-MS Instrumental Methods

All GC-MS analysis was performed on 7890B GC coupled to a 5977B MSD (Agilent Technologies, Cheadle, UK). Sample chromatographs for general screening were processed through MassHunter Unknowns B.08.00 (Agilent Technologies, Cheadle, UK) and compared against three mass-spectral libraries: NIST14 (Gaithersburg, MD, USA), SWGDRUG 3.9 (August 2019, VA, USA)[78] and an in-house library, before confirmation with reference material. For sample quantification, chromatographs were processed through MassHunter MS Quantitative Analysis B.08.00 (Agilent Technologies, Cheadle, UK).

GC-MS Method (general screening): 0.5 µL injection split 50:1 with an inlet temperature of 265 ^{o}C and a split flow of 20 mL/min a purge flow of 3 mL/min. Temperature started at 175 ^{o}C ramping to 300 ^{o}C at 30 ^{o}C/min with a hold time of 1 minute, for a total run time of 5.167 minutes. 1.2 mL/min of He through a HP5-MS (30m x 250 µm x 0.25 µm) column into the MS through a 300 ^{o}C transfer line. MS was set to scan across 40 – 550 amu with a solvent delay of 2.5 minutes. Extended method increased the hold time to 10 minutes.

GC-MS Method (cocaine quantification): 1.0 µL injection split 50:1 split with an inlet temperature of 280 ^{o}C and a split flow of 20 mL/min a purge flow of 3 mL/min. Sample wash contained 3 post and prewashes in methanol followed by 3 post and prewashes in hexane. Temperature starts at 180 ^{o}C, holds for 5 minutes before ramping to 290 ^{o}C at 25 ^{o}C/min with a hold time of 0.6 minute, for a total run time of 10 minutes. 1.2

mL/min of He through a HP5-MS (30m x 250 µm x 0.25 µm) into the MS through a 300 ^{o}C transfer line. MS was in SIM (selective ion monitoring) mode with the respective groups and ions outlined within results and discussion.

2.2.4. IMS Instrumental Methodology

All IMS spectroscopy analysis was performed on a IONSCAN 600 (Smiths Detection, Hemel Hempstead, UK) running software 9824012.5.2.11. All of the default settings were used for analysis. Each sample plasmagram consisted of 22 scans, (11 positive polarity and 11 negative polarity), which the instrumentation refers to as segments. Throughout these segments, the front inlet temperature ramps from 135 ^{o}C to 240 ^{o}C. Each of these segments is combined and gaussian peak picking within the software identifies peaks over a set threshold of 200 intensity units. K_0 values are calculated within the software, reducing the variability from changes in pressure and temperature, through comparisons with the nicotinamide calibrant peak. Attempts to alter most parameters within the instrument resulted in little change to the K_0 values as a result of this. Library compound matching is achieved by matching K_0 values from identified peaks, with known reference material in the active control parameters (CP) set. The CP set is the source of library matches and instrumental parameters. Before every analysis, the instrument was "verified", which consisted of spiking a swab with a test solution provided with the instrument and analysed automatically through the software. This provides a test to ensure the results from the instrumentation are reproduceable and that it is functioning correctly.

2.3. Sample Analysis

2.3.1. NMR Qualitative Sample Analysis

Bulk Powders: 10 - 15 mg of sample was dissolved in 0.6 mL of DMSO-d_6 from an eVol XR syringe sonicated for 5 minutes, syringe filtered and transferred to an NMR tube (14.3 – 21.4 mg/mL) for analysis using the benchtop NMR.

Herbal Samples: 50 – 100 mg of sample was dissolved in 2.0 mL of DMSO-d_6 from an eVol XR syringe, sonicated for 20 minutes, syringe filtered and transferred to an NMR tube (25 – 50 mg/mL) for analysis.

Tablet Analysis: All tablets seized from the same sample were crushed with a pestle and mortar and combined to ensure homogeneity. 10 - 15 mg of the resulting powder was analysed the same as bulk powder samples.

2.3.2. NMR Quantitative Analysis

T_1 Relaxation Determination Experiment: Relaxation times of specific ^1H environments were calculated using an inversion-recovery experiment consisting of the application of a 180° pulse, a variable time delay (τ) and then a subsequent 90° pulse. Values of 0.1 to 60 seconds were used for τ. Spectra were acquired on the benchtop Pulsar system using 8 scans relaxation delay (RD) of 60s, in addition to 2 dummy scans. Each experiment took 160 minutes. Spectra were processed though MestReNova (Version 9.1, Mestrelab Research, Santiago de Compostela, Spain) to calculate the relaxation time (T_1) of specific peaks within each sample. Each sample was prepared in the appropriate deuterated solvent as required for quantification at a typical qualitative sample concentration (20 mg/mL). Samples containing binary mixtures were produced as a 50:50 % wt. (10 mg/mL each component).

External Calibration: A stock solution of TMSP in D_2O (1.0 mg/mL) was produced and used as the solvent for extraction for the calibration points and the samples. A blank calibration point was prepared by using only the TMSP solution with no analyte extraction. Five further calibration plots were produced between 5.0 – 25.0 mg/mL by weighing out accurately the required amount of cocaine hydrochloride reference material and adding 1.0 mL of TMSP solution using an eVol XR syringe. These solutions were then sonicated for 10 minutes, syringe filtered and then transferred to high field NMR tubes for analysis. Each sample was ground with a pestle and mortar to maximise homogeneity before accurately weighing 20 mg of sample for extraction into the TMSP solution. This solution was then sonicated for 10 minutes before being syringe filtered and transferred to high field NMR tubes for analysis (20 mg/mL). The analysis for cocaine freebase was identical except the use of $CDCl_3$ as a solvent and TMS as an internal standard, which was spiked using volume rather than mass, using an electronic syringe. Adulterant calibrations were prepared the same way in their respective solvents and internal standard. The TMSP / TMS peak was used both as a reference peak (0.0 ppm) and to calculate analyte / internal standard ratios for analysis.

Single point calibration (External): Once the calibration curve was identified as linear for both cocaine hydrochloride and freebase, only a single reference point standard (20 mg/mL) spiked with the TMSP solution (1 mg/mL) used *in lieu* of a full calibration series.

Internal ratio calculation: The TSP peak was used both as a reference peak (δ0.0 ppm) and to quantify using integral ratios. The ratio of the integrals was used between the environment of interest and the spiked TSP peak and sample content was calculated using Equation 2.

$$Content\ (\%) = \frac{Int\ (x)}{Int\ (IS)} \times \frac{N\ (IS)}{N\ (x)} \times \frac{MM\ (x)}{MM\ (cal)} \times \frac{W(cal)}{W(x)} \times 100\%$$

Equation 2 - qNMR internal ratio quantification. N = number of hydrogens, MM = molecular mass (g/mol) w = mass (mg)

Reference standard solubility experiments: Solutions of varying concentrations between 0 – 10 mg/mL of internal standard in respective NMR solvent were produced and sonicated for 10 minutes. Solutions were split in two, one half transferred directly to a tube for analysis, the other half syringe-filtered and then transferred. The amount of volume in each NMR tube was pipetted equally to ensure consistent shimming. The spectral acquisition was not performed in triplicate and was not held in the probe any longer than the stated spectral acquisition time.

Number of scans: Multiple ^{1}H NMR experiments on the same reference standard tube was used with varying number of scans, between 1 – 128 were acquired to select the number of scans ideal for quantification. The SINO's were calculated using an automated script in MestReNova (Version 14.0, Mestrelab Research, Santiago de Compostela, Spain). The spectral acquisition was not performed in triplicate and was not held in the probe any longer than the stated spectral acquisition time.

Reference stability determination: A sample containing reference material (20 mg/mL) was produced using the method for external samples. This sample remained at room temperature and reanalysed multiple times across 14 days using a JEOL 500 NMR spectrometer.

2.3.3. GC-MS Qualitative Analysis

Bulk Powders: 5 mg of sample was extracted into 5 mL of eicosane (99.0%, Sigma-Aldrich, Gillingham, UK) solution (200 µg/mL) in MeOH, which was used as an internal standard for the analysis. The sample was then sonicated for 5 minutes before dilution, 1 in 10, directly into a GC vial (100 µg/mL) for analysis.

Herbal Samples (and cannabis resin): 25 - 50 mg of herbal material (or cannabis resin) was crushed using a pestle and mortar, then extracted into 10 mL of eicosane solution (200 µg/mL) in MeOH, which was used as an internal standard for the analysis. The sample was then sonicated for 20 minutes before dilution, 1 in 10, directly into a GC vial (0.25 – 0.5 mg/mL).

Tablet Analysis: All tablets seized from the same sample were crushed with a pestle and mortar and combined to ensure homogeneity. 5 mg of the resulting powder was analysed as per the procedure for bulk powder samples as noted above.

Derivatisation for Non-volatile Samples: 5 mg of sample was dissolved in 5 mL of MeCN. 20 µL of MTBSTFA (N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide) containing 1% TMS (tetramethylsilane) solution was added to a 200 µL aliquot, sealed in a vial and heated at 60 ºC for 1 hour. Once cooled, the reaction was quenched with excess MeOH and reconstituted in 5 mL of MeOH. The solution was then spiked with methanolic eicosane solution (200 µg/mL) before dilution, 1 in 10, using an eVol XR syringe directly into a GC vial for analysis.

2.3.4. GC-MS Quantitative Analysis

Cocaine Quantification: A calibration stock solution containing benzocaine, caffeine, ketamine hydrochloride, tetramisole hydrochloride, procaine, paracetamol, phenacetin and cocaine hydrochloride (alternately, cocaine freebase) was accurately weighed by difference (0.1 mg/mL). Calibration stock solution was sonicated for 10 minutes before further dilution. Six calibration points between 2 – 12 µg/mL were prepared from dilutions of the stock solution and each point spiked with methanolic methyl stearate (5 µg/mL) used as an internal standard. A blank calibration point was prepared by only spiking blank MeOH with the methyl stearate internal standard solution. This methyl stearate solution was also used for column conditioning before analysis. Three extra points were produced at 33%, 50% and 66% (4 µg/mL, 5 µg/mL and 6 µg/mL

respectively) to measure the uncertainty of the calibration curve as a measure of linearity. Each sample was accurately weighed (10 mg) and extracted into methanol with sonication for 10 minutes, before a 2 mL aliquot was removed and syringe filtered. The filtered aliquot was diluted further (10 μg/mL) and spiked with methanolic methyl stearate (5 μg/mL), before being transferred to a GC vial for analysis. A QC (quality control) solution stock solution was prepared using the same method to the samples, except it was diluted to produce three evenly spaced points along the calibration curve at low, medium and high concentrations (2.5 μg/mL, 5.0 μg/mL and 7.5 μg/mL respectively), all previous injections were noted as reliable providing the following QC concentration was within 5% of the expected concentration.

"Putylone" Quantification: Separation was achieved with a capillary column (HP5 MS, 30 m x 0.25 mm x 0.25 μm) using helium as the carrier gas at a constant flow rate of 1.0 mL/min. The initial oven temperature was set to 190 ^{o}C prior to be ramped to 280 ^{o}C in 35 ^{o}C/min intervals. A hold time of 0.5 min was used at 280 ^{o}C to give a total run time of 10 min. A 0.5 μL aliquot of the sample was injected with a split ratio of 50:1. The injector was maintained at 280 ^{o}C and the GC interface temperature maintained at 300 ^{o}C. The MS source and quadrupole temperatures were set at 230 ^{o}C and 150 ^{o}C respectively. Mass spectra were obtained in full scan mode (50–550 amu; qualitative analysis) and SIM (quantitative analysis) using three specific fragment ions for each analyte. A base ion fragment was used for quantification with the remaining two ions used as qualifiers (Table 60). Stock solutions for quantification were prepared to 0.1 mg/mL and then diluted further to six concentrations between 0 – 12 μg/mL containing methyl stearate (20 μg/mL). Samples were prepared in the same manner, diluted to 10 μg/mL and spiked with methanolic methyl stearate solution, acting as an internal standard, to a final concentration of 20 μg/mL.

2.3.5. IMS Analysis

General sample dilution for IMS analysis: All reference standard analysis and street sample analysis were diluted to final mass loadings between 500 – 1000 ng. This requires dissolution of the bulk samples, dilution and drop casting a small amount (1 – 5 μL). Specific volumes are outlined in their respective descriptions. The low mass loadings (500 – 1000 ng) required for analysis, in comparison with other techniques, (NMR: mg/mL scale, GC-MS: μg/mL scale and FT-IR: mg bulk sample) is due to the very

low LoD of IMS (Table 46) coupled with a poor linear range causing the detector to be prone to saturation.[79] Bulk sample analysis is described within the manufacturer's guidelines as "swab-to-swab" method, where a PTFE swab is used to sample powder but then transferred to another swab for analysis. There is no simple way to quantify the amount of substance submitted for analysis using this method, thus sample dilution is used.

Reference Material Analysis for Library Additions: 1 – 2 mg of reference material was weighed and dissolved in 20 mL of MeOH, measured out using a variable volume 10 mL piston pipette (VWR international, Lutterworth, UK). 2.5 µL was then transferred to a swab using a 20 µL variable piston pipette (Eppendorf research, Stevenage, UK) (500 ng mass loading) and allowed to dry before insertion into the instrument for analysis. 20 replicate swabs were analysed in total (5 replicates each day over 4 days) to identify a representative potential range of K_0 values for each analyte.

Sample Screening for IMS: 1 – 2 mg of sample was weighed and dissolved in 5 mL MeOH. 1.25 µL was then transferred to a swab using a 20 µL Eppendorf research variable piston pipette (1000 ng mass loading) and allowed to dry for insertion into the instrument for analysis.

Sample Swab Stabilities: To measure intra-day stabilities, 3 replicate sample swabs containing reference material (500 ng mass loading) were prepared and analysed evenly across 8 hours. To measure inter-day stabilities, 13 replicate sample swabs reference material (500 ng mass loading) were prepared and analysed in triplicate across 12 days, separately for each compound. Prepared swabs were stored at room temperature in a clean sealed container and care was taken to ensure minimal handling before analysis.

Live event analyst method testing: 10 samples obtained at a live testing event, were selected to assess 3 different strengths of the technique, 3 samples which are commonly found within the main prevalence, 3 samples which are uncommon and 3 isomers, to assess real time isomeric identification. One sample was used as a control to ensure instrumentation consistency. The samples were then prepared using the method outlined in "Sample Screening For IMS" and labelled for the duration of the assessment. Across 3 days of the event, 11 volunteer analysts were selected to process

these samples *via* IMS analysis and record the result without any interference. The analysts were unable to report back to the rest of the group their results or discuss any aspects of the test until after the event. A small selection of analysts was asked to perform repeat analysis across multiple days, to assess inter-day analysis.

Analyte limit of detection: Mass loadings of 500, 250, 50, 25, 5, 2.5 and 0 ng of reference material was pipetted onto PTFE swabs in triplicate, for analysis using IMS. The magnitude of each peak was then plotted against concentration. 3.3 times the standard deviation of the blank responses was calculated against the regression equation to identify the limit of detection for each analyte, in accordance with the ICH (International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines.[80]

2.3.6. High-Resolution Mass Spectrometry

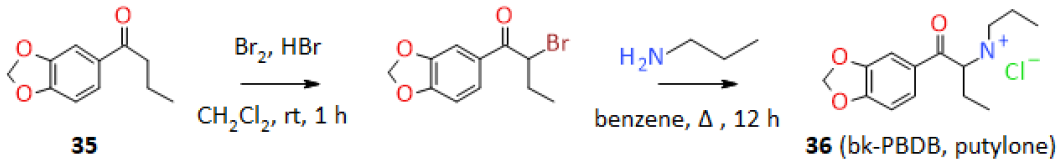
High-resolution mass spectrometry (HRMS) data was obtained on an Agilent 6540 LC-QToF spectrometer (Agilent Technologies, Cheadle, UK) running MassHunter MS Qualitative Analysis (Version B.08.00, Agilent Technologies, Cheadle, UK), in positive electrospray ionization mode.

2.4. Synthesis of "putylone"

The reference standard of 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one hydrochloride ("putylone") (**36**) was synthesized, purified and obtained as a stable, colourless powder using an adaptation of the protocols reported by Santali *et al.* and Ogawa *et al.*[81, 82]

To a solution of 3',4'-(methylenedioxy)butyrophenone (**35**, 19.2 g, 100 mmol) in dichloromethane (50 mL) was added one drop of hydrobromic acid (48% aqueous solution) and one drop of bromine. The mixture was stirred at room temperature until the bromine colour was discharged (*circa*. 30 s) and additional bromine (5.1 mL, 100 mmol total including the original drop) was introduced dropwise with stirring. The mixture was stirred for 1 h and then concentrated *in vacuo* to reveal a dark brown oil which solidified on standing to give 2-bromo-3',4'-(methylenedioxy)butyrophenone as dark brown prisms and used without further purification. Immediately after, a stirred solution of 2-bromo-3',4'-(methylenedioxy)butyrophenone (2.71 g, 10 mmol) and *N*-propylamine (1.64 mL, 20 mmol) in benzene (75 mL) was heated under reflux for 8 h

and then allowed to cool. The colourless precipitate (*N*-propylamine hydrochloride) that resulted was filtered off and washed with benzene (2 x 20 mL). The filtrate and washings were combined and concentrated *in vacuo* to give a yellow oil. The oil was redissolved in diethyl ether (25 mL), and 3 M HCl in CMPE (6.67 mL, 20 mmol) was added until the solution was acidic (pH 1). The resulting mixture was concentrated *in vacuo* and triturated with acetone to give 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one hydrochloride (**36**, "putylone") as a colourless powder (0.660 g, 23% overall yield from **35**). The synthetic route for "putylone" was summarised in Scheme 1.



*Scheme 1 – Reaction scheme for the synthesis of **36**, which was synthesised following acidification using HCl (3M) in CMPE*

^{1}H NMR (Figure 45, 500 MHz, DMSO-*d*$_6$): d (ppm) = 9.20 (br s, 1H, H12), 8.92 (br s, 1H, H12), 7.75 (dd, 1H, $^{3}J_{HH}$ = 8.3 Hz, $^{4}J_{HH}$ = 1.8 Hz, H6), 7.57 (d, 1H, $^{4}J_{HH}$ = 1.8 Hz, H4), 7.14 (d, 1H, $^{3}J_{HH}$ = 8.3 Hz, H7), 6.19 (s, 2H, H1), 5.19 (app. quin., 1H, $^{3}J_{HH}$ = 5.1 Hz, H9), 2.90 (app. q, 1H, $^{3}J_{HH}$ = 9.1, H13), 2.76 (app. q, 1H, $^{3}J_{HH}$ = 9.2, H13), 1.87-2.03 (m, 2H, H10), 1.63-1.74 (m, 2H, H14), 0.90 (t, 3H, $^{3}J_{HH}$ = 7.5 Hz, H15) and 0.77 (t, 3H, $^{3}J_{HH}$ = 7.6 Hz, H11); ^{13}C(^{1}H) NMR (Figure 47, 125 MHz, DMSO-*d*$_6$): d (ppm) = 194.0 (C8), 152.9 (C2), 148.2 (C3), 128.5 (C5), 125.9 (C6), 108.6 (C7), 107.8 (C4), 102.6 (C1), 61.3 (C9), 47.5 (C13), 23.2 (C10), 19.1 (C14), 10.9 (C15) and 8.4 (C11); ATR-FTIR ν_{max}/cm^{-1} (Figure 48): 2490–2965, 1672, 1603, 1261 and 1030; GC-EI-MS (Figure 49, +ve, 70 eV): t_R = 7.34 min; *m/z* = 149 (7.9), 121 (5.2), **100** (100.0) and 58 (12.5%); HRMS (ESI+, 70 eV) calculated for [M+Na] $C_{14}H_{19}NNaO_3$: 272.1257, found: 272.1260 (mass error = 0.95 ppm

3. Chapter 3 – Qualitative Screening and Comparison of Analytical Techniques

3.1. Introduction to On-site / Off-site Testing Techniques

Illicit substance (principally drug) screening is typically performed at / or during large events, such as festivals, to aid in drug intelligence and harm reduction. There are two ways in which illicit drugs are primarily screened, on-site and off-site. Off-site testing refers to when the drug samples are taken away from the location they're seized from and tested in a laboratory environment.[83] On-site illicit drug screening is performed at the location in which they are used (at the event).[83] The main benefit of on-site testing is the ability to start analysis as soon as possible since the samples do not have to travel to another location. The time that is saved may allow medical staff to administer the correct treatment sooner, potentially saving an individual's life. The main issue with on-site testing is the lack of standard laboratory instrumentation and equipment available to the analysts. This is mainly due to the mobility of the off-site lab setup. If an instrument is to be used, then it needs to be portable and durable enough to withstand being moved many times between testing locations. Also, testing on-site is performed at a cost to the venue / event, so a large number of on-site testing staff as fully trained chemists may prove costly. In some instances, volunteers or other staff members that are not typically trained chemists is potentially all that is available; resultingly, analytical methods that are robust and simple to perform are required for drug testing. Identification of the main component and any potential adulteration is typically reported back either to the venue and / or specifically to the individual who submitted the sample, so they can then make an informed decision before administration of the sample.

A number of research groups and organisations have performed on-site testing, using a variety of different techniques and feedback methods. McCrae *et al.,*[17] conducted testing at four music festivals in Canada (Jul - Sep 2018), using Fourier Transform-Infrared spectroscopy (FT-IR) for identification of the main component and adulteration, in combination with fentanyl strips to detect fentanyl in low concentrations. A small portion of each sample was used for analysis, with 2-3 mg used for FT-IR and 1 - 2 mg dissolved in water (30 mL) for use with the fentanyl test screening kits. A health worker was then on-site to discuss the results, with users offered the sample back if they wished. Out of the 336 samples that were analysed

during the event, 42.0% were identified as MDMA, 18.5% as cocaine and 18.2% as ketamine. Small numbers of 2C-family compounds, methylenedioxyamphetamine (MDA) and methamphetamine samples were also identified. Out of the samples identified as psychedelics, 72.5% were identified as the compound that was expected, 11.6% contaminated with an adulterant or diluent and 15.9% not as advertised to the user. For the stimulants, 62.1% were unadulterated, 36.4% adulterated and 1.5% contained no active material. Although the paper never states explicitly, the FT-IR is assumed to have been using a multi-component search function or spectral subtraction method, since it was implied to identify multiple components within samples.

Mema *et al.*,[84] performed an on-site drugs checking service at an electronic dance music festival across five days in British Columbia (Canada). Users submitted samples to an amnesty bin and results of high importance were displayed electronically on a screen at the event, visible to all users. 2683 samples were analysed in total, split across a number of techniques. 2387 samples were analysed using common colour tests, 79.2% matched what was advertised, 6.5% did not match what was advertised, with the remaining 14.3% giving an unknown result. 1971 samples were screened using fentanyl test strips, with 1.6% providing a positive result. 1022 samples were analysed by a portable Raman spectrometer and 76 were analysed by a portable GC-MS. The results of both GC-MS and Raman spectroscopy were not disclosed within the report.

Valente *et al.*,[85] performed on-site testing at a music festival (Portugal, 2016). Sample collection occurred from 7pm – 2am each day of the festival within a harm reduction hub. Samples were collected and analysed using two techniques, one station performed colour tests on the samples and three further stations analysed samples using thin layer chromatography (TLC). 753 samples were collected and analysed with 41.0% of seizures believed to be MDMA, 27.4% lysergic acid diethylamide (LSD) and 8.6% cocaine. The caveat for these results is that the samples were not analysed by a confirmatory technique and although colour tests and TLC are useful techniques, there is a lack of accuracy and specificity for compounds outside of the main prevalence identified within this study. At the bare minimum these techniques should be capable of identifying if a sample has adulteration. 90% of MDMA samples were unadulterated and 9% of these samples were synthetic cathinones, mis-sold as MDMA. For the cocaine samples, “some” of these samples were adulterated with cathinones and

lidocaine, although specifics on the percentages were not stated. 9.2% of cocaine samples were unadulterated, 6.2% were mis-sold as ketamine, 52.3% contained only lidocaine with the remaining containing adulterated cocaine.

Measham *et al*.,[86, 87] reported on work conducted by a drug testing organisation called 'The Loop' which regularly performs on-site drugs testing at festivals across the UK. One report from July 2016 described how samples were analysed in a large tent located within a welfare setting at the event.[86] Users would submit a small portion of their samples (around 5 mg) whilst receiving a consultation from healthcare staff. The users would be given a unique ID and told to come back in an hour, after analysis has been performed, to get their results. Three techniques were used for sample analysis: FT-IR was used primarily, with acquired spectra matched against the SWGDRUG spectral library, using the 'default' search algorithm. If the sample was not identified by FT-IR, colour tests were then used to aid in identification. If further analysis did not provide a clear identification, samples were then dissolved in methanol and filtered. The filtrate is then concentrated and reanalysed using FT-IR, allowing a spectrum to be recorded of the main API (Active Pharmaceutical Ingredient) with a potentially reduced amounts of dilutants / adulterants. This approach is to be referred to as mass loss analysis and is used by the group to quantify MDMA tablets. 230 samples were analysed across four days using all described techniques in tandem, 57.0% were identified as MDMA, 13.5% ketamine, 10.0% cocaine and for 7.0% only an adulterant or dilutant was identified. After the results of the individual sample analysis was returned to the users, 1 in 5 (20%) individuals chose to dispose of the sample.

A later report by Measham *et al.*,[87] performed analysis across three music festivals, two in 2019 and one in 2021. The sample collection and user service were the same as the previously outlined work from this group. Analysis was performed using FT-IR acquisition of 16 scans, searching against the SWGDRUG library. If a clear identification with a high match score was not found, then the sample was reconstituted in methanol, to remove diluents or fillers from the sample. The prevalence of MDMA samples identified from 2019 to 2021 reduced from 92.8% to 54.6%, with the number of MDMA samples adulterated with caffeine increased from 1.0% to 21.2% and the number of synthetic cathinones identified increased from 0.2% to 19.4%. Other

compounds identified within tablets were 4-chloromethcathinone (4-CMC), 3-methylmethcathinone (3-MMC), 4-methylmethcathinione (4-MMC) and eutylone.

The majority of the literature outlines the use of non-confirmatory techniques such as, FT-IR and colour tests, for on-site harm reduction testing. Off-site analysis takes advantage of more confirmatory techniques such as GC-MS. Despite the differences in the instrumentation used, no reports used only a single technique, maximising the potential reliability of the data.

The samples acquired and tested described in this chapter, were analysed off-site in a laboratory setting, utilising FT-IR, ^{1}H NMR and GC-MS in unison for qualitative sample identifications. Samples were seized and obtained from surrender bins at a Manchester based festival. Potentially harmful samples were displayed on social media, reported to the event organisers and local police force. These samples were analysed with the dual purpose of not only for harm reduction purposes but to assess the accuracy and reliability of these three techniques typically used for off-site routine analysis, for both single component and samples containing mixtures.

3.2. Results and Discussion

3.2.1. Analysis of Samples

318 samples were analysed in total, seized from central Manchester (UK), between the 24th to the 29th August 2019, using FT-IR, low-field ^{1}H NMR (60 MHz) and GC-MS in tandem. Average match scores for qualitative identifications for each analysis were obtained and used to cross-validate each result. GC-MS, hailed as the 'gold-standard' for forensic chemistry,[88] is used as the reference point for the "correct" identification with all but a small number of exceptions. Samples were seized from a festival setting within Greater Manchester, UK.

Eight samples were not analysed using any of the techniques described due to insufficient material. From the remaining samples 259 were found to contain a single component (83.5%), 47 were binary mixtures (15.2%) and 4 samples were tertiary mixtures (1.3%).

3.2.2. Prevalence

Typical festival seizure prevalence differs from the usual England and Wales and European prevalence. There is assumed to be a bias of the samples seized, to a more 'party focused' group of compounds, which grants euphoric effects to the users. The overall prevalence for seizures across Europe (2019 – 2020) are shown in Figure 6.

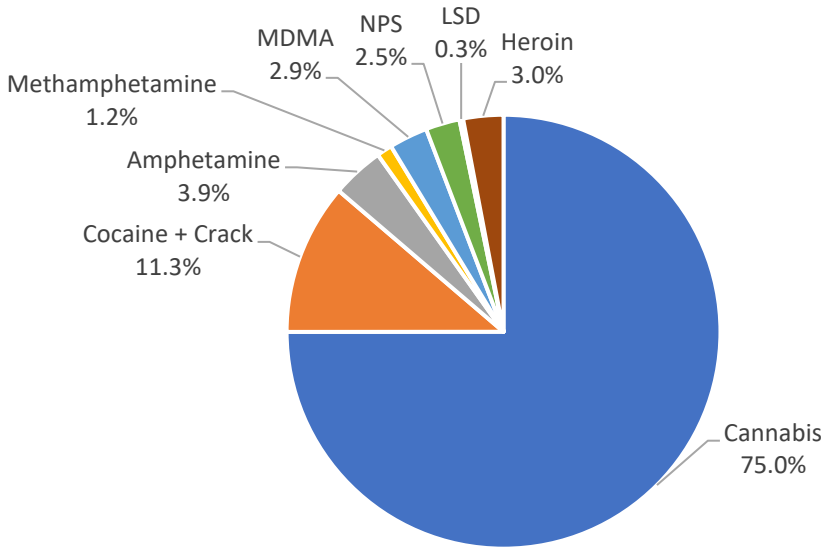


Figure 6 - Prevalence for the illicit drug seizures between 2019 - 2020 within Europe.[89]

One other category was outlined containing DMT (dimethyltryptamine), mushrooms, ketamine and gamma hydroxybutyrate (GHB) but the seizures were not described due the low number across Europe.

The overall prevalence for seizures across England and Wales (2019-2020) are shown in Figure 7,[90] with details of the "Other" category detailed in Table 2.

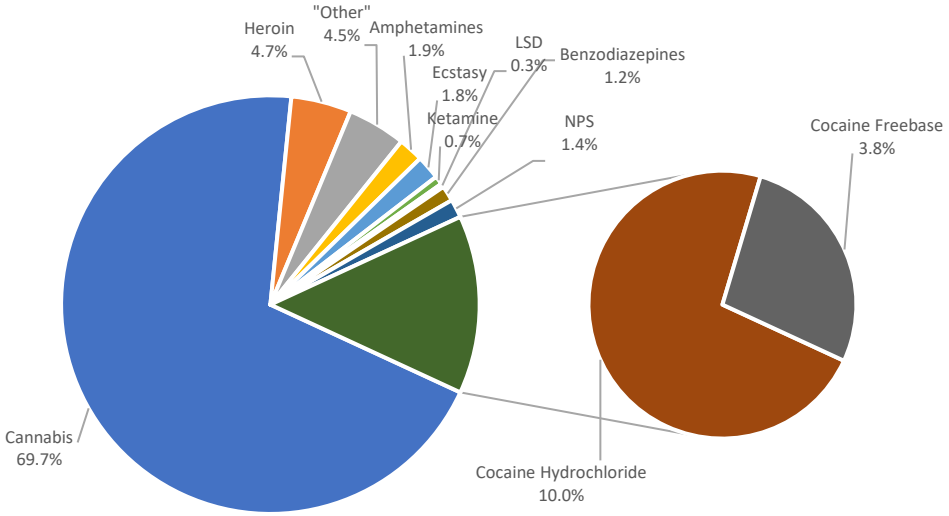


Figure 7 - Prevalence for the illicit drug seizures between 2019 - 2020 within England and Wales.[90] The expanded pie chart showcases the form in which cocaine was seized (13.8%).

Table 2 - Details of the substances within the "Other" category which do not fit the normal prevalence.

Substance	Number Seized	Percentage (%)
"Other" Class B's	4883	2.21
"Other" Class C's	617	1.30
Methadone	427	0.19
Morphine	163	0.07
Non-injection Methadone	76	0.03
Fentanyl / Analogues	32	0.01

The “other” category consists of substances which do not fit the main prevalence of the data. Specific numbers of which, are not released by the Office of National Statistics.[91] Class B compounds are not specifically identified. Class C compounds consist of prescription only medication (PoM), GHB and Khat, which is a plant commonly found in countries around the Red Sea which contains cathinone and cathine.[92] The remaining opioid based compounds are not outlined due to their low prevalence.

Across all of the 310 samples tested within this chapter, the most prevalent drug identified is cocaine (66.8%), followed by MDMA (15.8%) then ketamine (6.5%). This data is shown in Figure 8.

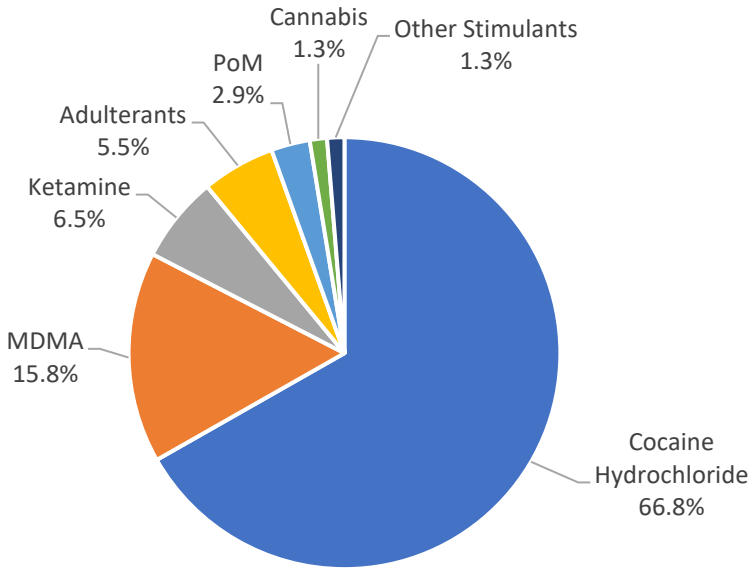


Figure 8 - Prevalence for the August 2019 festival based seizures within central Manchester.

A comparison of each of the illicit drug seizures prevalence between continental (European), national (England and Wales) and local seizures is shown in Table 3.

Table 3 - Prevalence comparison between European, England and Wales and central Manchester based festival seizures - 2019 - 2020

	Prevalence (%)		
Substance	Europe	E & W	Pride 19
Cannabis	75.0	69.7	1.3
Cocaine + Crack	11.3	13.8	66.8
Amphetamines	5.1	1.9	-
MDMA	2.9	1.8	15.8
Heroin	3.0	4.7	-
LSD	0.3	0.3	-
NPS	2.5	1.4	-
PoM	-	1.2	2.9
Ketamine	-	0.7	6.5
"Other"	-	4.5	1.3
Adulterants	-	-	5.5

Between continental and national seizures, the data is consistent and comparable with the top two most seized substances being cannabis followed by cocaine. The third most prevalent substance shifts from amphetamines to heroin from continental to national but the percentages are similar. The differences in the range of substances are mainly due to the detail contained in the reports. Perscription only Medications (PoM), ketamine and the inclusion of an “other” category for the national data, shows the national data to have a wider variety of substances seized. In actuality, the continental data does not report on compounds which does not fit the main prevalence as seizure data is unreliable due to low amounts.

Between the samples seized from the Manchester-based festival compared to the national and continental seizures, the prevalence shifts from cannabis, cocaine and heroin / amphetamines to cocaine, MDMA and ketamine. These compounds are assumed to be prevalent due to the ‘euphoric’ effects they give the users, opposed to the depressant effects granted by cannabis and heroin. The prevalence of ketamine (6.5%) at this event compared to the very low prevalence described throughout continental and national data (0.7%) suggests that ketamine is primarily used as a ‘party’ substance as it is rarely seen throughout general seizures.

GC-MS analysis lacked the discriminatory power to differentiate between cocaine free-base and cocaine hydrochloride, whereas both FT-IR spectroscopy and ^{1}H NMR

spectroscopy was able to do so. All of the samples that were identified as cocaine were in their hydrochloride form. This differs from the usual prevalence found in England and Wales shown in Figure 6. To repeat, this may be due to the 'euphoric' nature of the festival event, making cocaine within its freebase form unattractive to users due to very intense but short term effects.[93] No comparison can be made with continental data due to the lack of discrimination between cocaine hydrochloride and freebase for European seizure data.[89]

Outside of the usual main prevalence are outliers to the main trends which make up 11% (n = 34) of the overall prevalence. Adulterants are described as samples which only have a pharmacologically active compound but does not contain any illicit material.[94] In this case, 13 samples contained only paracetamol, one contained only aspirin, two contained only caffeine and a single sample contained a mixture of Benedryl® (diphenhydramine) and paracetamol. These were not seen throughout the continental and national data, since they are not classed as illicit substances

Nine samples contained PoM as the only identifiable active component. Four of the samples returned as oxandralone, two as diazepam, two as pregabalin and a single sample contained metformin.

Four samples containing herbal material, were identified as cannabis, since common cannabinoids were found during analysis. GC-MS returned dronabinol, which is the generic name for Δ^9-tetrahydrocannibol. ^{1}H NMR returned cannabis directly and FT-IR returned THCA-A (tetrahydrocannbinolic acid A) for three of the four samples, which is a precursor to Δ^9-tetrahydrocannibol. The remaining sample returned basil leaf following FT-IR analysis.

Other stimulants identified includes: two samples of 4-MMC and one of n-ethyl-pentylone (NEP), which are both synthetic cathinones with similar effects to MDMA.[95, 96] One sample was found to contain 2-bromo-4,5-dimethoxyphenethylamine (2-Br-4,5-DMPEA), a compound belonging to the 2-C-X family and an isomer of 2C-B, granting MDMA like effects with hallucinogenic properties.[97]

3.2.3. Single Component Samples

The identifications, number of samples and median match scores for each of the single component samples is shown in Table 4.

Table 4 - Single component samples and their respective median match score for each technique

Substance	No. of samples	FT-IR	^{1}H NMR	GC-MS
2-Br-4,5-DMPEA	1	–	–	-
4-MMC	2	0.98	0.94	0.96
Aspirin	1	0.99	0.99	-
Caffeine	2	0.88	0.90	0.96
Cocaine	158	0.96 (n = 149)	0.96	0.98
Diazepam	2	–	0.94	0.88
Dronabinol	4	0.90 (n = 3)	0.95	0.98
Ketamine	20	0.92	0.95	0.98
MDMA	48	0.99	0.98	0.98
Metformin	1	0.78	0.97	–
NEP	1	0.92	0.93	0.98
Oxandrolone	4	–	0.93	0.85
Paracetamol	13	0.99	0.96	0.99
Pregabalin	2	0.92	0.84	0.96

3.2.4. FT-IR Analysis

149 samples containing a single component of cocaine correlated with the identification from ^{1}H NMR and GC-MS analysis (94.3%). The remaining nine samples returned hits that did not correlate with the other two techniques. Inadequate material remained for FT-IR analysis for four samples, the remaining five samples were identified as creatine hydrate (n = 3), boric acid (n = 1) and chlorhexidine (n = 1) with match scores ranging from 0.54 to 0.97. Spectral subtraction did not aid the identification of cocaine for these samples.

The ^{1}H NMR spectrum of creatine hydrate is relatively simplistic, with only two environments appearing within the solvent region of the spectrum, resulting in the peaks of interest being not analysed during the qualitative analysis step. This is one of the downfalls of the automated analysis of the NMR drugs detector, which automatically processes all spectra, cutting out a specific region around the DMSO-d_6 solvent peak. This renders creatine hydrate as not being identifiable using the NMR drugs detector. Creatine hydrate is also GC-MS inactive without derivatisation.[98]

Boric acid has a strong FT-IR absorbance spectrum, especially within the O-H region (3300 – 2800 cm^{-1}) providing an overpowering spectrum within samples mixtures,

allowing easy identification. However, ^{1}derivatisation.[100] This explains why FT-IR can only identify boric acid in these samples.

Chlorhexidine acetate is a broad-spectrum antiseptic which is FT-IR, ^{1}H NMR and GC-MS active without derivatisation.[101, 102] Since chlorhexidine was not identified using GC-MS, it is most likely a misidentification by the FT-IR library search caused by an unknown diluent. It is unlikely that the cocaine sample would contain any chlorhexidine, since it is only typically available to purchase within an aqueous gel for application.

The other samples misidentified by FT-IR, was a tablet containing 2-Br-4,5-DMPEA misidentified as cardboard, two samples containing diazepam misidentified as Ambien, one cannabis sample misidentified as basil leaf and four samples containing oxandrolone misidentified as vitamin K.

The sample identified as cardboard, contained 2-Br-4,5-DMPEA when identified *via* GC-MS. This misidentification is likely due to the tablet containing a high percentage content of filler (cellulose), since the tablet was quantified to be 9.9% (% w/w) *via* quantification using GC-MS. It was initially assumed that this sample contained 2C-B, since only GC-MS could produce an identification. However, this was not confirmatory due to the low match score (0.78). Evaluation of the EI-MS spectrum shows no molecular ions [*m/z* = 259.0 (^{79}Br-M^{+}) and 261.0 (^{81}Br-M^{+})]. The presence of the base ion peak [*m/z* = 180.0], indicates the presence of the isomer 2-Br-4,5-DMPEA, which has been previously identified in Dutch tablets between 2015 – 2020.[103] This sample was later confirmed against reference material.

For ^{1}H NMR, the 2-Br-4,5-DMPEA was also misidentified as 2C-B. Unlike the difference in the EI-MS spectra between the two isomers, distinguishing the differences between ^{1}H NMR spectra using the NMR drugs detector is more difficult. Reference spectra shows that 2C-B contains two peaks in the aromatic region at 7.20 and 6.98 ppm. For 2-Br-4,5-DMPEA, these peaks have shifted up-field to 7.14 and 6.96 ppm. Due to other peaks not being included in the fingerprint or class regions of the spectrum, the aromatic region is the main point of identification for these compounds. Included in the algorithm is a peak shift tolerance of $\pm$0.06 ppm. Since both of these peaks shift by

a small and similar amount between the isomers, distinguishing these isomers is difficult using the NMR drugs detector.[104]

The sample identified as 'Ambien' was identified as diazepam by both GC-MS and ^{1}H NMR. Since 'Ambien' is used as a brand name for zolpidem, tablets which typically contain 5 mg of active material bound together with tablet fillers including but not exclusive to lactose. Diazepam tablets also typically contain 2 - 10 mg of active material with 152 mg of lactose used as a tablet filler.[105] FT-IR analysis identified the major component (lactose), opposed to the small percentage of active material.

The cannabis sample, despite repeated spectral acquisitions, identified consistently as 'basil leaf' opposed to a cannabinoid like the other of the samples, despite cannabinoids being previously identified from both GC-MS and ^{1}H NMR. The potency of the cannabis could be lower than the other samples, or the active material may have degraded or been removed. Care has to be taken with samples of this nature, as synthetic cannabinoids are typically imprinted on herbal material but the active material may not have been present in high enough concentration to be detected by FT-IR. However, in the case of this sample, synthetic cannabinoids should have been identified during GC-MS analysis. As a result, this sample was not determined to contain "spice".

The four misidentified oxandrolone tablets, were correctly identified by ^{1}H NMR and GC-MS; it is important to note that the GC-MS analysis was injected without any dilution. Even though the content of the tablet was unable to be directly quantified, it is assumed to have an extremely low content % (w/w) of active material, since routine analysis produced a chromatograph with a low peak area. Even without dilution, the peak area of the oxandrolone peaks were still low, reducing the average match scores of the samples due to the low number of ions reaching the MS.

One sample, containing only aspirin, was identified by FT-IR and ^{1}H NMR but was unable to be detected by GC-MS. Aspirin, without derivatisation is GC inactive and the chromatograph contained a peak for the internal standard only.

The remaining 242 single component samples correctly correlated with the identifications from GC-MS and ^{1}H NMR giving an overall correlation rate of 94.9%. For the most prevalent seizures, FT-IR can identify quickly and efficiently. Coupled with the

simple and non-destructive nature of the instrumentation, showcases FT-IR as a useful tool for most bulk sample screening of single component samples. It suffers for samples containing a low percentage content of active material or samples which contain a diluent or adulterant with overpowering influence of the spectral data. In this case, FT-IR has the advantage of being able to identify GC and NMR inactive compounds, such as dilutants and adulterants identified within these misidentified cocaine samples.

For ^{1}H NMR, the only uncorrelated sample was a tablet containing 2-Br-4,5-DMPEA. The remainder of the single component mixtures were identified correctly matching the result from the GC-MS, giving a total percentage of 99.6%. This technique is helpful for single component sample analysis for the vast majority of compounds but suffered due to the close spectral similarities between these two isomeric compounds.

Metformin is typically GC-MS inactive as it requires derivatisation to be detected.[106] The sample was derivatised using MTBSTFA and the compound was confirmed using MS spectral data from previous work performed by Goedecke *et al*.[106] The extra derivatisation step is time consuming, expensive and hazardous to apply for all samples during bulk routine analysis. As an alternative, the use of three separate techniques can alert analysts when a non-volatile compound is present and derivatisation, if necessary, can be used to allow GC-MS to be used as a confirmatory technique.

The median match scores for the analysis of the single components are shown in Table 4. For GC-MS analysis, the match scores remained the highest overall, with an average of 0.95, showing a strong correlation with the library spectral data, for all but oxandrolone, due to the low content of active material within the tablets. 2-Br-4,5-DMPEA and metformin sample were confirmed by eye from reference material and literature. The high match scores are most likely due to the chromatography aspect of the instrumentation, separating each compound in a sample mixture ensuring each library match will be of the 'pure' component.

For ^{1}H NMR analysis, the match scores were high with an average of 0.94, with the lowest correlations for pregabalin and caffeine, both compounds with poor solubility in DMSO, resulting in low signal to noise ratio within the peaks and thus lower match scores.

For FT-IR analysis, the match scores were the lowest overall with an average of 0.93 but still high enough to show good correlation with library spectra. The higher correlation scores are for the most prevalent compounds but lower match scores were obtained for caffeine, cannabis, metformin and pregabalin. The cannabis sample consists of the herbal material ground up to ensure homogeneity and then tested directly with no extraction. The lack of sample extraction means the herbal material is included in the spectrum, which will lower the match score of the sample due to matrix complexity. Metformin and pregabalin were both seized in tablet form, which contains tablet fillers and excipients such as lactose and magnesium stearate.[30] Multiple components within a sample cause spectral complexity, lowering the match score of the sample. Similarly, the caffeine sample is assumed to have other diluents present, lowering the match score.

3.2.5. Binary Mixtures

47 binary mixtures were analysed in tandem by FT-IR, ^{1}H NMR and GC-MS. 45 of these consisted of adulterated cocaine hydrochloride samples. The remaining two were MDMA adulterated with caffeine and Benadryl® (diphenhydramine) with paracetamol. The full identifications, with their respective content % (w/w) is shown in Table 5.

Table 5 - Binary mixtures full identifications and composition % (w/w)

Sample	Adulterant	Number	Percentage (%)	API Content Range % (w/w)	Median API content % (w/w)
Cocaine	Levamisole	19	40.4	79.9 - 99.5	94.5
	Benzocaine	9	19.1	11.0 - 91.4	62.5
	Caffeine	7	14.9	8.3 - 93.8	85.3
	Phenacetin	7	14.9	70.6 - 90.1	85.2
	Paracetamol	2	4.3	64.0 - 94.7	79.3
	Diphenhydramine	1	2.1	97.8	97.8
MDMA	Caffeine	1	-	78.9	78.9
Diphenhydramine	Paracetamol	1	-	96.0	96.0

The GC-MS analysis, was set as the benchmark for this analysis, as being a chromatography technique, should be able to separate and identify each GC active component in the binary mixtures.

Complete quantification was not performed on these samples. Peak area normalisation was used, where the ratios of the peaks within the GC-MS chromatograph were used as guide to the composition of each sample. This has reduced accuracy compared to full quantification against reference standards and does not account for GC inactive diluents. This applies throughout this chapter unless stated otherwise.

For the cocaine samples, it was found that levamisole was the most prevalent adulterant, followed by benzocaine, phenacetin and caffeine. Compared to previous work by Núñez *et al.,*[44] (Figure 3) over a study performed in Spain between 2007 – 2014, consisting of 43,196 samples, levamisole was the most prevalent (46.9%) which matches the results shown from this small number of samples. Likewise, is the prevalence for caffeine (13.2%) and paracetamol (4.1%) as adulterants. However, the percentage of samples to contain phenacetin (36.3%) and benzocaine (2.6%) are much higher and lower respectively than the values reported from this dataset.

Results from the Swiss based study by Broséus et al.,[74] (Figure 4) showcased phenacetin (80%), levamisole (65%) and lidocaine (47%) as the most prevalent adulterants from western Switzerland. This differs by the order of prevalence for both the Spanish study and the samples analysed in this chapter, except the inclusion of lidocaine is usually unseen in Manchester.

Results from the Manchester based study by Antondies *et al.,*[41] based on 432 samples seized between 2017 - 2018, found levamisole to be the most prevalent cocaine adulterant (46.2%). The other adulterants identified in the study are also identified within this work but the prevalence percentages do not match.

FT-IR analysis showed agreement with at least one component in each sample with the exception of one sample which identified as lactose by FT-IR and cocaine and caffeine by GC-MS. The peak areas of both cocaine and caffeine are low respective to other samples, hinting that the major component could be the diluent lactose, which is unable to be detected by GC-MS since it is GC inactive. Out of the correctly identified samples, in four samples, the major component was identified over the illicit component. These samples consisted of three cocaine samples, with cocaine content between 8.3 – 26.4 % (w/w) and one sample containing diphenhydramine with 4.0 % (w/w) content. Understandably, FT-IR being a spectroscopy technique, the library search function is expected to identify the major component. However, care must be taken with samples such as these, in that the samples are not incorrectly assumed non-illicit and that further confirmatory analysis occurs. A summary of the ^{1}H NMR binary mixtures analysis is shown in Table 6.

Table 6 - ^{1}H NMR binary mixture identifications summary

^{1}H NMR Match	**Number**	**Percentage (%)**
Complete match	25	53.3
Major component identified	17	36.2
Missed illicit component	4	8.5
Misidentified adulterant	1	2.1

The drugs detection algorithm is capable of library matching two components within the collected ^{1}H NMR spectra. This allows complete matches for samples which are binary mixtures. In more than half of the binary mixtures, both components were identified. This is helpful from a harm reduction perspective to highlight any potentially toxic components.

For 17 of the samples, only the major illicit component was identified. Although not as useful as a full identification, if the illicit component is identified then a general treatment can be applied to any individual that may be unwell and the sample can be included in prevalence data.

For four of the samples, the illicit component was not able to be identified. For the same reasons as described for FT-IR, ^{1}H NMR is a spectroscopy technique and the major component will overpower and overlap similar distinguishing features within the sample spectra causing the algorithm to miss both components. For example, aromatic protons in a major component may overlap similar aromatic protons. Other protons environments should remain visible, e.g., aliphatic protons.

One sample, which was identified as cocaine and paracetamol by GC-MS, was identified as cocaine hydrochloride and 2,3-dimethyldiphenidine by ^{1}H NMR analysis. Diphenidines in general are GC-MS active and should have been identified by GC-MS.[107] The reference spectra between 2,3-dimethyldiphenidine and paracetamol in DMSO-d_6 on a low field instrument have similar features, with aromatic protons that possess second order effects in the same regions. With the cocaine hydrochloride spectra overlayed, the algorithm has understandably mismatched the adulterant. The spectra of 50:50 % (w/w) mixture containing both cocaine hydrochloride and paracetamol and cocaine hydrochloride and 2,3-dimethyldiphenidine are shown in Figure 9.

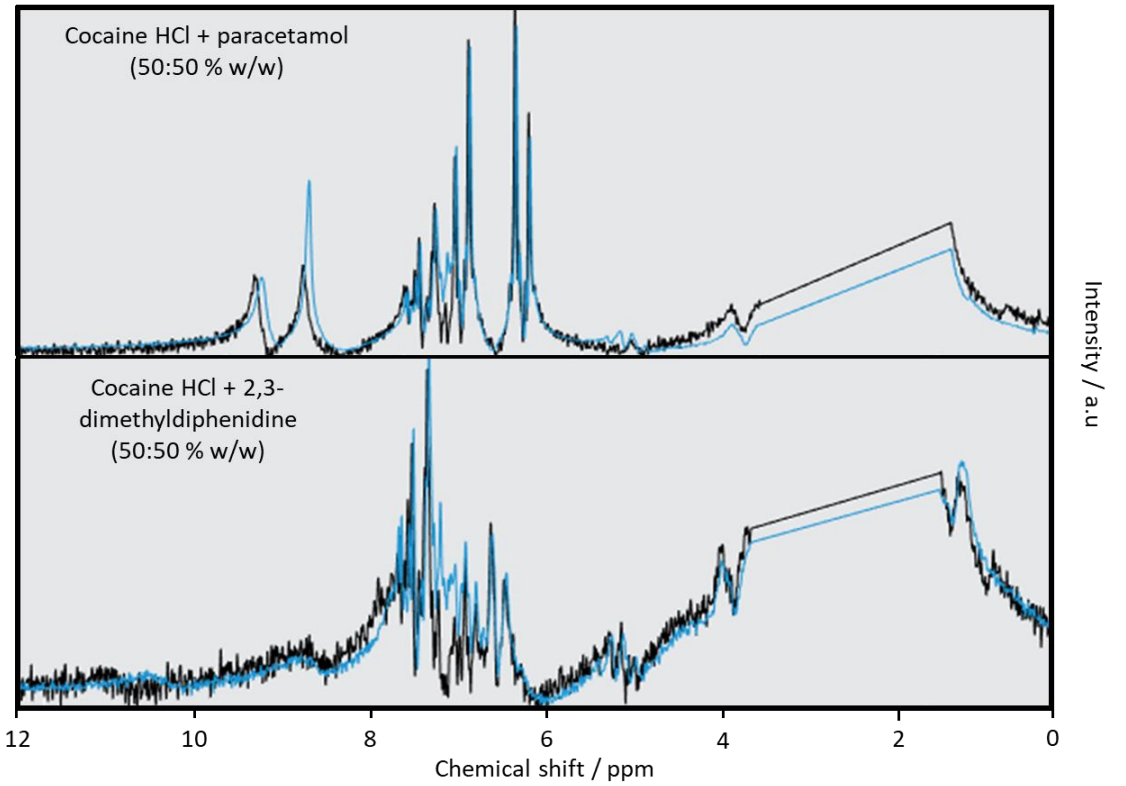


Figure 9 - ^{1}H NMR Benchtop drugs detector output of reference spectra of both 50:50 % (w/w) cocaine hydrochloride : paracetamol (top) and 50:50 % (w/w) cocaine hydrochloride : 2,3-dimethyldiphenidine (bottom)

3.2.6. Tertiary Mixtures

Four tertiary cocaine samples were identified. Details of each sample and their respective content % (w/w) is shown in Table 7 and the qualitative analysis from each technique is shown in Table 8.

Table 7 – Tertiary cocaine sample content % (w/w)

	Tertiary Sample component ratios % (w/w)					
Sample	Component 1	% (w/w)	Component 2	% (w/w)	Component 3	% (w/w)
1	Cocaine HCl	18.3	Benzocaine	80.6	Levamisole	1.1
2	Cocaine HCl	35.8	Benzocaine	61.7	Caffeine	2.5
3	Cocaine HCl	54.3	Benzocaine	44.1	Caffeine	1.7
4	Cocaine HCl	64.8	Paracetamol	32.7	Levamisole	2.5

Table 8 - Tertiary sample identifications by instrumentation

	Instrumental identifications					
Sample	FT-IR	Match Score	^{1}H NMR	Match Score	GC-MS	Match Score
1	Cocaine HCl	0.93	Benzocaine & Cocaine HCl	0.92	Benzocaine + Levamisole + Cocaine	0.99 / 0.88 / 0.98
2	Benzocaine	0.77	Benzocaine & Cocaine HCl	0.84	Benzocaine + Caffeine + Cocaine	0.99 / 0.95 / 0.98
3	Cocaine HCl	0.90	Benzocaine & Cocaine HCl	0.87	Benzocaine + Caffeine + Cocaine	0.99 / 0.85 / 0.98
4	Not enough sample	-	Paracetamol	0.92	Paracetamol + Cocaine + Levamisole	0.98 / 0.97 / 0.89

Three out of four of the tertiary samples were analysed in unison by each technique, with the exception of sample four which did not have enough sample material for a full analysis (FT-IR analysis was not conducted).

FT-IR analysis initially identified the major component in samples 2 and 3 and one of the minor products in sample 1. The actual percentage content of cocaine within the sample is possibly higher than described from the peak area ratios, especially since benzocaine has a large response factor compared to cocaine using GC-MS.

^{1}H NMR analysis identified both cocaine hydrochloride and benzocaine in samples 1 – 3, which is expected since these components make up almost all of the sample, with the tertiary adulterant consisting of very low amounts (1.1 – 2.5%). For sample 4, only

paracetamol was identified. Although not being the major component within the sample mixture, the ^{1}H NMR spectrum for paracetamol consists of two sets of second order doublets within the aromatic region, which is the predominant feature for the sample spectra in which the algorithm identified as paracetamol.

GC-MS analysis identified all three components with the tertiary mixtures and is used as the benchmark for these comparisons.

The percentage of samples which identified the major component with the sample has been outlined in Table 9.

*Table 9 - Percentage of samples in which the major component was identified. * = GC-MS acts as the benchmark for this analysis, which does not account for GC-MS inactive components. - = Spectral subtraction was not applied for single component samples.*

Instrument	Single Component	Binary Mixtures	Tertiary Mixtures
FT-IR	94.9%	95.7%	66.7%
FT-IR (Spectral Subtraction)	-	100.0%	100.0%
NMR	99.6%	91.6%	100.0%
GC-MS	99.6%	100.0%*	100.0%*

GC-MS was used as the benchmark for comparison with other techniques. This is inherently a poor assumption as a large percentage of samples may contain GC-MS inactive fillers / diluents.[74] A comprehensive identification of each diluent is beyond the scope of this comparison.

3.2.7. Qualitative Detection Threshold % (w/w)

The qualitative detection threshold for both FT-IR and ^{1}H NMR were analysed, showcasing the minimum percentage weight % (w/w) needed for adulterant detection within simulated samples. This will be specifically referred to as the Detection threshold to differentiative from instrumental and method Limit of Detection (LoD). GC-MS was not analysed, as the chromatography of the instrumentation would separate the main API from the active adulterant and is assumed to show both components for each sample. These samples are produced using reference material and the percentage content of each % (w/w) is based on the weight of each component in each mixture. These samples of cocaine and caffeine, benzocaine or

levamisole were separately analysed by FT-IR and ^{1}H NMR; Detection threshold data following analysis is presented in Table 10, Table 11 and Table 12 respectively.

Table 10 - Cocaine hydrochloride & caffeine - Detection threshold comparison FT-IR and ^{1}H NMR

Cocaine hydrochloride & caffeine	Content of cocaine hydrochloride % (w/w)		
Component identified	**FT-IR (Normal)**	**FT-IR (Spectral Sub)**	**^{1}H NMR**
Only cocaine hydrochloride	100.0 - 50.0	100.0	100.0 - 81.4
Cocaine hydrochloride & adulterant	-	88.8 - 9.7	70.3 - 28.6
Only adulterant	40.2 - 0.0	0.0	20.0 - 0.0

Table 11 - Cocaine hydrochloride & benzocaine - Detection threshold comparison FT-IR and ^{1}H NMR

Cocaine hydrochloride & benzocaine	Content of cocaine hydrochloride % (w/w)		
Component identified	**FT-IR (Normal)**	**FT-IR (Spectral Sub)**	**^{1}H NMR**
Only cocaine hydrochloride	100.0 - 40.6	100.0	100.0 - 79.6
Cocaine hydrochloride & adulterant	-	90.2 - 9.9	70.3 - 41.7
Only adulterant	30.5 - 0.0	0.0	31.0 - 0.0

Table 12 -Cocaine hydrochloride & levamisole - Detection threshold comparison FT-IR and ^{1}H NMR

Cocaine hydrochloride & levamisole	Content of cocaine hydrochloride % (w/w)		
Component identified	**FT-IR (Normal)**	**FT-IR (Spectral Sub)**	**^{1}H NMR**
Only cocaine hydrochloride	100.0 - 39.6	100.0	100.0
Cocaine hydrochloride & adulterant	-	89.7 - 10.7	89.7 - 69.5
Only adulterant	30.3 - 0.0	0.0	60.3 - 0

FT-IR typically identified cocaine hydrochloride within the sample mixtures, the match score decreased until around 40% cocaine % (w/w). At this point, the adulterant was identified instead and the match score increased until 100% adulterant was analysed. This was consistent for all three mixtures apart from the switch over percentage, this varied between 50.0 – 30.3% dependant on the adulterant used. The identifications with their respective match scores are shown in Table 13, Table 14 and Table 15

.

Table 13 - Cocaine hydrochloride and caffeine - Detection threshold FT-IR sample analysis - Full subtraction data

Mass in samples (mg)		Percentage % (w/w)		Result Before	Match Score	Result After Subtraction		Subtraction Factor	Result After Subtraction		Subtraction Factor
Cocaine	Caffeine	Cocaine	Caffeine			(of) Cocaine	Match Score		(of) Caffeine	Match Score	
20	0.0	100.0	0.0	Cocaine HCl	0.9939	-	-	-	Cocaine HCl	0.9664	0.3204
45.4	5.7	88.8	11.2	Cocaine HCl	0.9853	Caffeine	0.7836	0.9104	Cocaine HCl	0.9701	0.3652
39.9	9.8	80.3	19.7	Cocaine HCl	0.9721	Caffeine	0.8051	0.8597	Cocaine HCl	0.9717	0.3948
70.4	30.2	70.0	30.0	Cocaine HCl	0.9323	Caffeine	0.8082	0.8976	Cocaine HCl	0.9690	0.5083
60.8	40.9	59.8	40.2	Cocaine HCl	0.8775	Caffeine	0.7816	0.6331	Cocaine HCl	0.9644	0.4113
50.7	50.6	50.0	50.0	Cocaine HCl	0.7706	Caffeine	0.8457	0.8346	Cocaine HCl	0.9662	0.6626
41	61.1	40.2	59.8	Caffeine	0.7251	Caffeine	0.8261	0.6662	Cocaine HCl	0.9590	0.6102
29.5	70.6	29.5	70.5	Caffeine	0.7848	Caffeine	0.8257	0.5293	Cocaine HCl	0.9429	0.5349
19.9	81.2	19.7	80.3	Caffeine	0.8982	Caffeine	0.8642	0.5863	Cocaine HCl	0.9283	0.721
9.7	90.3	9.7	90.3	Caffeine	0.9682	Caffeine	0.8642	0.4974	Cocaine HCl	0.8047	0.7246
0	20	0.0	100.0	Caffeine	0.9886	Caffeine	0.8729	0.5521	-	-	-

Table 14 - Cocaine hydrochloride and benzocaine - Detection threshold FT-IR samples analysis - Full subtraction data

Mass in samples (mg)		Percentage % (w/w)		Result Before	Match Score	Result After Subtraction		Subtraction Factor	Result After Subtraction		Subtraction Factor
Cocaine	Benzocaine	Cocaine	Benzocaine			(of) Cocaine	Match Score		(of) Benzocaine	Match Score	
20	0	100.0	0.0	Cocaine HCl	0.9940	-	-	-	Cocaine HCl	0.9249	0.2671
90.3	9.8	90.2	9.8	Cocaine HCl	0.9912	Benzocaine	0.7335	1.0905	Cocaine HCl	0.9169	0.3155
80.1	20.3	79.8	20.2	Cocaine HCl	0.9718	Benzocaine	0.7100	1.0510	Cocaine HCl	0.9059	0.3396
68.9	30.5	69.3	30.7	Cocaine HCl	0.9397	Benzocaine	0.7555	1.1209	Cocaine HCl	0.9092	0.3922
60.1	39.6	60.3	39.7	Cocaine HCl	0.9118	Benzocaine	0.7528	1.1826	Cocaine HCl	0.9139	0.4355
50.9	50	50.4	49.6	Cocaine HCl	0.8133	Benzocaine	0.7489	1.1353	Cocaine HCl	0.9071	0.4654
40.5	59.2	40.6	59.4	Cocaine HCl	0.7520	Benzocaine	0.7679	1.3158	Cocaine HCl	0.9100	0.5701
30.5	69.6	30.5	69.5	Benzocaine	0.8756	Benzocaine	0.7581	1.1799	Cocaine HCl	0.8750	0.5908
19.4	79.5	19.6	80.4	Benzocaine	0.9434	Benzocaine	0.7559	1.0292	Cocaine HCl	0.8044	0.5652
9.8	89.6	9.9	90.1	Benzocaine	0.9776	Benzocaine	0.7854	1.3428	Cocaine HCl	0.7565	0.8076
0	20	0.0	100.0	Benzocaine	0.9980	Benzocaine	0.8014	1.4888	-	-	-

Table 15 – Cocaine hydrochloride and levamisole - Detection threshold FT-IR samples analysis - Full subtraction data

Mass in samples (mg)		Percentage % (w/w)		Result Before	Match Score	Result After Subtraction		Subtraction Factor	Result After Subtraction		Subtraction Factor
Cocaine	Levamisole	Cocaine	Levamisole			(of) Cocaine	Match Score		(of) Levamisole	Match Score	
20	0	100.0	0.0	Cocaine HCl	0.9934	-	-	-	Cocaine HCl	0.9738	0.5105
90.1	10.4	89.7	10.3	Cocaine HCl	0.9803	Styrofoam	0.6622	0.8248	Cocaine HCl	0.9676	0.5817
79.6	20.1	79.8	20.2	Cocaine HCl	0.9729	Styrofoam	0.6921	0.8048	Cocaine HCl	0.9727	0.6159
69.5	30.5	69.5	30.5	Cocaine HCl	0.9521	Levamisole HCl	0.7833	0.8184	Cocaine HCl	0.9765	0.7011
60.5	39.8	60.3	39.7	Cocaine HCl	0.9187	Levamisole HCl	0.7963	0.7471	Cocaine HCl	0.9749	0.7325
50.3	49.9	50.2	49.8	Cocaine HCl	0.8245	Levamisole HCl	0.8195	0.6808	Cocaine HCl	0.963	0.8111
39.4	60.2	39.6	60.4	Cocaine HCl	0.7396	Levamisole HCl	0.7558	0.5109	Cocaine HCl	0.9441	0.7243
30.2	69.4	30.3	69.7	Levamisole	0.8391	Levamisole HCl	0.8306	0.535	Cocaine HCl	0.9223	0.9330
20.2	79.4	20.3	79.7	Levamisole	0.8732	Levamisole HCl	0.7990	0.4588	Cocaine HCl	0.8653	0.8588
10.8	90	10.7	89.3	Levamisole	0.9632	Levamisole HCl	0.8208	0.3417	Styrofoam	0.6558	0.8643
0	20	0.0	100.0	Levamisole	0.9902	Levamisole HCl	0.7507	0.3349	-	-	-

^{1}H NMR identified both components when the cocaine % (w/w) was between 80 – 40%. This differed slightly for each adulterant, as certain hydrogen environments will be more distinguishable within the overall sample spectra. Levamisole is identified at a lower percentage content % (w/w). and prevents detection of cocaine hydrochloride at a lower percentage content than both caffeine and benzocaine. This is most likely due to similarities between the cocaine hydrochloride and levamisole spectra, with both the aromatic and the single hydrogen on both ring systems overlapped. Both caffeine and benzocaine have ^{1}H NMR spectra which are easily distinguished from cocaine hydrochloride, explaining why the range in which both components are identified is centred around 50% % (w/w) of each component as features from both components are equally visible; it is only until the cocaine hydrochloride peaks are lost to the noise within the spectrum, does it become unidentifiable. From a harm reduction perspective, being able to detect both components within a binary mixture is a huge advantage, as to be able to identify potentially toxic compounds within samples.

The detection threshold for cocaine hydrochloride and other adulterants was assessed for just ^{1}H NMR. Results have been summarised in Table 16.

Table 16 – Cocaine hydrochloride and various adulterants - Detection threshold for ^{1}H NMR

	Content of cocaine HCl % (w/w) in samples containing adulterant					
Component identified	**Phenacetin**	**Paracetamol**	**Procaine**	**Ketamine**	**Aspirin**	**Diphenhydramine**
Only cocaine hydrochloride	100.0 - 41.6	100.0 - 80.4	100.0 - 80.4	100.0 - 60.8	100.0 - 60.2	100.0 - 69.7
Cocaine hydrochloride & adulterant	29.3	68.5 - 29.6	71.9 - 39.6	49.5	51.5 - 19.0	61.0
Only adulterant	22.0 - 0.0	19.8 - 0.0	27.7 - 0	38.2 - 0.0	8.0 - 0.0	50.0 - 0.0

For the remaining six adulterants, the percentage content range in which both components are identified varies. For paracetamol, procaine and aspirin, the range is large. For phenacetin, ketamine and diphenhydramine, the range is notably less ($\pm$10 % w/w). This is due to the characteristics of each adulterant's spectra and the algorithms' ability to distinguish the more prominent spectral features.

With the exception of binary mixtures containing cocaine HCl and levamisole or diphenhydramine, when cocaine is the major component within binary mixtures, it should always be detected over the minor component using the NMR drugs detector.

Cocaine could be identified in a potentially wider range of samples, dependant on the average content % (w/w) of cocaine HCl in the sample.

Comparing these results to the real binary samples analysed, the median percentage content, shown in Table 5, falls within the region of detection which at least cocaine hydrochloride should be identified.

For the 20 samples in which both components were not identified, 16 match the percentage content assessment described in Table 16 with 4 samples between 0.6 – 4.8% content difference between the assessment and the sample content. This difference is most likely due to the samples used to assess the detection threshold having a more accurate composition produced from reference material then compared to the peak area ratios used from the main sample analysis.

3.2.8. FT-IR Spectral Subtraction

FT-IR being a spectroscopy technique, cannot physically separate different components within a mixture unlike chromatography techniques. When a sample containing two or more components is analysed, the resulting sample spectrum will be a mix of both components, sometimes masking and overlapping each other. If the library search does not have a search function, programmed specifically to identify multiple components, then illicit components or potentially toxic compounds may not be identified. Spectral subtraction allows the removal of one component from a mixtures spectral data, to identify other potential components within the sample, allowing the FT-IR to identify multiple components. This works since absorbance is additive. This technique is most commonly used to remove water peaks from aqueous samples and other matrixes.[108, 109]

Once a sample is analysed by FT-IR, the spectrum is searched against the spectral library. The reference spectrum for the identified compound with the highest match score is then subtracted from the sample spectrum, leaving only the spectrum of the other component, which is then re-compared against the spectral libraries. If the search produced a match score with good agreement (> 0.97) then it is likely every component is identified. If not, then another subtraction should occur using reference spectra from the newly identified component. This continues until either the same

component subtracted, is identified by the search, or only results with a bad correlation are identified (< 0.60).

Spectral subtraction typically uses a ‘subtraction factor’ which is an indication of the amount of absorbance which should be subtracted. This usually has a default option set by the software but should be altered to achieve the best results. When choosing a subtraction factor, the analyst should have a basic knowledge of the more prominent features of the anticipated spectrum, in order to remove the highest amount of secondary component possible. Care must also be taken not to set the factor too high, essentially ‘imprinting’ the subtracting component onto the new spectrum.

Exemplar subtractions are shown in Figure 10, showcasing 50:50 % (w/w) cocaine hydrochloride and benzocaine sample with benzocaine and cocaine hydrochloride reference spectra for comparison. The default subtraction factor was set too high by for this subtraction and the resulting spectrum has some benzocaine spectrum peaks ‘imprinted’ on the result. The subtraction result, after alteration of the subtraction factor, is also shown. The aim of these subtractions is to remove the adulterants spectral features, obtained from reference data from the sample spectrum.

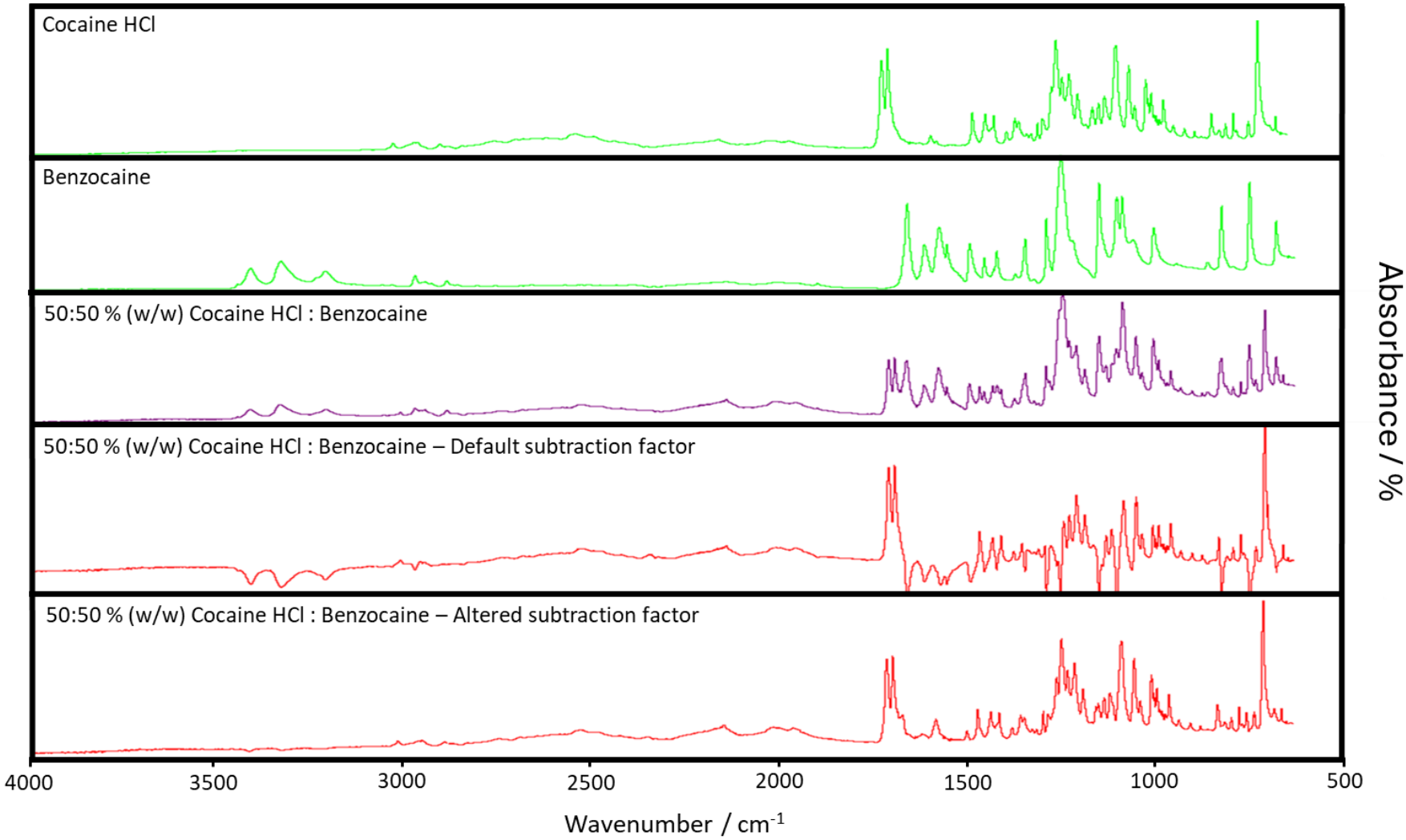


Figure 10 - FT-IR Spectral Subtraction of a 50:50 % (w/w) cocaine HCl : benzocaine sample, including subtraction at both the default and altered subtraction factors. Reference spectral data are shown for comparison purposes.

The detection threshold analysis was performed using this method with the result shown previously in Table 10, Table 11 and Table 12 compared to standard FT-IR and ^{1}H NMR. Both components in every mixture are identified. This shows that even in extremely low percentage content (> 10% (w/w)) both components can be identified. This is a huge advantage when using FT-IR for the analysis of binary mixtures and should be incorporated within further FT-IR analysis. Simulated tertiary mixtures have not been assessed, however, three actual seized samples have been analysed.

The binary and tertiary mixtures were re-analysed by FT-IR using spectral subtraction, this analysis is shown in Table 17 and continued in Table 18. Eleven samples were not included due to insufficient sample.. The remaining binary mixtures were reanalysed following the method outlined earlier for spectral subtraction. Both components were identified in 24 out of 36 of the samples (67%). For the remaining 12 samples, nine did not identify an adulterant after the initial subtraction of cocaine hydrochloride and subtraction could not continue since no adulterant was identified (25%). This normally indicates that the sample is composed solely of cocaine hydrochloride but more than likely the percentage component of the adulterant is lower than the limit of detection for this method. These nine samples consisted of 84.9 – 99.5 % content for cocaine hydrochloride, even though the exact percentage composition of the samples were not accurately quantified, these samples can be assumed that they are close to the extremes (> 90% cocaine hydrochloride % (w/w)) and is present at a percentage composition that is too low to be detected by this method. Three samples identified another diluent, most likely the real major component within the sample, that is not GC active, essentially masking the spectrum. Although, cocaine hydrochloride was able to be identified in all of these samples, no further adulterants could be identified from further subtraction. Vitamin B8, specifically identified in sample 113, is more than likely a minor component within the sample, since it was not identified by FT-IR analysis. This theory cannot be confirmed using GC-MS without derivatization, since vitamin B8 is not GC-MS active.[110]

Table 17 – Binary mixtures analysed using FT-IR using spectral subtraction

IR Samples Subtractions			**Subtracting Cocaine hydrochloride**			**Subtracted one adulterant**				**Subtracted further adulterant**			
Sample	Before Subtraction	Match Score	Identification after subtraction	Sub Factor	Match Score	Adulterant Subtracted	Sub Factor	Identification	Match Score	Adulterant Subtracted	Sub Factor	Identification	Match Score
8	Cocaine HCl	0.9620	Cocaine HCl	0.6388	0.7777	No further analysis since no adulterant identified							
22	Cocaine HCl	0.9596	Levamisole	0.5863	0.4671	Levamisole	0.1276	Cocaine HCl	0.9642				
40	Cocaine HCl	0.9273	Phenacetin	0.4038	0.6499	Phenacetin	0.2536	Cocaine HCl	0.9504				
41	Cocaine HCl	0.9631	Levamisole	0.7098	0.6137	Levamisole	0.1373	Cocaine HCl	0.9677				
45	Cocaine HCl	0.9493	Phenacetin	0.4144	0.7788	Phenacetin	0.1654	Cocaine HCl	0.9716				
47	Cocaine HCl	0.9383	Phenacetin	0.5481	0.8453	Phenacetin	0.2837	Cocaine HCl	0.9768				
51	Boric acid	0.745	Boric acid	0.0668	0.749	Boric acid	0.2925	Lactose	0.6133	Lactose	0.1262	Cocaine HCl	0.639
69	Cocaine HCl	0.9448	Boric acid	0.6105	0.5145	Boric acid	0.2416	Cocaine HCl	0.9525				
73	Cocaine HCl	0.9683	Cocaine HCl	0.6691	0.4262	No further analysis since no adulterant identified							
74	Cocaine HCl	0.9354	Phenacetin	0.6267	0.8655	Phenacetin	0.2445	Cocaine HCl	0.9685				
77	Caffeine	0.9394	Caffeine	0.1414	0.9613	Caffeine	0.9127	Cocaine HCl	0.9105				
92	Benzocaine	0.6962	Benzocaine	0.3648	0.8423	Benzocaine	0.3377	Cocaine HCl	0.9167				
99	Cocaine HCl	0.9677	Levamisole	0.7324	0.5706	Levamisole	0.1357	Cocaine HCl	0.9715				
111	Cocaine HCl	0.9179	Benzocaine	0.4931	0.7684	Benzocaine	0.1193	Cocaine HCl	0.9574				
113	Cocaine HCl	0.9393	Vitamin B8	0.5269	0.7481	Vitamin B8	0.1036	Cocaine HCl	0.8117				
125	Cocaine HCl	0.9561	Black Cohosh Extract	0.6808	0.6306	No further analysis since no adulterant identified							
129	Cocaine HCl	0.9762	Polyester	0.7212	0.4833	No further analysis since no adulterant identified							
132	Cocaine HCl	0.9359	Levamisole	0.1008	0.5523	Levamisole	0.1008	Cocaine HCl	0.9844				
174	Cocaine HCl	0.9622	Cocaine HCl	0.8313	0.5097	No further analysis since no adulterant identified							
190	Cocaine HCl	0.9754	Toluene	0.6579	0.5524	No further analysis since no adulterant identified							
194	Cocaine HCl	0.7597	Phenacetin	0.6075	0.9011	Phenacetin	0.6861	Cocaine HCl	0.9674				

Table 18 - Binary mixtures analysed using FT-IR using spectral subtraction (Continued)

IR Samples Subtraction			Subtracting Cocaine hydrochloride			Subtracted one adulterant				subtracted further adulterant			
Sample	Before Subtraction	Match Score	Identification after subtraction	Sub Factor	Match Score	Adulterant Subtracted	Sub Factor	Identification	Match Score	Adulterant Subtracted	Sub Factor	Identification	Match Score
225	Cocaine HCl	0.918	Phenacetin	0.6066	0.885	Phenacetin	0.3506	Cocaine HCl	0.9708				
229	Cocaine HCl	0.9487	Benzocaine	0.8705	0.746	Benzocaine	0.1188	Cocaine HCl	0.9584				
235	Cocaine HCl	0.6812	Caffeine	0.3079	0.9124	Caffeine	0.5865	Cocaine HCl	0.9412				
247	Cocaine HCl	0.9531	Benzocaine	0.9316	0.5891	Benzocaine	0.0786	Cocaine HCl	0.9555				
252	Cocaine HCl	0.8976	Black Cohosh Extract	0.5839	0.5941	No further analysis since no adulterant identified							
259	Cocaine HCl	0.9781	Levamisole	0.4015	0.5523	Levamisole	0.1008	Cocaine HCl	0.9844				
268	Cocaine HCl	0.9476	Benzocaine	0.8849	0.629	Benzocaine	0.037	Cocaine HCl	0.9505				
269	Cocaine HCl	0.9753	Levamisole	0.693	0.6133	Levamisole	0.1319	Cocaine HCl	0.9815				
272	Benzocaine	0.7771	Benzocaine	0.2447	0.8223	Benzocaine	0.791	Cocaine HCl	0.7284				
273	Cocaine HCl	0.9643	Levamisole	0.4521	0.4436	Levamisole	0.2087	Cocaine HCl	0.9698				
285	Cocaine HCl	0.7412	Benzocaine	0.2332	0.7972	Benzocaine	1.0366	Cocaine HCl	0.6307				
287	Cocaine HCl	0.8312	Paracetamol	0.2446	0.9533	Paracetamol	0.5524	Cocaine HCl	0.9174				
294	Cocaine HCl	0.9694	Levamisole	0.7038	0.6259	Levamisole	0.1828	Cocaine HCl	0.9752				
296	Cocaine HCl	0.9689	Cocaine HCl	0.7439	0.5546	No further analysis since no adulterant identified							
305	Cocaine HCl	0.967	Toluene	0.4808	0.3969	No further analysis since no adulterant identified							

Spectral subtraction of the tertiary mixtures is shown in Table 19. For each of the three samples, only two components were identified, each component making the majority of each sample (> 95%). The component not identified, was present in extremely low amounts (1.1 – 2.5% content) and has too little presence within the initial sample spectrum to be identified during spectral subtraction.

Table 19 -Tertiary mixtures analysed using FT-IR using spectral subtraction

Sample	Before Subtraction	Match Score	Identification after subtraction	Sub Factor	Match Score	Adulterant Subtracted	Sub Factor	Identification	Match Score
293	Cocaine HCl	0.7392	Benzocaine	0.419	0.7666	Benzocaine	0.3021	Cocaine HCl	0.8034
271	Benzocaine	0.7732	Benzocaine	0.4146	0.8197	Benzocaine	0.2090	Cocaine HCl	0.9508
173	Cocaine HCl	0.9177	Benzocaine	0.5387	0.8278	Benzocaine	0.1292	Cocaine HCl	0.9608

3.3. Conclusions

318 samples obtained over the period of 24th – 29th of August 2019 were analysed *in situ* by FT-IR, ^{1}H NMR and GC-MS. 259 samples were identified as single components, 47 identified as binary mixtures and four identified as tertiary mixtures. Eight samples could not be analysed using all three techniques in tandem. Cocaine hydrochloride was the most prevalent sample seized accounting for 61% of all single components, 96% of binary mixtures and all of the tertiary mixtures.

The analysis of the single component samples showed good agreement between the three techniques. Exceptions to this, include samples which contain other tablet fillers or dilutants for FT-IR analysis and non-GC active components for GC-MS analysis.

The analysis of the binary and tertiary samples resulted in lower agreement between the three techniques than compared to the single components as FT-IR and ^{1}H NMR did not always identify all of the components. Full matches were identified in 53.3% of the binary mixtures by ^{1}H NMR and none of the tertiary samples. Whereas FT-IR could only identify one component unless spectral subtraction is employed. Once spectral subtraction was employed, the number of full matches rose to 66.7%, with the illicit component identified in the remaining 33.3%. The tertiary mixtures had the two major components identified by both ^{1}H NMR and FT-IR when using spectral subtraction, with the minor component (1.1 – 2.5% (w/w)) not identified.

The limit of detection for ^{1}H NMR and FT-IR using spectral subtraction was also assessed. NMR was able to identify both the illicit component and the adulterant within samples containing binary mixtures, typically 70 – 40% (w/w) content (cocaine hydrochloride) but varies slightly dependant on adulterant. A smaller number of adulterants was assessed using FT-IR, which did not identify any binary mixtures initially, with cocaine hydrochloride identified from typically 100 – 40% (w/w) and the adulterant identified between 30 – 0% (w/w) cocaine hydrochloride content. Implementing spectral subtraction identifies all of the mixtures tested, showing that between 100 – 10% (w/w)cocaine hydrochloride , both cocaine and the respective adulterant can be identified. This was representative of most of the samples, with the exception of the very extremes (1.1 – 2.5% (w/w)) in the tertiary samples.

From a harm reduction perspective, FT-IR is the most rapid in terms of data acquisition and analysis (~2 minutes for full spectral subtraction analysis), then ^{1}H NMR (5 minutes) and then GC-MS (7 minutes plus sample preparation). However, the rapid nature of the analysis must be considered against the accuracy of the analysis. In this respect, GC-MS with the ability to separate complex mixtures, can typically separate and identify each GC active component within a mixture which reflects in this report with only one sample unable to be identified. Both FT-IR and ^{1}H NMR are on par for their ability to identify samples and ease of use, each with their own respective pros and cons for each technique. Furthermore, this study highlights the necessity for more than one technique to be used, during routine large scale bulk sample analysis.

4. Chapter 4 – Quantification of Cocaine Samples using Nuclear Magnetic Spectroscopy

4.1. Introduction

4.1.1. Background

Nuclear magnetic resonance (NMR) spectroscopy is useful technique for the analysis of illicit drug samples due to its inherent advantages. General advantages and disadvantages of the instrumentation have been outlined previously in section 1.1.4. Specifically, for the analysis of cocaine samples, NMR enables the differentiation between the hydrochloride and freebase form of cocaine.[41] This differs to GC-MS, which gives a single retention time and mass spectral data for both forms. The major disadvantage of low-field instrumentation is the lack of resolution, which can cause signal overlap for samples which contain complex mixtures.[36] For a number of previously identified samples which do not typically contain large amount of adulteration, quantification, using NMR, is simple and has been shown in a number of instances.[42, 111, 112] However, cocaine samples are typically adulterated and as such, represent a challenge for NMR to quantify successfully.[44]

A number of different groups have quantified cocaine samples using high field NMR instrumentation using different methods of quantification in tandem including NMR,[113-115], GC-MS,[116] GC-FID (gas chromatography – flame ionisation detector),[116] LC-MS-MS,[117] as well as partial least square approaches using Raman spectroscopy and FT-NIR.[118, 119] To the writer's knowledge, there has been no cocaine sample analysis performed using benchtop NMR. Thus, the quantification of cocaine samples by low-field (60 MHz) NMR is the focus of this chapter.

4.1.2. qNMR Literature Examples

Rocha *et al.,*[113] analysed 34 cocaine samples for both cocaine and five common adulterants, lidocaine, caffeine, phenacetin, procaine and benzocaine content % (w/w), using no-D qNMR (quantitative NMR) on a 400 MHz Varian Agilent spectrometer. Despite the report showcasing one of the advantages of qNMR - the ability to not need a cocaine reference standard - samples were analysed against a calibration curve using reference material and a full method validation was performed. Five solutions for each

analyte were prepared between 0 – 20 mg/mL for cocaine (assumed hydrochloride), 0 – 5 mg/mL for caffeine and 0 – 15 mg/mL for the other adulterants, with no explanation of the differences in concentration. Several robustness experiments were performed showcasing LoD and LoQ (Limit of Quantification) values between 0.06 – 0.67 mg/mL and 0.20 – 2.22 mg/mL respectively. Accuracy values between 91.8 – 127.1%, RSD (Relative Standard Deviation) values ranging between 0.00 – 4.83% with stability experiments showing consistent results seven days after analysis. Cocaine samples were extracted in 500 μL of MeOH at a concentration of 20 mg/mL, transferred into an NMR tube containing a coaxial tube with 80 μL of 15 mg/mL maleic acid (MA) in D_2O solution. The MA peak was used to normalise integrals against a fixed peak. T_1 experiments were performed prior to analysis to ensure complete longitudinal relaxation and ^1H acquisition was performed with 16 scans with a relaxation delay of 43.47 s. Between 1 – 3 individual regions were used for each analyte within the aromatic region of the spectra (6.27 – 8.53 ppm) with no overlapping peaks, showing good selectivity. 34 samples were analysed showing cocaine content between 0.0 – 94.9% with an average and median content of 17.5% and 13.3% respectively. Adulteration within the samples ranged between 0.5 – 73.6% (w/w) with the average and median content reported as 9.6% and 5.6% respectively. Samples were not verified against any other techniques.

Benedito *et al.*,[114] performed qNMR analysis, using a 600 MHz Bruker Avance spectrometer, for the quantification of cocaine hydrochloride / freebase with the common cocaine alkaloids, cis / trans-cinnamoylcocaine, within Brazilian cocaine samples. This method employs the use of pulse length-based concentration determination (PULCON), which in essence, requires a reference solution containing a known amount of internal standard and a sample solution to be analysed using identical conditions. The resulting integral values for each solution are then directly compared for quantification. Reference solutions contained either MA (20 mg/mL) or dimethylsulfone (DMS) (20 mg/mL) for samples prepared in D_2O or DMSO-d_6 respectively. Samples containing cocaine hydrochloride were prepared in D_2O spiked with TMSP (0.01% (w/w)) and cocaine freebase prepared in DMSO-d_6 spiked with TMS (0.01% (w/w)). 50 mg of sample was extracted into 600 μL of solvent before being vortexed for 1 minute to ensure complete dissolution then transferred to an NMR tube for analysis. ^1H NMR acquisition was achieved using 4 dummy scans, 32 scans with a

relaxation delay of 15 seconds for a total analysis time of 11 minutes and one environment was chosen for each analyte, free of overlap from adulteration. T_1 experiments were performed prior to analysis to ensure complete longitudinal relaxation. Several method validation experiments were performed, showing good accuracy (0.43% - 3.41% relative error), good precision over three replicates (0.73% - 2.69% RSD) and good sample stability over 24 hours (0.25% deviation). 15 cocaine hydrochloride (66.74 – 93.83% (w/w)) and 11 cocaine freebase (65.03 – 76.81% (w/w)) samples were analysed and cross-validated with analysis performed by GC-FID showing good correlation (0.9811 – 0.9928 R^2).

Mehr *et al*.,[115] produced an algorithm named *NMRquant.* This algorithm was used to quantify a selection of 15 illicit drug samples. The algorithm first identifies and separates out components within the mixtures by comparing the mixture spectra to a set of library spectra containing 30 commonly found drugs and adulterants. The spectrum with the highest similarity is then subtracted from the mixture with the algorithm. This was repeated until no further close library matches are found. Each mixture has a set of predetermined regions which are used for quantification. Peaks within this quantification region are deconvoluted to minimise peak overlap and shimming issues before being used for quantification against the reference library spectra, to obtain a preliminary purity value for the API within the sample. The algorithm then adjusts the reference shift of the spectrum, up to a tolerance of $\pm$0.08 ppm, calculating the mean and standard deviation purity values for each reference shift. The most consistent values are used and reported to the end user. ^{1}H NMR sample and reference acquisition were performed on a Bruker ascend 400 MHz instrument, using 8 scans with a relaxation delay of 30 s. 5 mg of reference material was dissolved in 600 μL of D_2O for the majority of library compounds, although $CDCl_3$ was used for water insoluble compounds. 15 samples were distributed and analysed between four different laboratories to validate the algorithm, showing high consistency between results (typically within 3% deviation).

These three groups showcase three alternative methodologies used in qNMR analysis: calibration curves generated using reference material, PULCON method using a set of internal standards within a co-axial tube and an automated script method, automatically analysing sample spectra against previously analysed reference material.

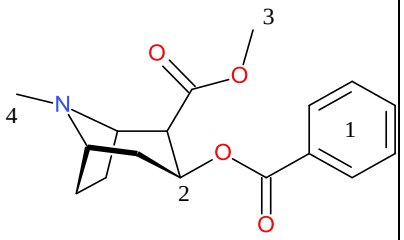
Each of these methods has their own advantages and disadvantages associated with them. The work described within this chapter showcases a combination of these methods. Including concentration determination using a calibration curve using reference material and a direct comparison against a spiked internal standard.

4.2. Results and Discussion

4.2.1. T_1 Relaxation Values

For accurate quantification using qNMR, the hydrogen environments must be completely relaxed to obtain accurate intergrals.[120] The relaxation times of the environments used for quantification are measured using a T_1 inverse-recovery experiment.[114] Typically 7 times the T_1 value calculated is used for 99.9% relaxation.[114, 120] T_1 values for each region of quantification for both cocaine hydrochloride and cocaine freebase, in the presence of adulteration is shown in Table 20 .

Table 20 – Low-field ^1H qNMR environments and their respective T_1 relaxation values for cocaine hydrochloride / freebase in the presence of D_2O / $CDCl_3$ soluble adulterants
† = splitting of signal, m = multiplet, q = quartet, s = singlet. Unadulterated cocaine was analysed at 20 mg/mL whereas adulterated analytes were 10 mg/mL for both cocaine and adulterant. 1 – 4 refers to the regions of quantification for cocaine hydrochloride, outlined on Figure 10.

Analyte	Shift (ppm)	Environment†		T_1 in the presence of adulterant as indicated (s) (10 mg/mL)					Structure (freebase form shown)
				None	Ketamine HCl	Procaine	Levamisole	Paracetamol	
Cocaine hydrochloride (D_2O)	7.32 – 8.17	(ArH) (m)	1	3.08	-	-	-	3.33	
	5.30 - 6.01	(CH) (q)	2	1.46	0.87	1.34	-	1.52	
	3.54 – 3.73	(OCH_3) (s)	3	2.02	1.42	-	1.13	1.68	
	2.66 – 2.92	($N\text{-}CH_3$) (s)	4	1.10	0.41	1.22	-	0.83	
Analyte	Shift (ppm)	Environment		None	Aspirin	Caffeine	Phenacetin	Benzocaine	
Cocaine freebase ($CDCl_3$)	7.82 – 8.29	(ArH) (m)	1	2.61	2.78	-	-	2.57	
	4.78 – 5.64	(CH) (q)	2	1.16	1.75	2.91	1.58	1.04	
	3.47 – 4.04	(OCH_3) (m)	3	1.74	-	1.86	2.09	-	

Since T_1 values can be dependent on temperature, concentration and solvent, multiple T_1 experiments were performed to ensure complete relaxation within binary mixtures. This includes extraction into two different solvents, both in concentrations typically seen for pure samples (20 mg/mL) and 50:50 binary mixtures (10 mg/mL). In adulterated samples, due to the low resolution compared to traditional high-field NMR, there are limited isolated signals for possible quantification. Fortunately, there is at least one environment present for the quantification of both forms of cocaine when present with each adulterant.

The addition of adulteration does not consistently alter the relaxation values, a consistent decrease in relaxation time is shown for ketamine, levamisole and benzocaine, whilst an increase in relaxation time is shown for aspirin, caffeine and phenacetin. Paracetamol shows a decrease in two of the four regions and an increase in the remaining two. The adulterated T_1 values were calculated using half the concentration of cocaine, compared to unadulterated references and since concentration effects relaxation times, no comparisons can be made due to differences in sample composition. For the purpose of method validation, the highest relaxation time calculated is for cocaine hydrochloride in the presence of paracetamol (3.33 s).

For the quantification of the adulterants present within cocaine samples, T_1 values must also be calculated for each environment used for quantification. This data is shown for adulterants soluble in D_2O and $CDCl_3$ in Table 21 and Table 22 respectively.

*Table 21 - Low-field ^1H qNMR environments and their respective T_1 relaxation values for D_2O soluble cocaine adulterants - * denotes a peak which was overlapped with solvent peak so cannot be determined † = splitting of signal, m = multiplet, q = quartet, s = singlet. 1 - 3 refers to the regions of quantification used for respective water-soluble analytes*

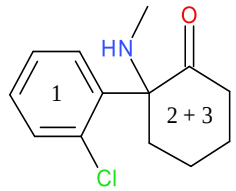
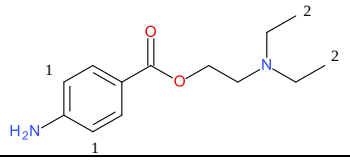
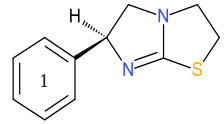
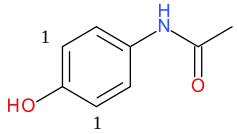
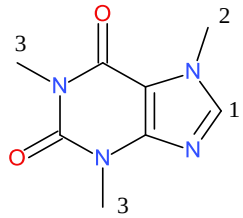
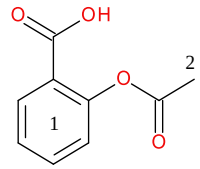
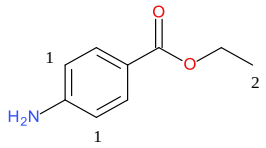
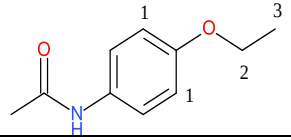
Analyte	Shift (ppm)	Environment†	DMSO-d_6	D_2O		Structure
			T_1 (s) (20 mg/mL)	T_1 (s) (20 mg/mL)	T_1 (s) (10 mg/mL)	
Ketamine HCl	7.36 – 8.25	1 (ArH) (4H m)	1.48	3.08	-	
	2.29 - 2.84	2+3 (5H m)	*	1.27	-	
	1.49 - 2.29	2+3 (5H m)	0.51	0.79	0.49	
Procaine	6.54 - 7.13	1 (ArH) (d)	1.34	3.44	3.66	
	1.05 - 1.67	2($2xCH_3$) (t)	0.85	1.66	1.74	
Levamisole	7.22 - 7.81	1 (ArH) (m)	2.43	4.38	-	
Paracetamol	6.67 - 7.06	1 (ArH) (d)	1.73	5.37	4.33	

Table 22 - Low-field ^1H qNMR environments and their respective T_1 relaxation values for $CDCl_3$ soluble cocaine adulterants † = splitting of signal, m = multiplet, q = quartet, s = singlet
1 – 3 refers to the regions of quantification for respective chloroform-soluble adulterants

Analyte	Shift (ppm)	Environment†	DMSO-d_6	$CDCl_3$		Structure
			T_1 (s) (20 mg/mL)	T_1 (s) (20 mg/mL)	T_1 (s) (10 mg/mL)	
Caffeine	7.36 - 7.67	1 (CH) (s)	4.97	3.49	-	
	3.84 - 4.21	2 (CH_3) (s)	1.89	2.28	2.16	
	3.01 - 3.69	3 (2xCH_3) (2s)	1.87	2.24	-	
Aspirin	7.88 - 8.41	1 (ArH) (4H m)	3.07	2.52	-	
	1.81 - 2.79	2 (CH_3) (s)	1.83	1.87	-	
Benzocaine	6.26 - 7.00	1 (ArH) (d)	1.56	2.78	3.19	
	1.01 - 1.56	2 (CH_3) (t)	2.09	2.24	2.47	
Phenacetin	6.53 - 7.06	1 (ArH) (dd)	1.73	2.67	-	
	3.73 - 4.33	2 (CH_2) (q)	1.38	2.12	-	
	1.81 - 2.39	3 (CH_3) (s)	1.00	1.91	-	

T_1 values were not calculated for environments (1 – 4*, Figure 11) which overlapped between cocaine and its respective adulterant, since quantification would not be possible using low field qNMR. This results in three adulterants, levamisole, aspirin and phenacetin which are unable to be quantified in the presence of cocaine using the low field qNMR outlined herein. Fortunately, in the presence of these adulterants, there are still environments which can be used to quantify cocaine, as shown in Table 20. This only applies to low-field qNMR; conventional high-field NMR (≥ 400 MHz) provides enough resolving power so that all sites of quantification are available within binary mixtures. Exemplar spectra showcasing binary mixtures containing cocaine hydrochloride and respective adulterants, which are readily soluble in D_2O, are shown in Figure 11, with the regions used for quantification outlined.

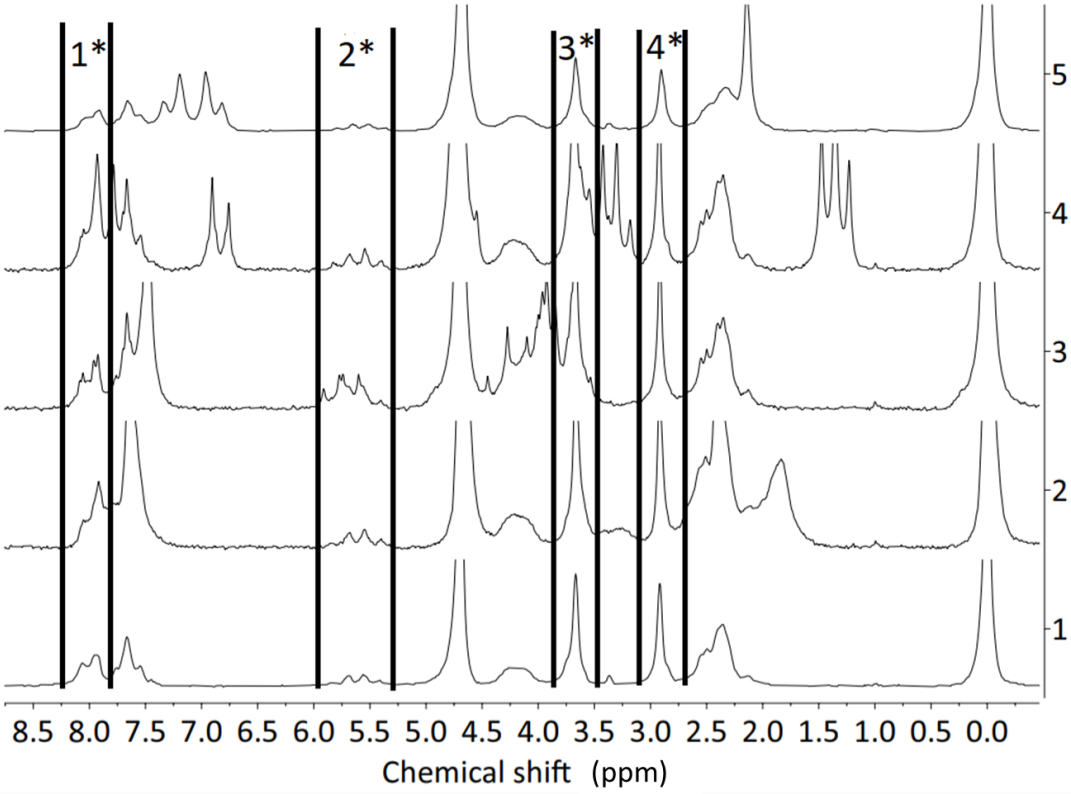


Figure 11 - Exemplar low-field NMR spectral data for cocaine hydrochloride (1) (20 mg/mL) and binary mixtures of cocaine hydrochloride (10 mg/mL) and water-soluble adulterants (10 mg/mL) consisting of ketamine hydrochloride (2), levamisole (3), procaine (4) and paracetamol (5). 1-4 represent regions of quantification used for cocaine hydrochloride. Peak at δ 0 represents TMSP (1 mg/mL).*

Similar exemplar spectral data is shown in Figure 12, for cocaine freebase in $CDCl_3$ and binary mixtures of cocaine freebase and $CDCl_3$ soluble adulterants.

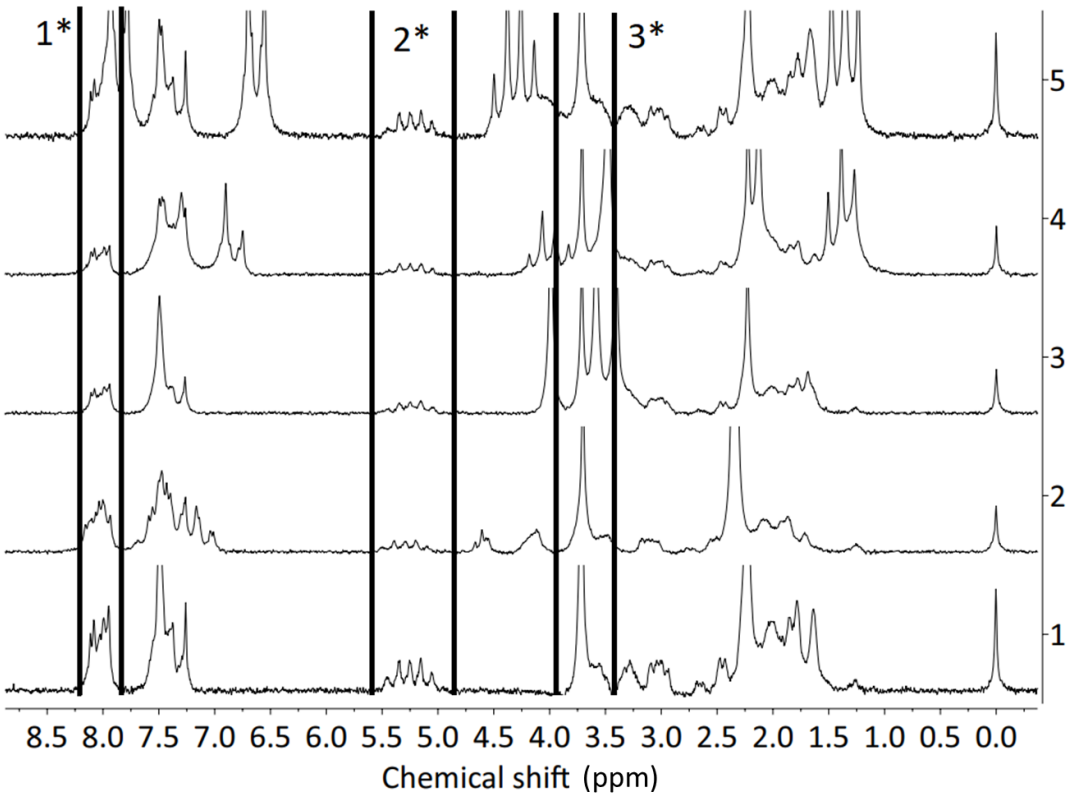


Figure 12 - Exemplar low-field NMR spectral data for cocaine freebase (1) (20 mg/mL) and binary mixtures of cocaine freebase (10 mg/mL) and chloroform-soluble adulterants (10 mg/mL) consisting of aspirin (2), caffeine (3), phenacetin (4) and benzocaine (5). 1-3 represent regions of quantification used for cocaine freebase. Peak at δ 0 represents TMS (1 mg/mL).*

Typically speaking, the more viscous the solvent used, the shorter the NMR relaxation times of nuclei environments.[121] This is reflected for the majority of signals, when comparisons are made between cocaine adulterants at the same concentrations in DMSO-d_6 (2.007 cP at 25˚C)[122] compared to less viscous D_2O (1.2514 cP at 20˚C)[123] and $CDCl_3$ (0.5357 cP at 25˚C)[124]. Comparisons cannot be made with cocaine in both hydrochloride and freebase form, due to inadequate solubility in DMSO-d_6. This general trend follows across all adulterants, with the exception of caffeine (environment 1) and aspirin (environment 1) which may have changed due to additional solvent interactions.

Aromatic hydrogen environments, for the compounds analysed, typically possess T_1 values of 2.52 – 2.78 s in $CDCl_3$ and 3.08 – 3.44 s in D_2O. Notable exceptions include levamisole (4.38 s) and paracetamol (5.37 s) which are notably longer than other similar environments. CH_3 and CH_2 groups across cocaine and all adulterants, typically range between 1.87 – 2.28 s in $CDCl_3$ and 1.66 – 2.02 in D_2O with two notable exceptions. The CH_2 groups with the 6 membered ring on ketamine, (environment 2

and 3) have lower relaxation times of 0.49 – 0.79 s, due to a complex system of coupling and potential hydrogen bonding interactions which causes these groups to appear as indistinguishable multiples in low-field NMR. The N-CH_3 group in cocaine hydrochloride has a lower relaxation time (1.10 s) in D_2O, this is likely due to the protonation of the nitrogen atom.

The environment with the longest T_1 relaxation (5.37 s) belongs to paracetamol when dissolved in D_2O. To accommodate for this, the relaxation delay for spectral acquisition should be set to a minimum of 37.6 seconds to ensure repeatable quantification. Quantification was initially planned in DMSO-d_6, due to the initial screening the qualitative "NMR drug detector" identification software, however, the solubility is not high enough for use for a full method validation. The use of two solvents was thus employed, for both the hydrochloride and freebase forms of cocaine, due to their high respective solubilities in D_2O and $CDCl_3$ respectively. Similar relaxation delays of 40 and 30 s was used by Rocha *et al.* and Mehr *et al.* for similar qNMR quantification of cocaine samples.[113, 115] Benedito *et al.,*[114] used a shorter relaxation delay of 15 s, assumingly due to the high concentration of analysis (83.3 mg/mL) causing shorter T_1 relaxation times.

A small study on both the hydrochloride and freebase forms of cocaine were performed to assess the difference in T_1 values dependant on temperature. This is important when applying the method for use in high-field spectrometers which typically stay around room temperature, whereas low-field spectrometers operate higher than room temperature and are more influenced by their surrounding temperatures.[125] This is important for off-site analysis, which could be warmer or colder than typical laboratory-controlled environments. T_1 experiments were repeated between 25 – 40 degrees on a high-field spectrometer. The high field was used due to the ability to control the temperature of the sample. This data is shown graphically in Figure 13.

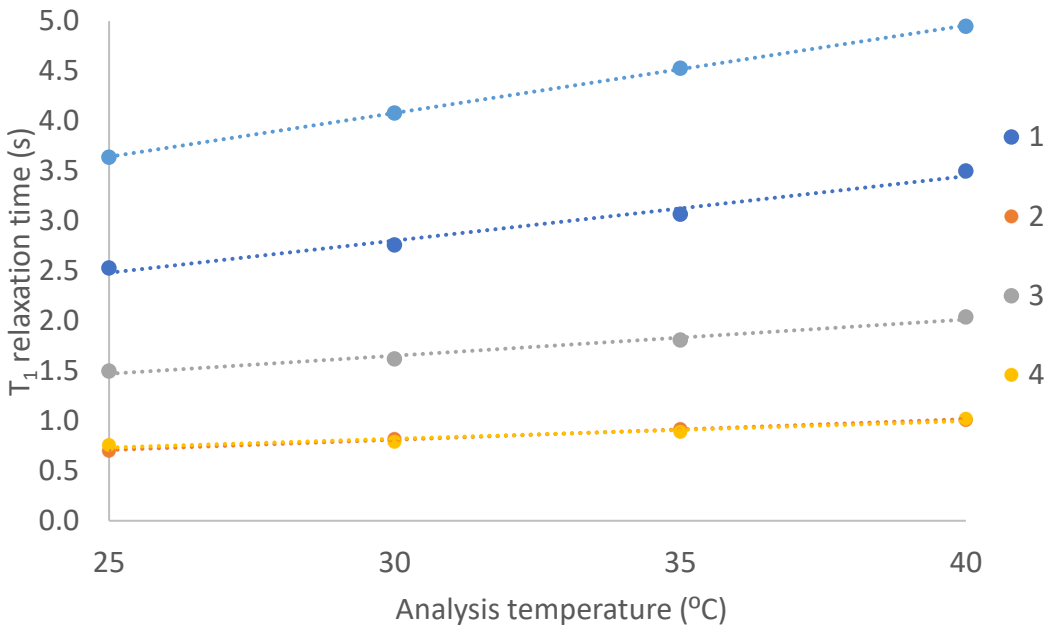


Figure 13 - T_1 relaxation times for cocaine hydrochloride in D_2O across variable temperatures (25 - 40 ˚C) 1 – 4 refers to the regions of quantification for cocaine hydrochloride, outlined on Figure 10.

The T_1 relaxation times for cocaine hydrochloride in D_2O increase in a linear relationship between 25 – 40˚C matching findings shown within literature.[126] The T_1 relaxation values for cocaine freebase were also assessed, which showed no significant difference between the relaxation values assessed between 25 – 40˚C. These differences are due to fewer interactions between non-polar chloroform and cocaine freebase, compared to polar water and cocaine hydrochloride. A full assessment of what is causing these differences, is outside of the scope of simply choosing a relaxation delay for quantification. Only a small range of temperatures were assessed based on typical laboratory temperature (25˚C) and up to 40˚C, limited by the boiling point of $CDCl_3$ (60.9 °C).[127]

From extrapolation using the trendline belonging to the signal with the longest relaxation values, which is TMSP for cocaine hydrochloride, the maximum temperature in which analysis can occur is 48.6˚C.

4.2.2. Number of Scans Determination – Signal to Noise Determination

To determine the number of scans needed for spectral acquisition, a number of ^1H NMR spectra were acquired with a varying number of scans and the SINO for each peak was calculated. On a sample containing reference standard cocaine hydrochloride and

reference cocaine freebase at the appropriate concentrations for analysis (20 mg/mL), acquisition was performed using a varying number of scans between 1 and 128, SINO of the environments used during quantification calculated. A graphical form of this data is shown for low-field analysis in Figure 14.

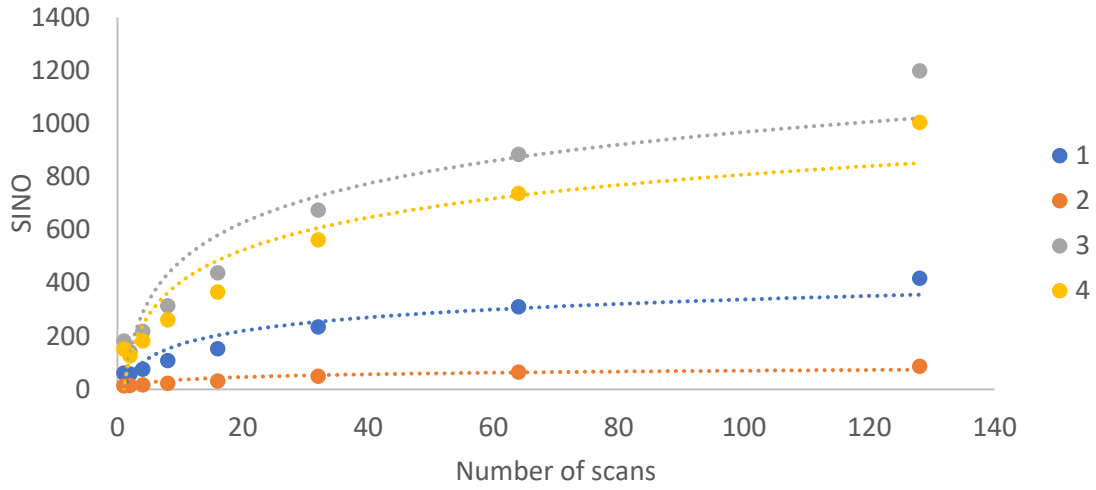


Figure 14 - Signal-to-noise ratio for low-field analysis of each signal used for quantification for cocaine hydrochloride. 1 – 4 refers to the environments used for quantification for cocaine hydrochloride, outlined on Figure 10.

The minimum signal to noise (SINO) required for reliable quantification should be at least 10 for most analytical methods following the ICH (International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines for method validation.[80] However for qNMR, literature guidelines show different minimum arbitrary SINO values, mainly between 100 – 250 since previous literature shows that SINO less than 86 produce data with RSD values greater than 1 % deviation.[120] For traditional high field NMR, typically the higher resolution and sensitivity produces signals with a much higher SINO compared to low field NMR. This means that, for high field NMR, even with a single scan, there is sufficient signal-to-noise for three out of four environments used for quantification for cocaine hydrochloride and all the environments for cocaine freebase. However, due to the employment of low field NMR, eight scans were chosen for analysis, since three out of the four of environments have a SINO ratio greater than 100. This brings total analysis time to roughly five minutes per spectral acquisition and due to the relationship between SINO and number of scans increasing with a factor of $\sqrt{x}$, the improvement in SINO would not warrant the additional acquisition time needed.

Acquisition time should also be kept to a minimum due to the use in large scale of samples within routine analysis.

4.2.3. TMSP Solubility Study – Internal Standard Concentration

When preparing the initial stock solution of spiked deuterated solvent, TMS and TMSP were chosen for their single singlet environment, which has a large relative integral and does not overlap with any of the environments of cocaine. When spiking $CDCl_3$ for the analysis of freebase samples, care must be taken during transfer since TMS is extremely volatile and volumes should be used opposed to weights, to minimise loss of material. For TMSP, since the material is a solid, initial spiking of the deuterated solvent is less troublesome, however, care must be taken to ensure the solid has suitably dissolved. Unlike other solids, where it may be more apparent if material is undissolved, TMSP appears close to translucent when submerged in liquid and can adhere to glass, making it difficult to spot. Therefore, a number of acquisitions were made using a series of solutions containing D_2O with a varying amount of TMSP spiked between concentrations of 0.1 – 10.0 mg/mL. These solutions were prepared in duplicate with one set syringe filtered before analysis and another set left unfiltered.

In general, across the concentrations of TMSP assessed, trendline equations have similar gradients. Between 0 – 2 mg/mL the trendlines overlap closely, from 2 mg/mL onwards, the difference between the two trendlines increases, implying that the solution starts to become saturated at concentrations higher than 2 mg/mL. 1 mg/mL was chosen as the concentration used for analysis, since TMSP is freely soluble at that concentration in D_2O and the height and integrals of the TMSP peak, are closely related to the reference material at the concentration of analysis (20 mg/mL). TMS was not assessed since it is a liquid and as such should not have issues with solubility; a concentration of 1 mg/mL of TMS was also chosen to keep consistency between the two internal standards.

4.2.4. Analyte Solubility

Due to the inherent sensitivity issue with NMR, analyte solutions should prepared to a much higher concentration compared to analysis using other techniques. For this, analytes need to be soluble at these high concentrations. Although the literature has reported solubilities on a wide range of substances commonly used as cocaine

adulterants,[31, 44, 128] a large number of the literature reports the solubility values as a descriptive term which falls into a range of solubility values. The definitions commonly used by the United States Pharmacopeia (USP) are shown in Table 23.

Table 23 - USP definition of solubility terms.[129]

Descriptive term	Solubility range (mg/mL)
Very soluble (vs)	≥ 1000
Freely soluble (fs)	100 – 1000
Soluble (s)	33 – 100
Sparingly soluble (sps)	10 – 33
Slightly soluble (ss)	1 – 10
Very sightly soluble (vss)	0.1 – 1
Practically insoluble (pi)	< 0.1

Due to the potentially large range of concentrations associated with these terms, small solubility studies were carried out to assess which solvents would be suitable for each analyte. A number of dissolution experiments were performed between 0 – 30 mg/mL in increments of 5 mg/mL for each analyte in both water and chloroform. This data is shown in Table 24.

Table 24 - Solubility ranges of common cocaine component analytes in both water and chloroform (mg/mL)

Analyte	Max dissolution concentration (mg/mL)		Reference solubility at 25 ˚C
	Water	Chloroform	
Aspirin	0	25 - 30	"Soluble" ($CHCl_3$)[25]
Benzocaine	0	25 - 30	517.9 mg/mL ($CHCl_3$)[26]
Caffeine	0	25 - 30	183.3 mg/mL ($CHCl_3$)[27]
Cocaine freebase	0	25 - 30	1490 mg/mL ($CHCl_3$)[27]
Phenacetin	0	25 - 30	71.0 mg/mL ($CHCl_3$)[27]
Paracetamol	0	0	17.4 mg/mL (H_2O)[28]
Cocaine hydrochloride	25 - 30	0	2500 mg/mL (H_2O)[27]
Procaine HCl	25 - 30	0	"Very soluble" (D_2O)[130]
Tetramisole HCl	25 - 30	0	"Freely soluble" (D_2O)[29]
Ketamine HCl	25 - 30	0	"Freely soluble" (D_2O)[131]

With the exception of paracetamol, every analyte is soluble enough for complete extraction into either water or chloroform at the 0 – 25 mg/mL calibration range. Some of these analytes could be potentially more soluble than stated but this cannot be confirmed as no higher concentrations were tested. Judging from the solubilities of these analytes identified within the literature, all of the solubilities either match or are

higher than the solubility range required for a 0 – 25 mg/mL calibration range. In actuality, paracetamol solubility will not be 0 mg/mL which implies complete insolubility but less than 5 mg/mL due to 5 mg/mL increments used. Differences in solubility are most likely due to changes in acidity of the D_2O compared to H_2O used in literature and potential temperature differences.

4.2.5. Sample Stabilities

Both on-site and off-site drug sample testing benefit from immediate sample testing to ensure high analytical reliability and to report the sample identification to end users at events. Sample stabilities were assessed in the case of a "worst case scenario", either a large sample backlog, instrumentation failure or replicate analysis after the event.

A sample containing reference cocaine hydrochloride was produced at medium concentration (9.2 mg/mL) and was analysed each day to measure the stability of the solution. Quantification was achieved using molar ratios compared to the spiked TMSP internal reference standard, with more explanation on the purity calculation provided in section 4.2.6 - NMR Calibration Low verses High-field – Cocaine hydrochloride in D2O. Spectral acquisition was kept consistent throughout with the sample stored within the high field autosampler rack, exposed to light and laboratory room temperature throughout 11 consecutive days. This data is shown in Figure 15.

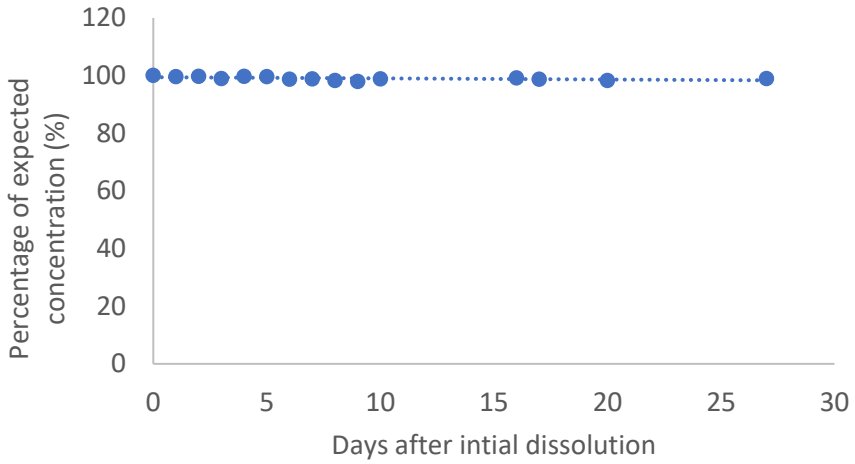


Figure 15 - Cocaine hydrochloride spiked with TMSP in D_2O, sample stability determination across 28 days

For the cocaine hydrochloride sample stability, the calculated concentration across 27 days shows a slight negative trendline, implying small sample degradation over time. The majority of the readings, with the error's bars showing RSD, show overlap across

the full 27 days, with the errors of analysis increasing noticeably after 10 days. The first instance of deviation from 100% expected concentration was on day 6. This showcases that analysis of these samples should be performed immediately but in the case of a large number of samples or instrumentation failure, samples will be stable for up to six days under laboratory conditions.

The same experiment was repeated for cocaine freebase, spiked with TMS in $CDCl_3$, the results of this are shown in Figure 16.

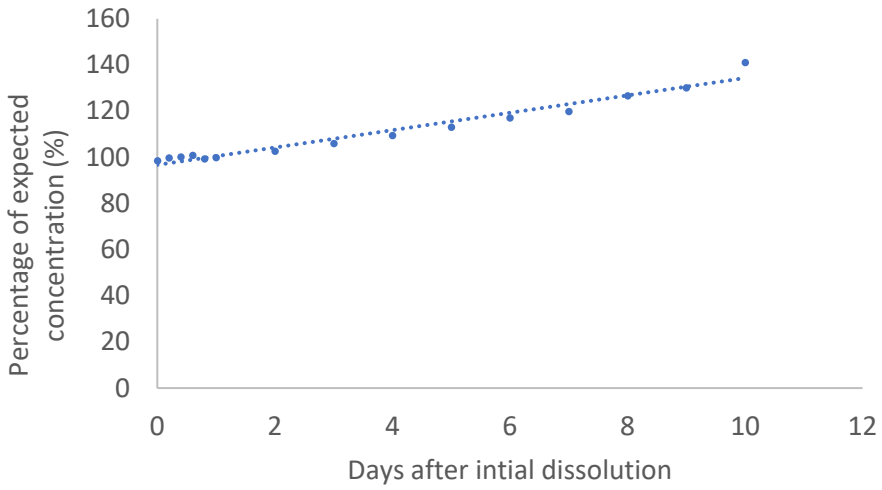


Figure 16 - Cocaine freebase spiked with TMS in $CDCl_3$, sample stability determination across 11 days.

The concentration determinations increased in a linear trendline across the 11 days of analysis with no significant change in errors throughout analysis. This increase is due to the chloroform within the sample evaporating over time, decreasing the volume of the sample and artificially increasing the concentration of the sample. To maintain consistency and accuracy to routine sample analysis, the sample showcased was capped with a standard NMR tube cap with no other provisions to prevent evaporation. Extra points of analysis were performed at equal spaces throughout the first day, showing consistent concentration calculations within errors. At the start of day 2, the percentage increased to 102.7% indicating that samples extracted into $CDCl_3$ should be analysed within the same day to ensure accurate data or contain samples within a sealed system e.g., a Young's capped tube to minimise solvent evaporation.

A more accurate method of confirmation is to achieve forced degradation of the sample and identify potential degradants. However, this is unsuitable for this type of work due to the inherent insensitivity of the NMR being unable to detect such low

concentrations of degradation products. Also, previous reports by Bijlsma *et al.,*[132] outlined the degradation products of cocaine in water, implying the major degradant is benzoylecgonine, which is formed from hydrolysis of the ester of cocaine but no evidence of this was identified, either due to the amount being smaller than the limit of detection, or being undetectable due to possessing similar NMR signals to those of cocaine hydrochloride. Liquid chromatography – quadrupole – time-of-flight mass spectrometry (LC-qTOF) analysis is typically required due to the low concentrations of degradants.[132]

A number of small experiments have been performed to assess the robustness of the analytical method. This can identify if small changes within sample analysis or sample preparation has any effects on the overall sample concentration determination.

Changes in temperature during analysis may occur which is more prominent for on-site testing which may not be within a temperature-controlled environment. On-site analysis can be subjected to a range of temperatures, influenced heavily by seasonal and weather changes. These changes are an issue especially for benchtop NMR spectrometers which are influenced by outside environmental temperatures.[125] These changes in temperatures may affect the temperature of the samples, which may change the relaxation times of the ^{1}H environments. Unfortunately, due to instrumentation restrictions of the benchtop NMR used for this analysis, with no simple method of variable temperature analysis, these robustness experiments were performed on high-field instrumentation.

A QC sample of medium concentration was selected from the calibration curve (9.9 mg/mL) and was repeatedly analysed against a single point reference standard, analysed at 25 ^{o}C, repeated at different temperatures and calculated against spiked TMSP. This data is shown in Table 25.

Table 25 - Analysis of a QC sample of medium concentration cocaine hydrochloride (9.9 mg/mL) at variable temperatures (25 - 40˚C) "External" purity values are quantified against reference material, "internal" purity values are quantified against an internal standard (TMSP)

	External		Internal	
Temp	Average	RSD (%)	Average	RSD (%)
25	100.8	0.66	99.45	0.85
30	100.43	0.86	99.87	0.11
35	100.7	0.52	100.14	0.64
40	100.58	0.83	100.02	0.43

Analysis between 25 – 40˚C shows no significant change of concentration determinations within this temperature range, since all values, calculated both externally and internally fall within the reported errors. An assessment of temperatures lower than 25 ^{o}C could be useful as the majority of benchtop NMR spectrometers are only designed for use between 18 – 30 ^{o}C.[133] Lower temperatures may cause shimming issues as permanent magnets are more susceptible to temperature changes.[133] Although a small selection of spectrometers are rated up to 60 ^{o}C, environmental temperatures greater than 40 ^{o}C would not only influence instrumental shimming but influence other analysis parameters.[133] As discussed in section 4.2.1 - T_1 Relaxation Values, temperatures greater than the calculated temperature 48.6 ^{o}C will cause incomplete relaxation of the TMSP internal standard for analysis in D_2O. This may cause inaccurate concentration determinations at higher temperatures. Analysis in $CDCl_3$ will also be affected as the boiling point of $CDCl_3$ is 60.9 ^{o}C.[127] Subjecting samples above the boiling point may cause faster solvent evaporation, an issue which has previously been outlined in Figure 16, solvent bubbling which will affect shimming quality and potential evaporation of the volatile TMS internal reference standard used for $CDCl_3$ analysis.

The influence that the quality of the NMR tubes used was assessed by comparing concentration determinations of identical samples analysed between 200 MHz economy tubes vs 400 MHz rated standard tubes, results of which are shown in Table 26.

Table 26 - Sample content determination for a reference solution of cocaine hydrochloride (20 mg/mL) in D_2O against a synthesised sample solution at medium (10.0 mg/mL) concentration – "Med" (10 mg/mL) calculated against both a reference standard - "Reference" (20 mg/mL) and against spiked TMSP (1 mg/mL)

Field Strength	Standard	Content % (w/w) - Std	Content % (w/w) - IS
400 MHz	Reference	100.00 ± 2.12	100.00 ± 2.13
	Med	95.40 ± 2.12	95.68 ± 3.30
200 MHz	Reference	100.11 ± 3.36	100.42 ± 3.52
	Med	95.82 ± 3.26	96.11 ± 3.38

The samples analysed between the different field strength rated tubes show no significant differences in concentration determination, outside of error values. This is expected since the resolution of the low-field instrumentation (60 MHz) is lower than the rating of the low-field tubes (200 MHz).

4.2.6. NMR Calibration Low verses High-field – Cocaine hydrochloride in D_2O

Quantitative analysis using NMR can be achieved using two different methods, the more traditional method producing a calibration curve is one method whilst the other method is comparing the integrals with a peak of known spiked reference material. This works since the integrals are proportional to the number of nuclei within the environment.[134] The initial calibration is plotted for the ratio between the integrals for the peak of quantification and the integral for TMSP against concentration of cocaine hydrochloride. Although NMR is a robust technique, the ratios of analyte : internal standard were used to minimise errors from the less sensitive low field instrumentation. A graphical plot of these calibration curves is shown in Figure 17, with a table of the respective validation parameters detailed in Table 27.

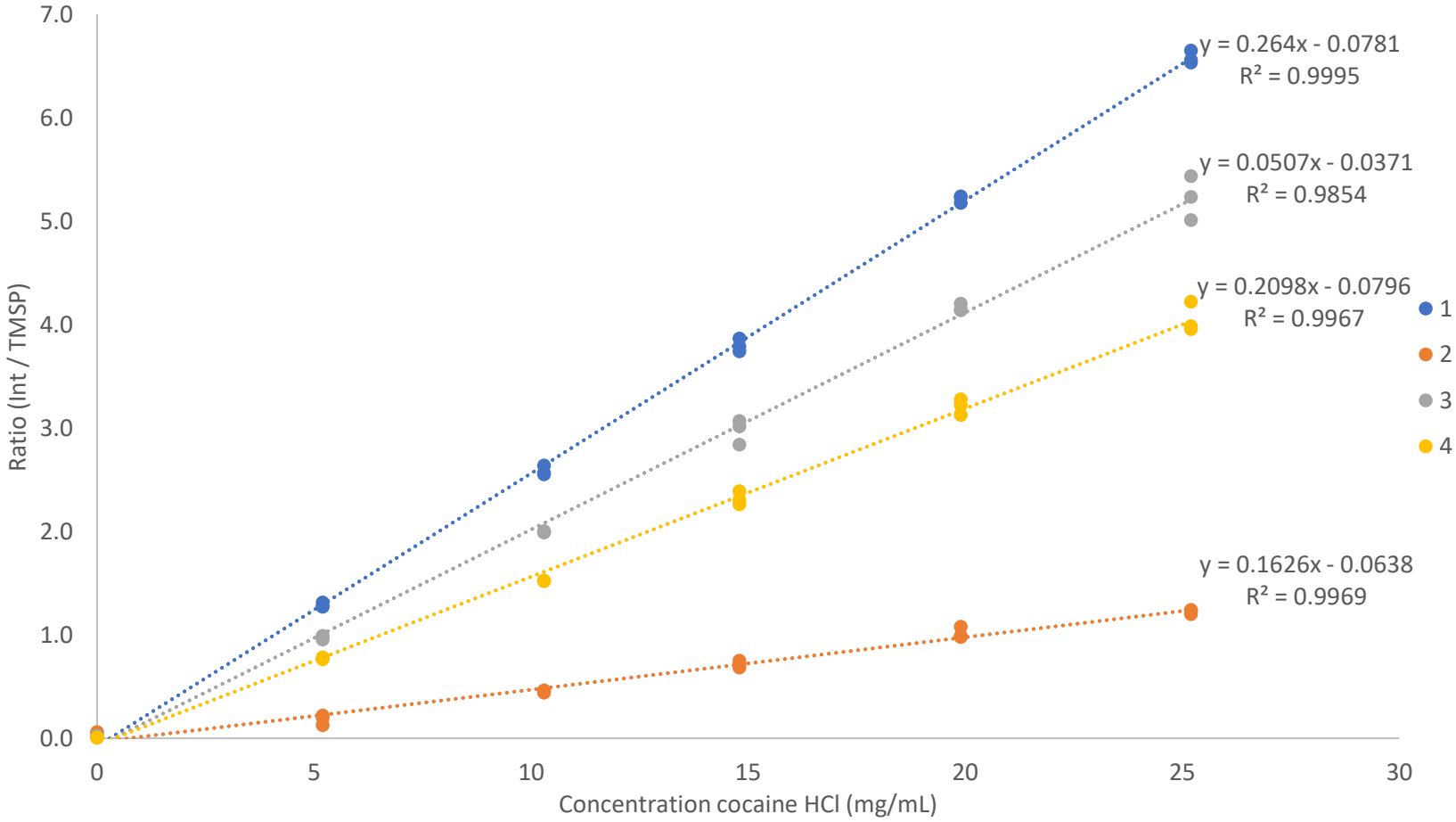


Figure 17 - Calibration plot of 0 - 25 mg/mL cocaine hydrochloride in D_2O analysed using low-field NMR. 1 – 4 refers to the regions of quantification for cocaine hydrochloride, outlined on Figure 10.

Table 27 - Calibration parameters for cocaine hydrochloride in D_2O analysed using low-field NMR. 1 – 4 refers to the regions of quantification for cocaine hydrochloride, outlined on Figure 10.

Environment		LoD (mg/mL)	LoQ (mg/mL)	R^2	Av. Accuracy (%)	Av. RSD (%)	Recovery range – Ext. (%)	Recovery range – Int. (%)
Aromatic	1	0.289	0.875	0.9995	99.52	1.53	88.61 – 102.25	87.30 – 100.74
Tropane CH	2	1.526	4.625	0.9854	96.25	11.17	91.57 – 110.95	79.91 – 110.09
O-CH_3	3	0.724	2.193	0.9967	98.66	3.14	87.12 – 105.97	89.25 – 108.56
N-CH_3	4	0.693	2.100	0.9970	98.92	3.08	86.37 – 108.54	95.30 – 119.76

Linearity values for all but the tropane CH environment are acceptable by the UNODC guidelines for method validation (> 0.99), aided by the inclusion of the internal standard between points to produce a ratio, minimising the spread of data produced from the low field instrumentation.[135] The average accuracy and spread of data on each calibration point has less than 5% deviation, except for the tropane CH environment, implying that this environment should be used only if no other alternative is available. This insensitivity is due to both low-field instruments having an inherent lack of sensitivity due to lower resolution as well as this environment consisting of just a single proton as well. The insensitivity is made worse due to the D_2O solvent peak, causing slight baseline interference.

The UNODC guidelines for validation currently define the LoD and LoQ for spectroscopy techniques such as NMR, as “the minimum concentration which can be defined with confidence”.[135] The UNODC guidelines state that 3.3 x SINO and 10 x SINO are given as the LoD and LoQ respectively.[135] The guidelines for if a calculated LoD and LoQ value is acceptable for a given method, is identified if it is ’fit for purpose’.[135] This may be open to interpretation by the analyst but ideally the majority of the samples which are analysed should be above the LoQ. European seizures (2022) described an average purity range between 52 – 83% (w/w), [61] whereas smaller studies have identified ranges in higher extremities (1 – 95% (w/w)).[62]

The minimum theoretical percentage content at the LoQ for this method, based on a 20 mg/mL sample solution, is between 4.38 – 23.12 % (w/w).[135] This is calculated from the LoD (mg/mL) converted to content of cocaine hydrochloride (% (w/w)) within a sample at concentration of analysis (20 mg/mL). Not including the tropane CH environment, which is not being used due to poor linearity, the LoQ range narrows to 4.38 – 10.97% (w/w). Table 33, showcased later within this chapter, outlines cocaine

sample purities used within this technique study. Out of the full dataset used, only two samples (2.2%) were below the LoQ range (10.97 % (w/w) cocaine content), showing good suitability for this methodology. The LoD and LoQ for this technique cannot be related to the LD_{50} for cocaine due to reasoning that adulteration, prior health conditions and drug interactions are typically the main cause of cocaine related deaths, as described in section 1.1.2 - Cocaine.[70]

QC samples were assessed at low, medium and high concentrations throughout the calibration curve (25%, 50% and 75% respectively) which show a range of analyte recovery values within the acceptable recovery range (80 – 120%). These recovery samples were prepared using reference cocaine hydrochloride material as well as a portion of lactose, to simulate adulteration. Selecting one specific adulterant or filler used to simulate cocaine samples is inherently difficult due to the wide range of potential compounds which could be used. Lactose was chosen as a filler for these QC samples, due to the similarity in appearance to cocaine. It is worth noting, that these recovery values are calculated using a single point reference standard similarly to how samples are analysed. This allows the recovery values to showcase errors which occur from use of a single point standard opposed to a full calibration series.

The concentration of the low, medium and high QC samples was also calculated against the molar ratio of the spiked TSMP internal standard, to assess the accuracy of the internal standard method. The sample purity (content % (w/w)) was calculated following Equation 3.

$$P_x = \frac{Int_x}{Int_{IS}} \times \frac{H_{IS}}{H_x} \times \frac{M_x}{M_{IS}} \times \frac{W_{IS}}{W_{Sample}} \times P_{IS}$$

Equation 3 – Sample content (% (w/w)) for qNMR analysis using direct molar ratios against spiked internal standard x = analyte, IS = internal standard, Int = integral area, H = number of hydrogen nuclei, W = mass (mg)

The main advantage of calculating purity (% (w/w)) of samples using this method is that a reference standard of the analyte is not required for analysis. However, with this method there is no way of identifying inaccuracies within analysis, since it is assumed that if a reference standard is not available, then QC samples cannot be accurately produced. The concentration determination for the QC samples, shown in Table 27, show acceptable agreement to the true value across the 25 – 75% of the calibration range.

Although the main focus of this research is the creation and validation of a method used for low-field NMR spectrometers, the method is transferable onto high-field instrumentation, which has many benefits including, typically autosampler access, higher resolution to distinguish between analytes in samples mixtures and a greater limit of detection. The same calibration solutions were analysed and the calibration is showcased in Figure 18 with the validation parameters outlined in Table 28.

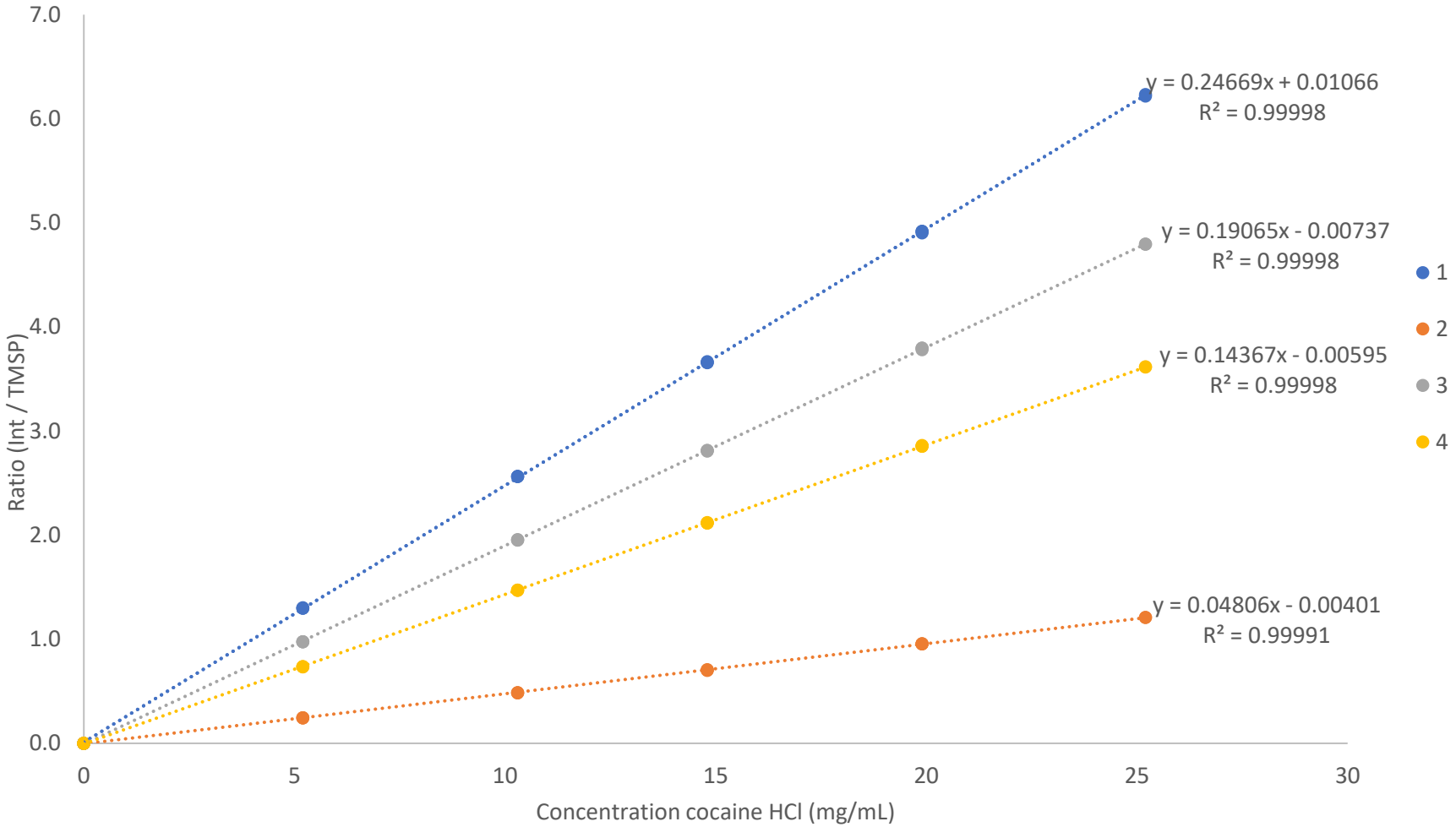


Figure 18 - Calibration plot of 0 - 25 mg/mL cocaine hydrochloride in D_2O analysed using high-field NMR. 1 – 4 refers to the regions of quantification for cocaine hydrochloride, outlined on Figure 10.

Table 28 - Calibration parameters for cocaine hydrochloride in D_2O analysed using high-field NMR. 1 – 4 refers to the regions of quantification for cocaine hydrochloride, outlined on Figure 10.

Environment		LoD (mg/mL)	LoQ (mg/mL)	R^2	Av. Accuracy (%)	Av. RSD (%)	Recovery range – Ext. (%)	Recovery range – Int. (%)
Aromatic	1	0.054	0.163	0.99998	100.18	0.31	87.97 – 101.13	88.23 – 101.43
Tropane CH	2	0.093	0.281	0.99991	99.72	0.63	87.85 – 101.17	85.18 – 98.10
O-CH_3	3	0.051	0.156	0.99998	99.81	0.39	87.03 – 100.06	84.58 – 97.24
N-CH_3	4	0.047	0.143	0.99998	99.85	0.35	87.38 – 101.17	88.10 – 102.00

The samples analysed by both low-field and high-field NMR analysis were identical, with the low-field analysis performed first followed by subsequent analysis by high-field the same day. Since these are the same sample solutions, any differences within the parameters, between Table 27 and Table 28, are assumed to be caused solely by the spectrometer used for analysis. The LoQ for the high-field analysis has around eight times more sensitivity compared to low field, reducing the minimum theoretical percentage content % (w/w), based on a 20 mg/mL sample, to 0.82 – 1.41%, which should be more than adequate for bulk sample analysis of this nature. Linearity for each environment is extremely linear and showcases an improvement compared to low-field analysis, this is due to the increase in precision across the three replicate spectral acquisitions for each data point. Average accuracy and RSD values for each calibration point are reflective of the increased precision of the high-field instrumentation compared to low-field, with accuracy values showing closeness to 100% and typically a small spread of data around each point. The recovery values for high-field analysis have a slightly smaller range compared to low-field analysis. This could be due to the decreased repeatability compared to high-field analysis, producing a wider range of concentration determinations, or could be due to over or underestimation from this technique, due to the use of unresolved peaks.

The single ^1H NMR environment on the tropane ring, which was deemed unsuitable for low-field analysis is more than adequate for high field analysis.

4.2.7. NMR Calibration Low verses High-field – Cocaine freebase in $CDCl_3$

The calibration series and validation parameters were repeated for reference cocaine freebase in $CDCl_3$, spiked with TMS instead of TMSP. The use of TMS as a reference standard for quantitative analysis is not ideal due to its high volatility. This can be minimised with careful spiking of the initial sample stock solution, by transfer of the TMS and measurements based on volume opposed to weight, directly into a partially filled volumetric, minimises the potential for evaporation.

A calibration curve containing cocaine freebase spiked with TMS for low-field analysis is shown in Figure 19, with the validation parameters detailed in Table 29.

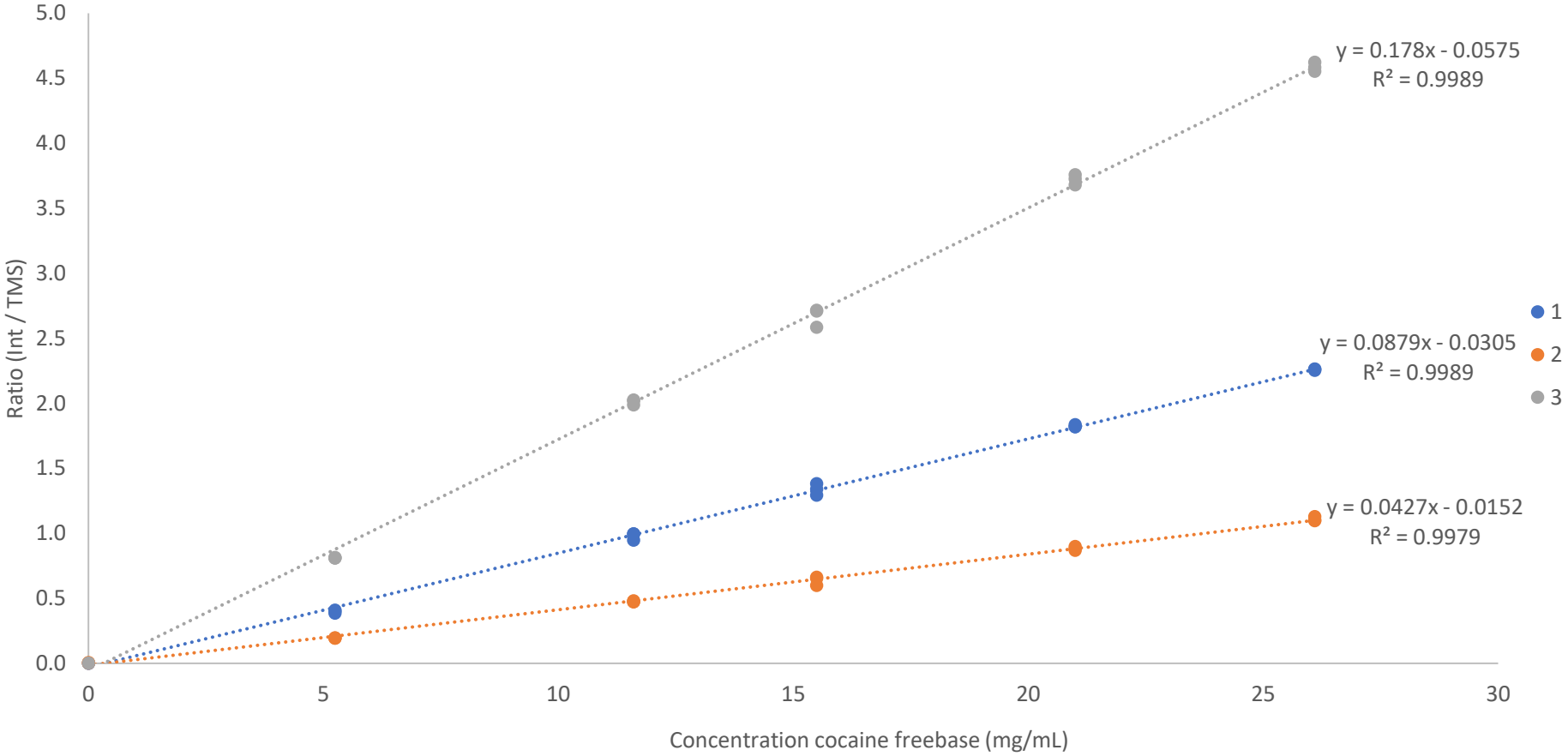


Figure 19 - Calibration plot of 0 - 25 mg/mL cocaine freebase in $CDCl_3$ analysed using low-field NMR. 1 – 3 refers to the regions of quantification for cocaine freebase, outlined on Figure 11.

Table 29 - Calibration parameters for cocaine freebase in $CDCl_3$ analysed using low-field NMR. 1 – 3 refers to the regions of quantification for cocaine freebase, outlined on Figure 11.

Environment		LoD (mg/mL)	LoQ (mg/mL)	R^2	Av. Accuracy (%)	Av. RSD (%)	Recovery range – Ext. (%)	Recovery range – Int. (%)
Aromatic	1	0.429	1.300	0.9989	98.66	3.51	92.89 – 111.54	93.32 – 112.04
Tropane CH	2	0.609	1.845	0.9979	98.41	3.54	103.24 – 108.14	101.62 – 106.45
O-CH_3	3	0.427	1.293	0.9990	98.61	3.27	94.97 – 107.40	98.34 – 111.21

The LoD and LoQ values for the analysis of cocaine freebase are similar to the analysis of cocaine hydrochloride with a minimum theoretical percentage content (% (w/w)) of 6.5 – 9.2%. The range of content values are less than the analysis of cocaine hydrochloride due to the decrease on the LoQ for the tropane CH environment. This is due to the lack of an interfering H_2O peak. Linearity values of the calibration curves are similar between the hydrochloride and the freebase form of cocaine, with the freebase values still residing within an acceptable range. The accuracy and precision, with the exception of the tropane CH environment, of each data point are similar between the two forms of cocaine. The recovery experiments of each show acceptable recoveries in a tighter range than the recoveries for cocaine hydrochloride.

The same sample solutions were analysed using high field instrumentation with the calibration series and validation parameters shown in Figure 20 and Table 30, respectively.

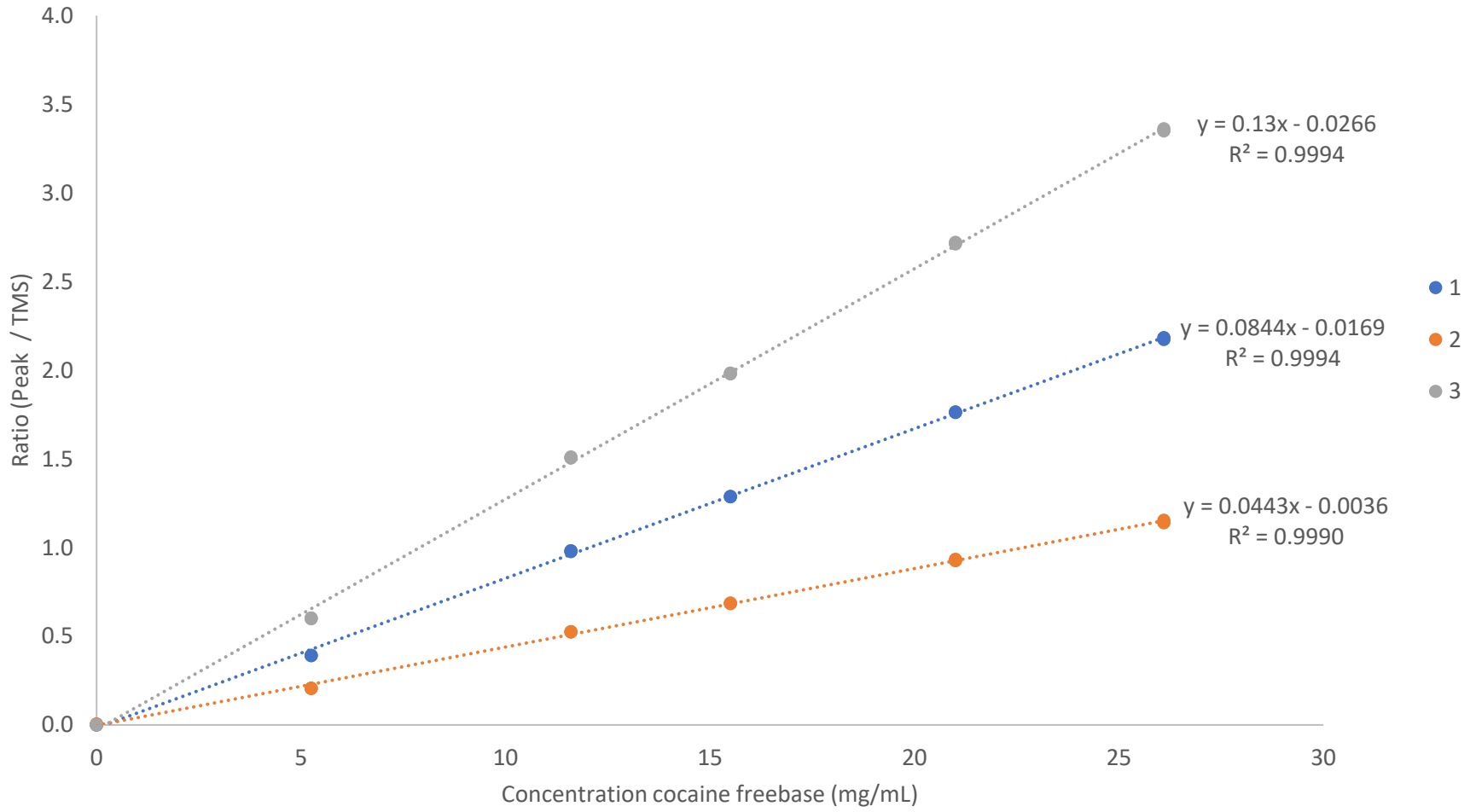


Figure 20 - Calibration plot of 0 - 25 mg/mL cocaine freebase in $CDCl_3$ analysed using high-field NMR. 1 – 3 refers to the regions of quantification for cocaine freebase, outlined on Figure 11.

Table 30 - Calibration parameters for cocaine freebase in $CDCl_3$ analysed using high-field NMR. 1 – 3 refers to the regions of quantification for cocaine freebase, outlined on Figure 11.

Environment		LoD (mg/mL)	LoQ (mg/mL)	R^2	Av. Accuracy (%)	Av. RSD (%)	Recovery range – Ext. (%)	Recovery range – Int. (%)
Aromatic	1	0.320	0.969	0.9994	98.71	3.71	92.92 – 102.67	93.21 – 103.00
Tropane CH	2	0.410	1.243	0.9990	98.65	4.74	93.01 – 103.04	92.15 – 102.08
O-CH_3	3	0.325	0.986	0.9994	98.68	3.74	93.28 – 103.11	94.35 – 104.29

Surprisingly, the LoD and LoQ values obtained for high-field analysis of cocaine freebase are similar to the values obtained for low field analysis, opposed to the 8-fold decrease observed between low and high-field analysis of cocaine hydrochloride. This is most likely due to the lower precision across the calibration curve, indicated by the higher RSD values, causing greater LoD and LoQ values. Linearity is slightly better for high-field analysis opposed to the low-field analysis for cocaine freebase and both show acceptable linearity. Recovery values show acceptable sample recovery percentages for each signal and both methods of concentration calculation, in a tighter range than low-field analysis.

Exemplar spectral data is shown in Figure 21 for each analysis, showcasing the differences between cocaine hydrochloride in D_2O against cocaine freebase in $CDCl_3$ for both low-field and high-field analysis.

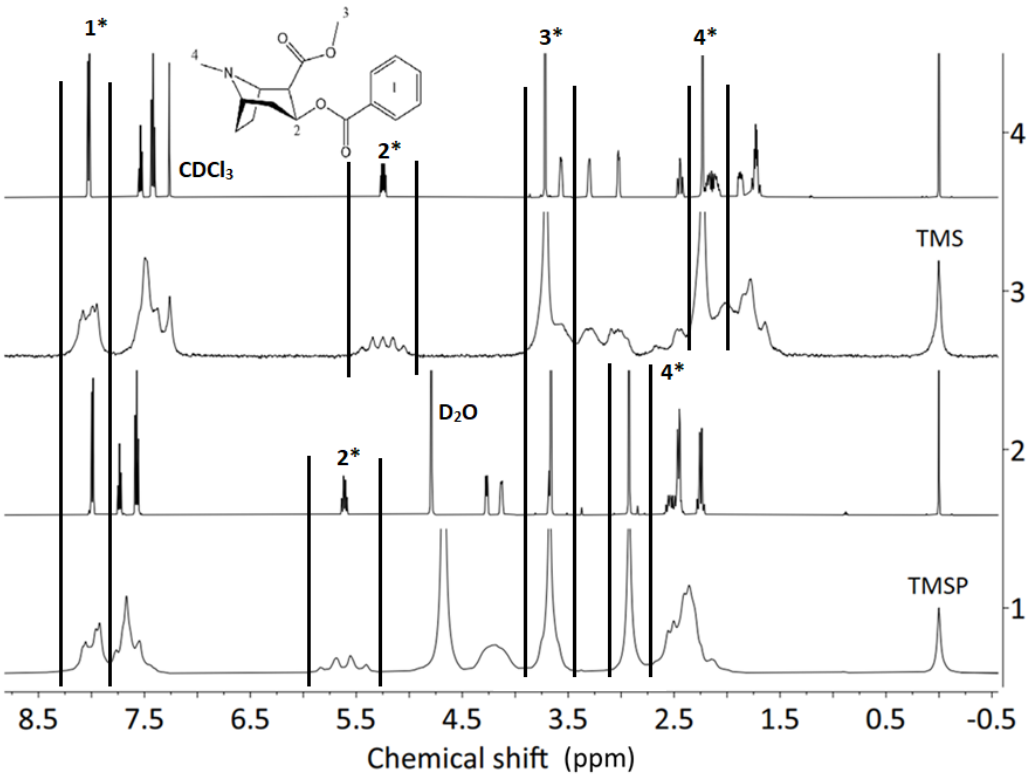


Figure 21 - Cocaine hydrochloride (20 mg/mL) in D_2O spiked with TMSP (1 mg/mL) analysed by low-field NMR (1) and high-field NMR (2). Cocaine freebase (20 mg/mL) in $CDCl_3$ spiked with TMS (1 mg/mL) analysed by low-field NMR (3) and high-field NMR (4) 1 – 4* refers to the regions of quantification for cocaine hydrochloride and cocaine freebase, shown in figure 10 and figure 11 respectively.*

4.2.8. GC-MS Method Validation Parameters

Previous literature has outlined the quantification of cocaine using GC-MS in various body fluids including urine, whole blood samples and saliva.[136-138] Similarly, a recommended method of analysis of bulk cocaine samples has been previously outlined by the UNODC (United Nations Office on Drug and Crime).[139] Due to this previously showcased literature, a large discussion will not be provided.

A representative chromatograph for the analysis of cocaine hydrochloride and seven commonly identified adulterants for samples seized around Manchester have been shown in Figure 22, with the validation parameters showcased in Table 31.[140] The same respective method was employed for the analysis of cocaine freebase samples except

chloroform is used for extraction, this posed no significant difference in validation parameters and therefore is not outlined.

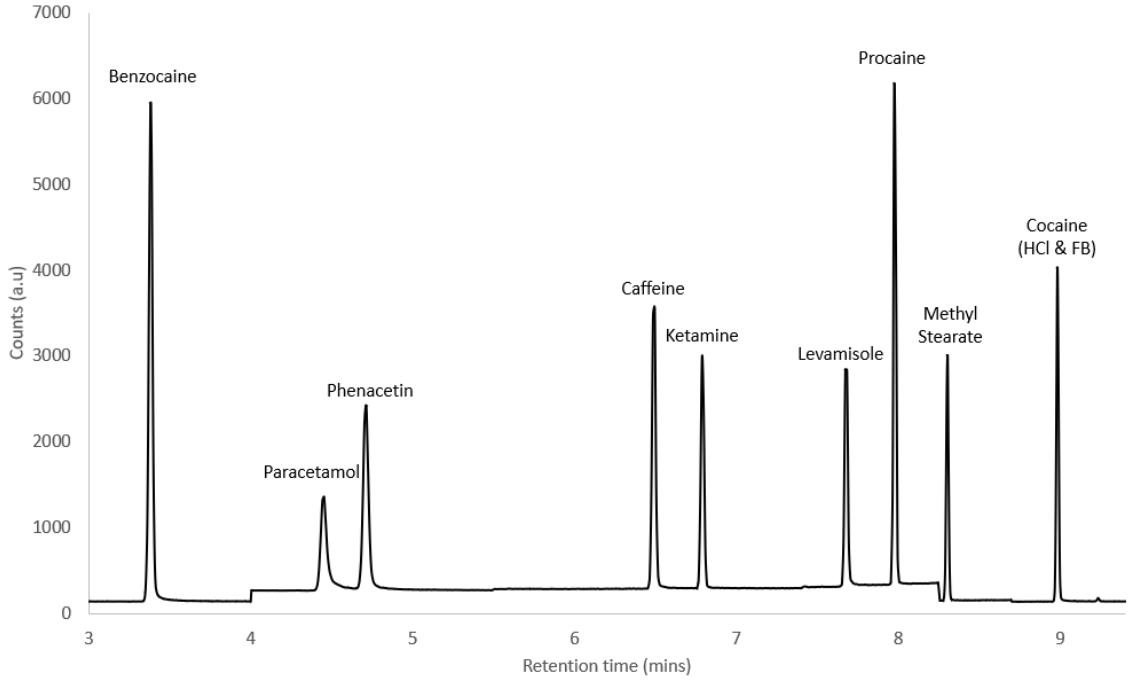


Figure 22 - Representative chromatograph for GC-MS analysis of cocaine and seven commonly found adulterants (0.10 µg/mL). Methyl stearate is used as an internal standard (0.10 µg/mL)

Table 31 - GC-MS validation parameters for the quantification of cocaine hydrochloride / freebase in bulk illicit samples. T_r = retention time, R_s = resolution between previously eluted peak, N = number of plates used for chromatographic separation, R^2 = linearity, accuracy = range of percentage determination of individual injections of calibration solutions. Methyl stearate is used an internal and is held at the same concentration (0.10 µg/mL) throughout the calibration. IB = SIM ion used for quantification, I1/I2 = SIM ions used as qualifiers

Analyte	Benzocaine	Paracetamol	Phenacetin	Caffeine	Ketamine HCl	Tetramisole	Procaine	Methyl Stearate	Cocaine
T_R	3.382	4.453	4.714	6.485	6.788	7.674	7.976	8.306	8.984
R_s	161.4	18.9	4.0	35.4	8.0	25.6	9.0	12.2	28.1
Tailing factor	0.9	1.8	1.0	1.3	1.2	1.2	1.0	0.9	1.0
N (Plates)	104142	61907	107946	370004	669644	720860	1042099	2130515	1997957
R^2	0.9995	0.9785	0.9987	0.9998	0.9993	0.9989	0.9995	-	0.9995
LoD (µg/mL)	0.103	0.632	0.147	0.065	0.112	0.160	0.100	-	0.098
LoQ (µg/mL)	0.312	1.916	0.445	0.198	0.337	0.434	0.304	-	0.298
Accuracy Range (%)	97.6 – 102.0	86.1 – 107.2	97.6 – 104.3	96.6 – 100.7	97.1 – 100.7	96.8 – 105.4	97.5 – 100.9	-	96.0 – 101.7
Recovery Range (%)	107.8 – 88.2	91.1 – 80.1	97.1 – 80.4	101.3 – 114.4	112.1 – 114.3	98.7 – 111.0	93.3 – 96.3	-	96.3 – 102.5
Gradient	0.3356	0.1576	0.1496	0.1965	0.1880	0.1004	0.2993	-	0.1113
Intercept	-0.0440	-0.1480	-0.0348	-0.0121	-0.0300	-0.0222	-0.0143	-	-0.0041
I B	120.0	179.1	109.0	194.0	180.0	148.0	86.1	74.1	82.1
I 1	165.0	132.1	151.0	109.0	209.0	204.0	99.1	87.1	182.1
I 2	92.1	149.9	82.0	67.1	152.0	73.0	120.0	143.1	303.1

4.2.9. Sample Analysis and Correlation with GC-MS

97 seized cocaine samples consisting of 87 cocaine hydrochloride (89.7%) and 10 cocaine freebase samples (10.3%) were analysed in tandem by low-field, high-field and GC-MS analysis.

A scatter plot, showcasing the correlation between the content (% (w/w)) determinations of low-field qNMR and GC-MS is shown in Figure 23, possessing a good positive correlation (R^2 = 0.9399). The data points are coloured to show the differences between the percentage content calculated between the two techniques, with green (0 – 5%), yellow (5 – 10%), red (10 – 15%) and purple (15%+).

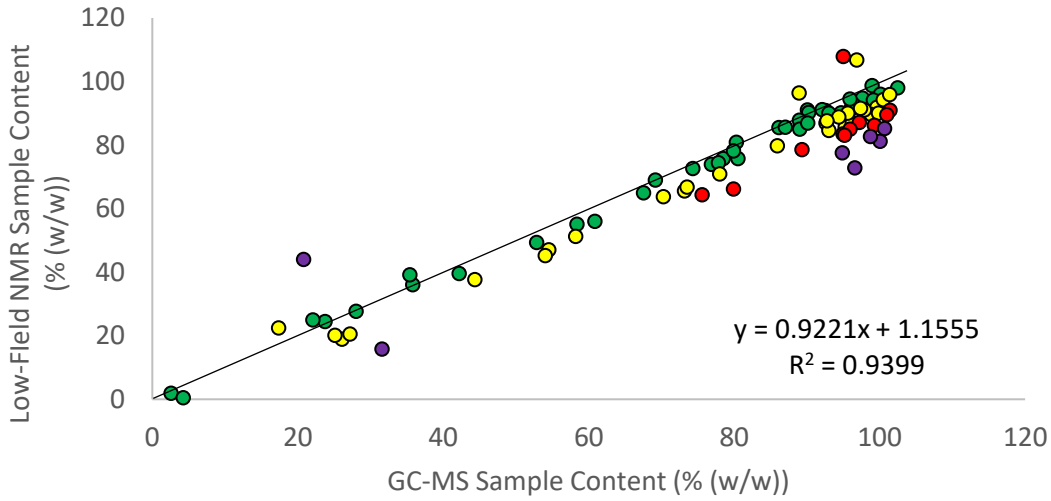


Figure 23 - GC-MS vs low-field NMR sample purity determination correlation, data values are percentage content of cocaine (% (w/w)) calculated for each sample. Samples (n= 88) contain 88.6% cocaine HCl (n = 78) and 11.4% cocaine freebase (n = 10)

As expected, the majority of the samples (79.6%) have 0 – 10 % difference, consisting of 0 – 5% (n = 41) and 5 – 10% (n = 29), showing close agreement between GC-MS and low-field NMR. The remainder of the samples which do not show close agreement (20.4%, n = 18), possess no clear indicator to explain these differences. There are no significant differences of correlation between cocaine hydrochloride and freebase samples, despite the small sample size of freebase samples (n = 10). The equation of the trendline does indicate that low-field NMR has a systematic underestimation, compared to GC-MS, of the samples accounting for -1.25 – 7.19% of the deviation. This underestimation is easily identified within the sample regression comparison as the

majority of the red and purple data points, which show 10%+ difference are under the regression trendline.

It is inherently unclear as to why these specific samples had variety in the content determination. Each batch of analysis had QC sample analysis included within the analytical run, which never deviated more than 5% from the expected value. Following this, the addition of adulteration did not affect analysis, as the majority of the red and purple data points did not contain any detected adulteration (89%, n = 16). It could also be that the GC-MS analysis was overestimating these samples, from other things such as matrix effects or poor chromatography.

Details of the correlation between high-field NMR and GC-MS were also made, outlined in Figure 24, following the same colour scheme for the data point as described for low-field analysis.

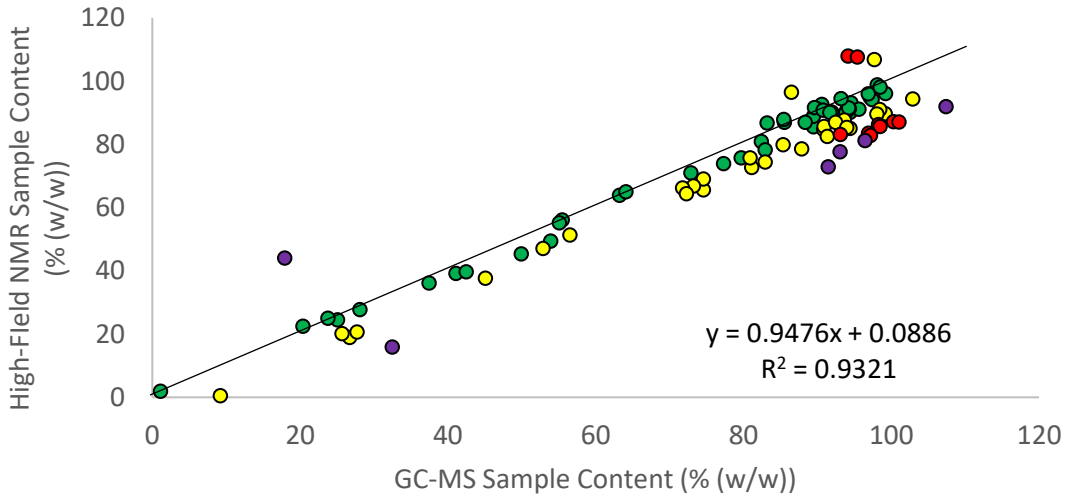


Figure 24 – GC-MS vs high-field NMR sample purity determination correlation, data values are percentage content of cocaine (% (w/w)) calculated for each sample. Samples (n = 91) contain 89.0% cocaine HCl (n = 81) and 11.0% cocaine freebase (n = 10)

The correlation co-efficient between GC-MS and high-field NMR shows a positive correlation with good agreement (R^2 = 0.9321). This is lower than the correlation achieved by Benedito *et al.*,[114] which compared cocaine analysis of a 600 MHz instrument with GC-FID (R^2 = 0.9811), which could be due to the lower number of samples analysed (n = 25) across a narrower range of percentage content (65.03 – 93.83 % (w/w)). Despite the increased precision and accuracy of the high-field instrumentation, a lower correlation co-efficient was identified compared to low-field comparisons with GC-MS (R^2 = 0.9399). This could be due to samples which deviate the

most from GC-MS, e.g., the red and purple colour coded samples, deviating higher than compared to low-field analysis.

A higher proportion (83.5%) of samples fall within 0 – 10% deviation from GC-MS analysis consisting of 0 – 5% (50.5%, n = 46) and 5 – 10 % (33.0%, n = 30), compared to low-field correlation (79.6%). The remaining samples deviated between 10 – 15% (9.9%, n = 9) and 15%+ (6.6%, n = 6), showing more correlation overall with GC-MS analysis compared to low-field NMR analysis. The regression equation was used to estimate differences across the majority of the calibration range, which showed a similar underestimation, in a smaller range, by high-field NMR compared to GC-MS across -0.1 – 5.43 %. This showcased a higher correlation between high-field NMR and GC-MS sample analysis compared to low-field NMR and GC-MS analysis, despite the lower correlation co-efficient.

Correlations between low-field and high-field analysis have been made, with the results of which shown in Figure 25, adopting the same colour scheme describing sample deviation throughout.

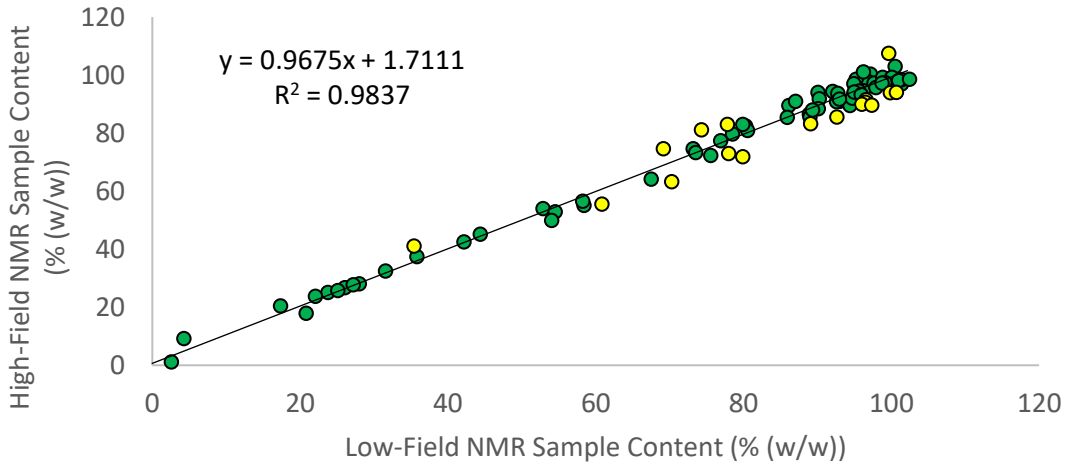


Figure 25 – Low-field vs high-field NMR sample purity determination correlation, data values are percentage content of cocaine (% (w/w)) calculated for each sample. Samples (n = 92) contain 89.1% cocaine HCl (n = 82) and 9.9% cocaine freebase (n = 10)

The samples between low-field and high-field NMR content were plotted which showed a high positive correlation between the different resolution instruments (R^2 = 0.9837). All of the sample dataset showed good agreement within 0 – 10% deviation, consisting of 0 – 5% (80.5%, n = 70) and 5 – 10% (19.5%, n = 17). This shows that between analysis performed on low-field NMR correlated highly with high-field NMR

analysis. This can be expected since the deviations in sample analysis are expected to be less, than when compared against a different technique, with a different sample preparation, instrumentation conditions etc. The regression equation for the trendline was plotted to estimate the differences across the calibration range, showing slight underestimation at low concentration and slight overestimation at high concentrations within a range of – 1.77 – 1.59 %. Due to this high correlation of samples between the two resolution spectrometers, low-field NMR spectroscopy can be showcased to be a viable alternative to high-field NMR spectroscopy for the quantification of cocaine samples.

A 3D scatter plot showing correlation between low-field, NMR high-field NMR and GC-MS samples content purities (% (w/w)) is shown in Figure 26.

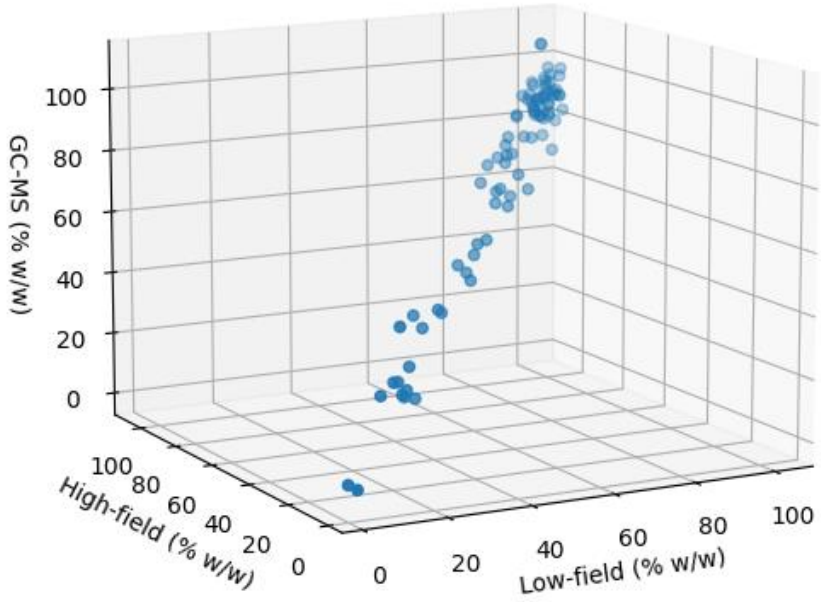


Figure 26 - 3D Scatter plot showing cocaine sample content (% (w/w)) correlation between low-field NMR, high-field NMR and GC-MS

A short comparison between the techniques is made by identifying the range, average and median content (% (w/w)) for each sample, with the results of this shown in Table 32.

Table 32 - Calculated percentage cocaine content (% (w/w)) sample ranges, averages and median values between low field NMR, high field NMR and GC-MS

	Cocaine content (% w/w) by technique		
	Low-Field	High-Field	GC-MS
Low Range	2.6	1.1	0.5
High Range	102.5	107.4	107.8
Average	77.3	77.1	73.4
Median	90.1	89.5	84.8

Compared to the regression trendline of the comparison data showing underestimation for low-field analysis and similar results for high-field analysis, the comparisons between content determination shows the opposite. Low-field and high field NMR analysis show an increase in average content of roughly 4% for the same sample dataset, with a median increase of roughly 5%. In this case, one specific technique cannot be assumed correct compared to the other since each technique has their advantages and disadvantages. However, despite these differences the results of the three techniques are comparable enough for analysis performed in harm reduction. For example, a cocaine sample showing high purity by low-field NMR, is expected to still be quantified as high purity by GC-MS, despite the potential 5% difference in content (% (w/w)) between techniques.

4.2.10. Adaption for no-D NMR – Cocaine hydrochloride

Despite the use of low-field NMR instrument having reduced operating costs compared to high-field analysis, due to lack of liquid coolant, the next biggest cost of analysis would be the use of deuterated solvents. Adaptation of the method to use non-deuterated solvents, i.e., deionised water and analytical grade chloroform, reduces the cost of each analytical run significantly. Outside of costs, the ease of analysis of using solvents which are typically found in large amounts within laboratories, makes analysis less restrictive and easier to perform for a large number of samples.

The same calibration and validation parameters as performed for the analysis of cocaine hydrochloride and freebase using deuterated solvents, has been outlined using deionised water and analytical grade chloroform for both cocaine hydrochloride and freebase respectively. The calibration series for cocaine hydrochloride in H_2O, is preferred to chloroform as it non-toxic and reduced cost. This calibration series for

cocaine hydrochloride in H_2O, using low-field NMR, is shown in Figure 27, with the validation parameters outlined in Table 33.

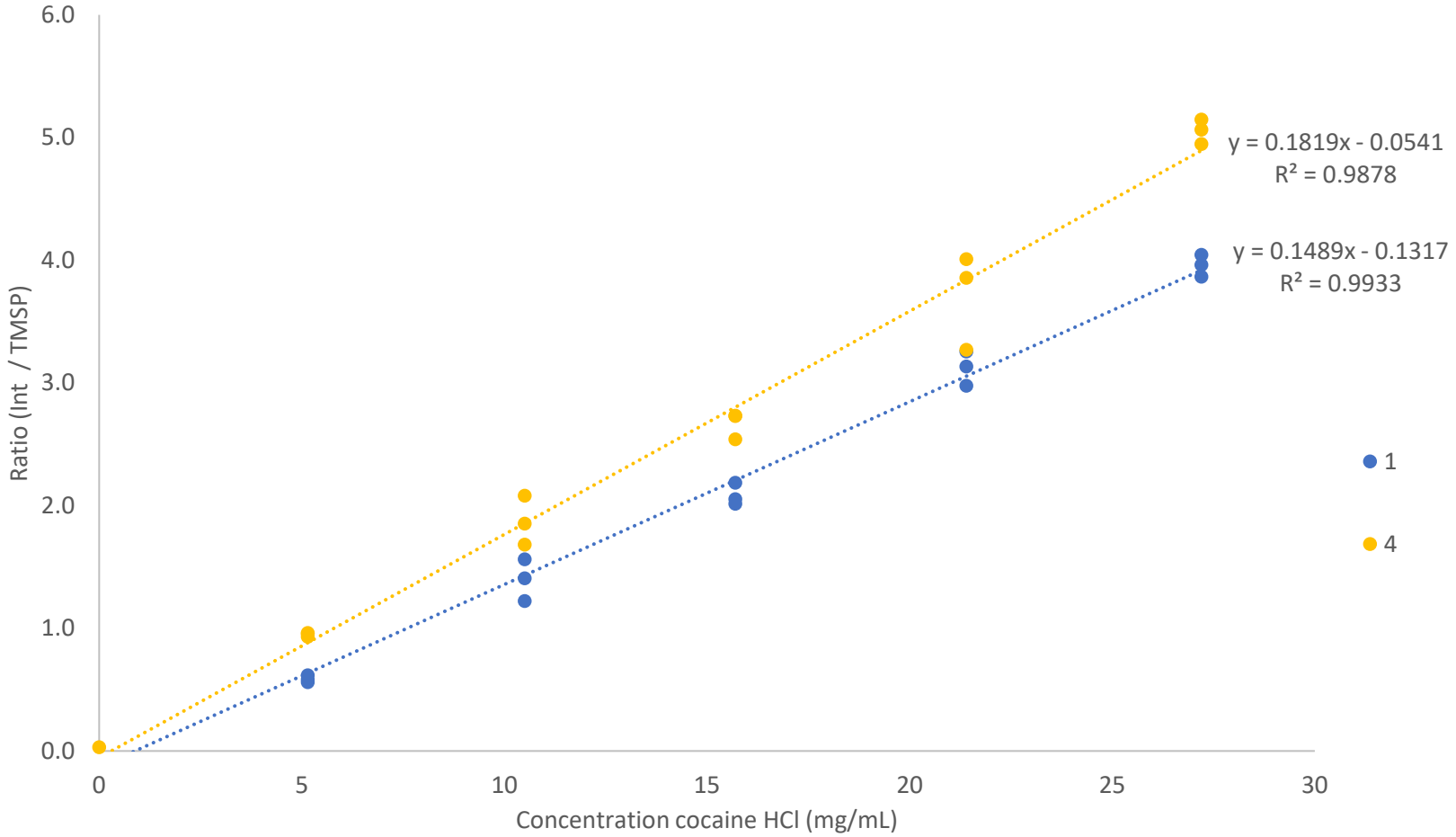


Figure 27 - Calibration plot of 0 - 25 mg/mL cocaine hydrochloride in H_2O analysed using low-field NMR. 1 – 4 refer to the regions of quantification for cocaine hydrochloride, outlined on Figure 30.

Table 33 - Calibration parameters for cocaine hydrochloride in H_2O analysed using low-field NMR. 1 – 4 refer to the regions of quantification for cocaine hydrochloride, outlined on Figure 30.

Environment		LoD (mg/mL)	LoQ (mg/mL)	R^2	Av. Accuracy (%)	Av. RSD (%)	Recovery range – Ext. (%)	Recovery range – Int. (%)
Aromatic	1	1.10	3.34	0.9933	97.87	6.15	87.2 - 112.5	89.9 – 125.4
N-CH_3	4	1.27	3.84	0.9878	101.24	7.47	95.1 – 113.3	87.2 – 107.0

For the analysis of cocaine hydrochloride by low-field NMR, the calibration parameters are similar to the analysis performed in deuterated solvent, except with decreased precision. The decrease in precision is due to the difficulty during spectral processing due to the large solvent peak and harsh baseline correction that is needed due to baseline interference. The decreased precision resulted in a numerically lower LoD and LoQ for this method, as calculation is based off the standard deviation of the response and slope. Linearity also decreased due to decreased precision but is still within acceptable ranges (> 0.99). The accuracy of the data points was similar and were unaffected by the decreased precision. Recovery of this method was assessed showing good recoveries for all but the high range standard for the aromatic region, which is outside of acceptable recovery range (120%). Fortunately, recovery values, determined against a reference standard, are still within an acceptable range (80 – 120%). Only two of the four potential signals used for quantification are viable for no-D analysis, due to the large solvent peak overlapping with the two remaining environments.

The same analysis solutions were analysed in tandem, using high-field NMR, to assess the differences between the techniques. The calibration series and validation parameters are showcased in Figure 28 and Table 34 respectively.

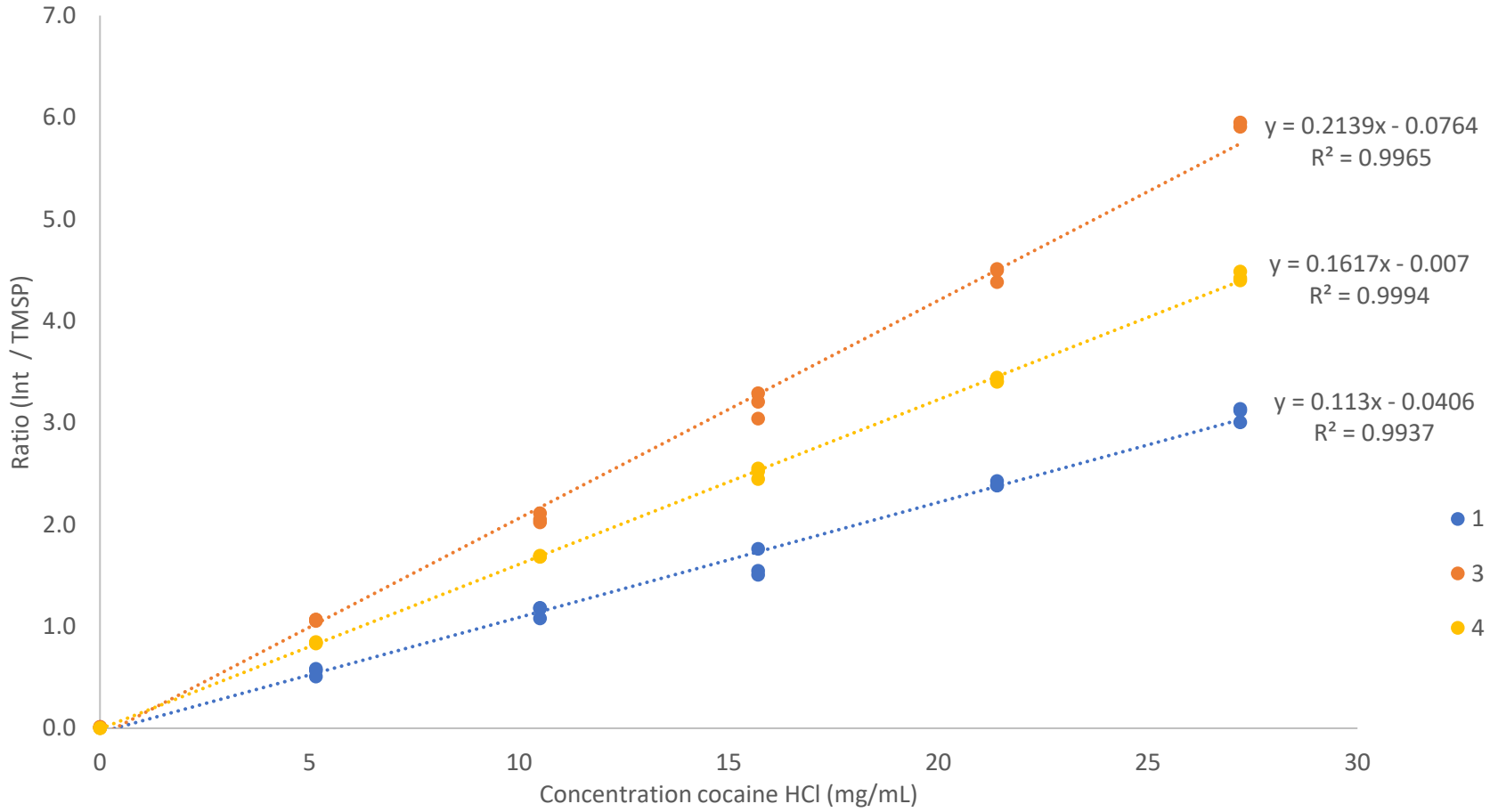


Figure 28 - Calibration plot of 0 - 25 mg/mL cocaine hydrochloride in H_2O analysed using high-field NMR. 1 – 4 refer to the regions of quantification for cocaine hydrochloride, outlined on Figure 30.

Table 34 - Calibration parameters for cocaine hydrochloride in H_2O analysed using high-field NMR. 1 – 4 refer to the regions of quantification for cocaine hydrochloride, outlined on Figure 30.

Environment		LoD (mg/mL)	LoQ (mg/mL)	R^2	Av. Accuracy (%)	Av. RSD (%)	Recovery range – Ext. (%)	Recovery range – Int. (%)
Aromatic	1	1.063	3.222	0.9937	99.47	5.72	84.8 – 125.4	83.1 – 127.2
O-CH_3	3	0.786	2.381	0.9965	99.27	3.92	94.9 – 121.2	88.6 – 116.8
N-CH_3	4	0.320	0.971	0.9994	100.08	1.50	90.1 – 123.1	88.7 – 121.6

The initial calibration series and analysis was performed in 100% H_2O spiked with TMSP. For low-field analysis, this is not an issue since the shimming remains consistent throughout the analytical run providing no changes in environmental temperature. For high-field analysis, the shimming is based off individual samples and since the lack of a deuterated solvent means the lock cannot start and thus the shimming suffers. Shimming can be turned off for high-field analysis to match low-field analysis conditions but the quality of the shim is not stable enough across a full analytical run and poor shimming occurs on later sample spectra. These samples were analysed in 10% D_2O : 90% H_2O (v/v) in order to allow the lock to detect a deuterated signal and improve the shimming of analysis. For high-field analysis, the increased resolution allowed quantification of each site, originally used in "normal" NMR analysis, since the H_2O peak does not overlap. However, the tropane CH signal is not used within no-D analysis of cocaine hydrochloride due to artifacts from the H_2O peak, causing instability of the baseline, affecting regions in close proximity. The LoD, LoQ and precision values for high-field no-D analysis of cocaine hydrochloride are lower than low-field analysis as expected. Linearity is acceptable (> 0.99) lowered slightly due to baseline interference from H_2O artifacts, with good accuracy across the calibration series (99.3 – 100.1%). Recovery values are slightly outside of acceptable ranges, mainly for the high concentration**.**

The no-D analysis for cocaine freebase, performed in $CHCl_3$ was also analysed with low-field and high-field NMR. The calibration series is shown in Figure 29, and the validation parameters detailed in Table 35 for low-field NMR analysis.

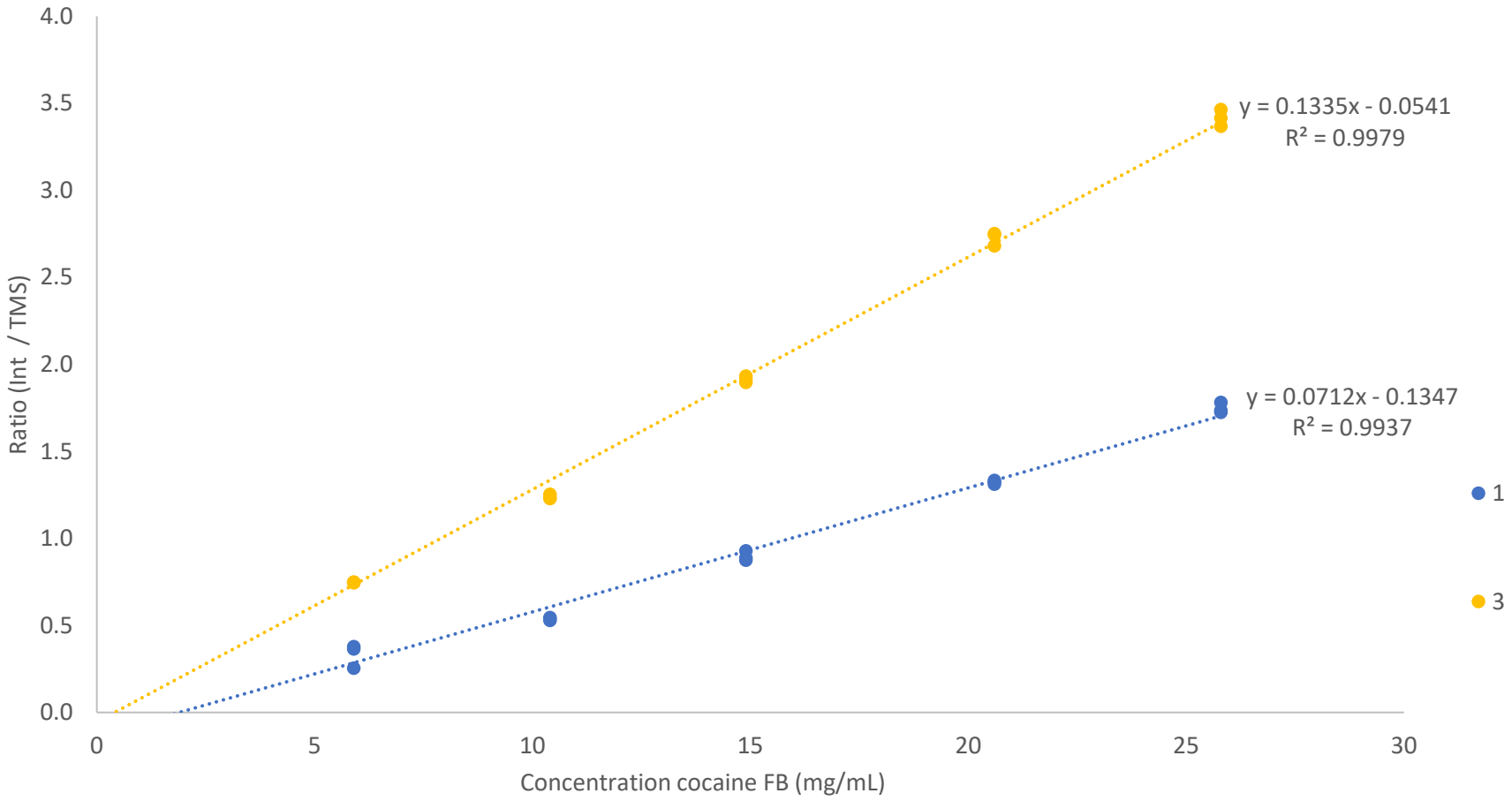


Figure 29 - Calibration plot of 0 - 25 mg/mL cocaine freebase in $CHCl_3$ analysed using low-field NMR. 1 – 3 refers to the regions of quantification for cocaine freebase, outlined on Figure 30.

Table 35 - Calibration parameters for cocaine freebase in $CHCl_3$ analysed using low-field NMR. 1 – 3 refers to the regions of quantification for cocaine freebase, outlined on Figure 30.

Environment		LoD (mg/mL)	LoQ (mg/mL)	R^2	Av. Accuracy (%)	Av. RSD (%)	Recovery range – Ext. (%)	Recovery range – Int. (%)
Aromatic	1	1.02	3.10	0.9937	100.1	9.29	106.8 – 116.0	92.1 – 122.3
O-CH_3	3	0.58	1.77	0.9979	99.1	3.38	101.4 – 117.0	103.6 – 126.1

The analysis for cocaine freebase in $CHCl_3$ showed similar results to the analysis in normal deuterated solvent, except with slightly less precision. This decreased precision increases the LoD and LoQ of analysis slightly for region 1 but remaining roughly the same for region 3. This decreased precision is due to the location of the $CHCl_3$ solvent peak which interferes with baseline resolution and has to be corrected for during the spectral processing. Both regions have acceptable LoD and LoQ values for bulk sample analysis, good linearity (R^2 = > 0.99), good accuracy (> 98%), good precision (<5 % RSD) with acceptable analyte recoveries for the majority of the analysis. The highest range of sample recoveries for the concentration determined using a spiked internal standard is higher than the typical acceptable range (80 – 120%). Fortunately, quantification using a reference calibration still shows acceptable recovery ranges. Tropane CH region is not used due to interference from a carbon satellite, omitting that region from analysis. Despite the interference with the tropane CH signal, the $CHCl_3$ peak, is smaller than H_2O, allowing for a flat baseline for the O-CH_3 region. The aromatic region is still slightlyaffected due to its close proximity. The resulting baseline is flat, opposed to analysis using D_2O, allowing for easier spectral processing and more repeatable integrals.

New sample solutions which contained 10% deuterated solvent, to aid with locking and shimming, for high-field analysis were analysed. The calibration series were plotted and are shown in Figure 30, with the validation parameters shown in Table 36.

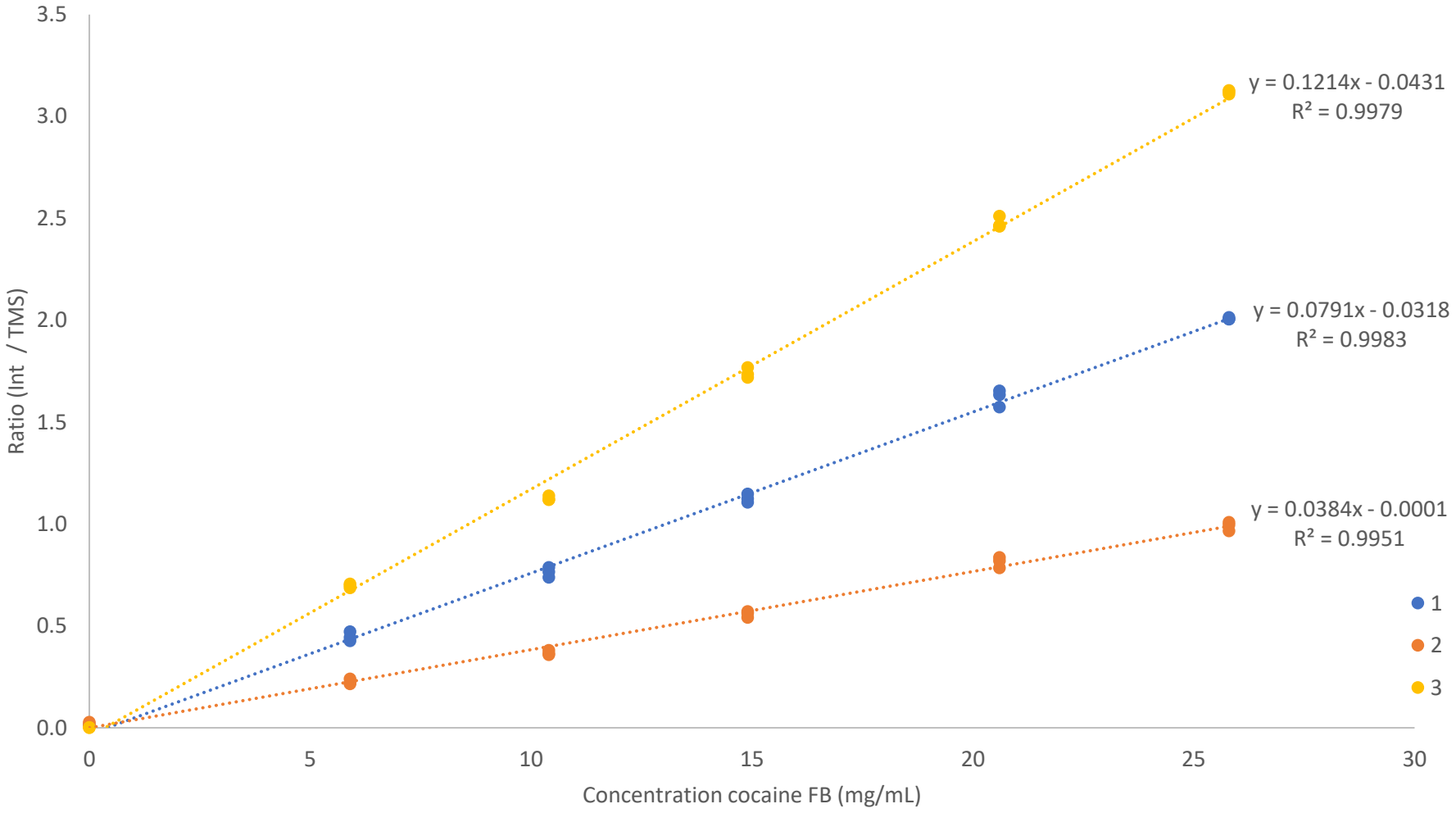


Figure 30 - Calibration plot of 0 - 25 mg/mL cocaine freebase in $CHCl_3$ analysed using high-field NMR. 1 – 3 refer to the regions of quantification for cocaine freebase, outlined on Figure 30.

Table 36 - Calibration parameters for cocaine freebase in $CHCl_3$ analysed using high-field NMR. 1 – 3 refer to the regions of quantification for cocaine freebase, outlined on Figure 30.

Environment		LoD (mg/mL)	LoQ (mg/mL)	R^2	Av. Accuracy (%)	Av. RSD (%)	Recovery range – Ext. (%)	Recovery range – Int. (%)
Aromatic	1	0.532	1.612	0.9983	99.87	3.33	105.8 – 113.8	103.3 – 113.3
Tropane CH	2	0.899	2.723	0.9951	98.59	4.85	103.6 – 114.8	102.4 – 113.5
O-CH_3	3	0.511	1.550	0.9979	98.86	3.90	104.8 – 114.6	104.1 – 117.3

Similarly, to the high-field analysis for cocaine hydrochloride, this analysis has been performed in 10% $CDCl_3$: 90% $CHCl_3$ (v/v) to allow a deuterated signal for the lock and shimming. The LoD and LoQ are acceptable for bulk sample analysis, with a minimum estimated percentage weight of 7.75 % (w/w) for quantification of cocaine hydrochloride. Analysis overall is similar to low-field no-D analysis, except with increased precision compared to low-field as expected. Recovery values are all within acceptable ranges, compared to low-field analysis, which has recovery values higher than 120% for quantification using the internal standard, this difference is due to the less inherent precision with the low-field instrumentation.

Exemplar spectra for each of the no-D analysis is shown in Figure 31, with the regions of quantification outlined.

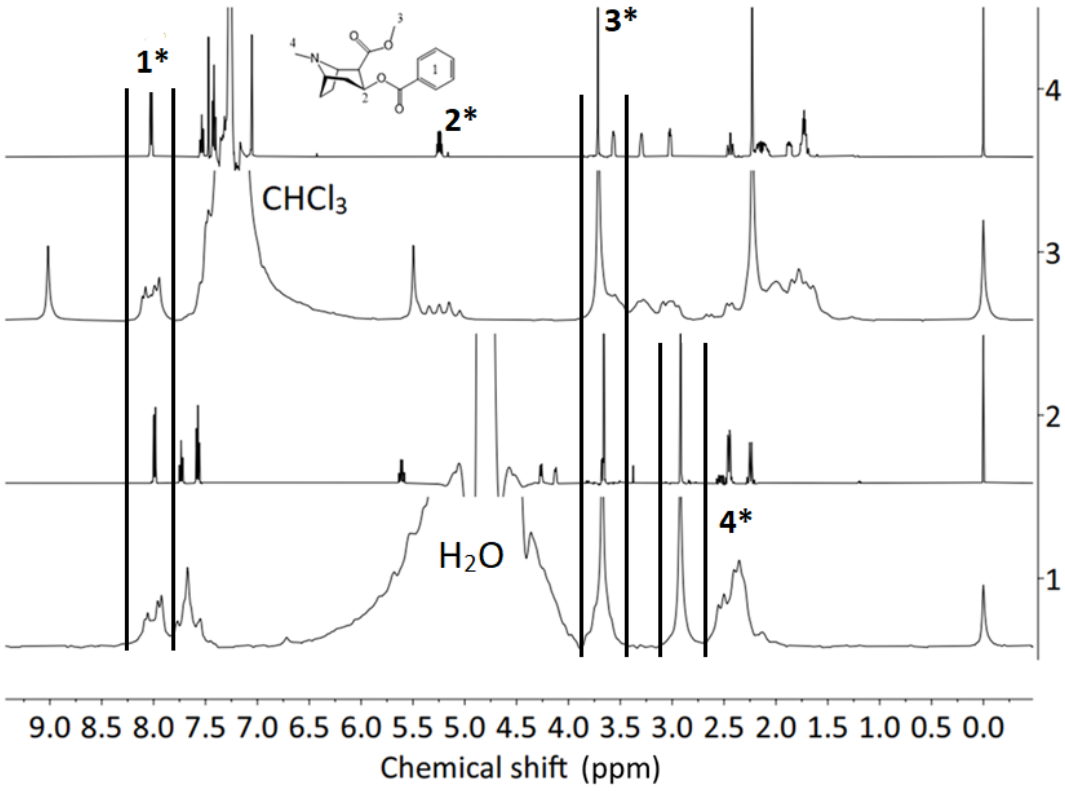


Figure 31 – (1) Cocaine hydrochloride (20 mg/mL) in H_2O spiked with TMSP (1 mg/mL) analysed by low-field NMR and (2) high-field NMR.(3) Cocaine freebase (20 mg/mL) in $CDCl_3$ spiked with TMS (1 mg/mL) analysed by low-field NMR (4) and high-field NMR.

A selection of cocaine hydrochloride (n = 13) and cocaine freebase samples (n = 5) were analysed using no-D analysis and the difference in percentage content determination (% (w/w)) of cocaine by no-D NMR, traditional NMR and GC-MS is shown in Table 37 to highlight differences within analysis.

Table 37 – Sample (n = 18) analysis for cocaine content (% (w/w)) comparison with low-field NMR, high-field NMR and GC-MS. Averages content (% (w/w)) calculated against both external and internal calibrants.

Analyte	Technique	Non – Deuterated solvents				Deuterated solvents				GC-MS				
		Low Field NMR % (w/w)		High Field NMR % (w/w)		Low Field NMR % (w/w)		High Field NMR % (w/w)						
	Sample	Average	±	Average	±	LF Ext	±	HF Ext	±	GC-MS	±	Adulterant	% (w/w)	±
Cocaine Hydrochloride	GM188	90.75	8.68	90.04	2.58	94.98	3.20	94.14	2.95	107.85	0.48			
	GM200	93.03	5.86	91.43	7.10	92.75	2.41	93.65	3.43	87.61	0.39			
	GM204	83.24	7.70	83.32	5.87	80.53	8.90	80.90	0.22	75.71	0.82	Levamisole	6.40	5.08
	GM206	42.28	5.38	43.31	2.52	42.18	4.60	42.48	2.28	39.62	0.68			
	Q19031	91.68	5.98	93.00	1.34	96.85	2.19	97.72	2.60	106.80	0.70	Caffeine	9.20	2.14
	Q19036	93.65	5.30	92.74	4.31	96.25	2.11	94.56	1.25	93.07	0.56			
	Q19041	77.19	9.08	72.92	6.93	76.89	3.42	77.32	0.94	73.90	0.69	Tetramisole	17.80	1.10
	Q19113	40.36	7.61	38.79	10.27	35.43	3.52	41.08	0.21	39.18	1.66	Caffeine	6.28	2.42
	Q19171	53.82	6.58	56.74	2.65	54.50	10.50	52.89	0.31	51.28	0.79			
	Q19273	85.62	10.85	79.60	2.97	77.83	2.41	82.93	0.61	74.40	0.67	Tetramisole	18.14	2.62
Cocaine Freebase	DC1 F	107.92	1.25	101.97	2.12	96.76	1.24	92.65	1.32	94.45	0.62			
	DC4 A	34.86	10.99	37.36	2.02	24.35	5.27	26.78	0.71	18.88	0.57			
	DC4 B	21.94	8.37	24.43	2.52	29.42	5.65	32.56	0.64	15.86	1.17			
	DC4 C	30.09	7.33	33.86	3.94	22.10	6.23	25.14	0.78	24.45	0.57			
	DC4 D	22.22	15.40	26.01	3.14	23.13	6.60	25.83	0.58	20.12	0.77			
	DC4 E	35.84	6.41	37.66	4.78	16.54	6.63	20.47	0.58	22.47	0.58			
	DC4 F	26.13	25.33	29.03	3.58	20.83	6.66	23.91	0.48	24.93	0.97			
	DC4 G	27.54	7.23	30.96	4.60	24.46	8.47	27.75	0.75	20.58	0.50			
Average		58.79	8.63	59.07	4.07	55.88	5.00	57.38	1.15	55.06	0.73		11.56	2.67
Median		48.05	7.47	50.03	3.36	48.34	4.94	47.69	0.73	45.45	0.68		9.20	2.42

No-D NMR analysis produces a slight overestimation compared to traditional NMR analysis typically between 3 – 4% as shown by the typically higher average and median values for this method. No-D analysis also affords higher RSD values compared to the deuterated counterpart, due to baseline interferences from large solvent signals. As showcased previously within the calibration and validation parameters, low-field analysis shows less average precision compared to high-field analysis and both techniques overestimate when compared to GC-MS analysis. There are also no consistent differences between the samples which had adulteration compared to unadulterated samples. This showcases no-D solvents as a viable alternative to traditional NMR solvents.

4.3. **Conclusions**

A method has been developed for the quantification of cocaine content % (w/w) using low-field NMR and compared to high-field NMR. T_1 experiments have been performed on both cocaine hydrochloride and cocaine freebase, as well as eight commonly identified adulterants which show quicker relaxation times when solvated in viscous solvents, coupled to more spins and when the analysis is performed at higher temperatures. The minimum relaxation delay for analysis was set at 40 seconds due to largest T_1 of 5.37 seconds for the aromatic signals of paracetamol. Eight scans were chosen producing a SINO ratio of at least 100 for three out of four signals used for quantification for low-field NMR analysis, producing a total method analysis time of 4.5 minutes. Solubility of TMSP internal standard was determined and 1 mg/mL was chosen due to excellent solubility and similar peak heights when analysed at the analyte target concentration (20 mg/mL). Stabilities of produced samples were assessed showing cocaine hydrochloride in D_2O should be analysed within six days of analysis and cocaine freebase in $CDCl_3$ should be analysed the same day to maintain less than 1% and 3% concentration deviation respectively. Robustness experiments showcase that between 25 – 40˚C, analysis temperature has no significant effect on analysis, and the use of lower quality, lower rated tubes does not affect low-field analysis, further reducing potential costs.

Method validations were performed on cocaine hydrochloride in D_2O and cocaine freebase in $CDCl_3$ showing good linearity (R^2 = 0.9967 – 0.9995), good precision (1.53 – 3.54% RSD), good accuracy (98.4 – 99.5%), adequate LoD (0.289 – 0.724 mg/mL) and

LoQ (0.875 – 2.193 mg/mL) values for bulk sample analysis with sample recoveries within acceptable ranges (87.3 – 111.0%). 97 cocaine samples were then analysed and compared using the three techniques. Low-field NMR shows a good correlation compared to GC-MS (R^2 = 0.9399) with slight underestimation (-1.25 – 7.19%) and high-field NMR shows slightly less correlation (R^2 = 0.9321) with GC-MS compared to less underestimation (-0.1 – 5.43%). Between low-field and high-field NMR, there is excellent correlation (R^2 = 0.9837) with no significant difference between analysis, showcasing low-field NMR as a cheaper, simpler alternative to high-field NMR for the quantification of cocaine samples. No-D analysis has been performed showcasing only two viable sites of quantification, with similar method validation parameters with the exception of decreased precision. 18 cocaine samples were analysed using no-D solvents showcasing an overestimation compared to traditional NMR solvents (3 - 4%) with decreased precision. However, for the purposes of harm-reduction, a 3 - 4% overestimation still enables an excellent insight into the weight-by-weight composition of a sample.. This allows the identification of samples which are within the extremes of purity content, allowing alerts to be made to the public.

5. Chapter 5 – Detection and Discrimination of Controlled Drugs by Ion Mobility Spectroscopy

5.1. Introduction

An introduction to IMS, showcasing how the instrumentation operates has been outlined previously in section 1.1.5 – Ion Mobility Spectroscopy.

5.1.1. Literature Examples

A number of groups have used IMS for illicit drug bulk sample screening. Armenta *et al.,*[141] analysed 38 purified samples of phenethylamine and synthetic cannabinoid derivatives using IMS. A Smiths Detection IONSCAN-LS was used in positive polarity mode for sample analysis. Eleven representative samples, encompassing a mix from each illicit drug group, were prepared in concentrations between 0.1 – 1.0 mg/L in isopropanol, from which 1 μL was pipetted on to a swab and allowed to dry before analysis. The average inter- and intra-day drifts of the K_0 values for these compounds were also reported, showing a K_0 RSD of 0.006% - 0.12% for intra-day analysis and 0.03% - 0.14% for inter-day analysis; no higher precision was reported since a region of 20 μs is automatically employed by the software for peak detection, which equates to a typical spread of 0.0050 $cm^2 V^{-1} s^{-1}$ dependent on the elution time after the calibrant. Limit of detection for these eleven compounds ranged between 20 – 50 ng. The general trend for the 38 compounds analysed was plotted; it was reported that the higher the molecular weight of the compound, the longer the drift time and resulting K_0 values.

Verkouteren *et al.,*[142] analysed a number of illicit compounds using IMS to determine their K_0 values. Sixteen reference compounds were analysed, with the average spread of the values (assessed using replicates) found to be $\pm$0.0005 $cm^2 V^{-1} s^{-1}$.[142] The K_0 values for five components were similar to previously published data, variabilities most likely caused by differences in the operational parameters between instrumentation.[142] Binary samples of four illicit compounds paired with four adulterants were prepared by co-doping sample swabs with between 3 – 97 % (w/w) of the illicit component. The adulterants were no longer identifiable at 5 – 25 % (w/w) of the mixture, dependant on the adulterant. Cocaine binary mixtures were analysed but were typically paired with

compounds not typically found in cocaine samples, except for procaine which was detected throughout until <10 % (w/w) of procaine in the mixture.

Gwak *et al.,*[143] used a commercial ^{63}Ni-IMS (IONSCAN 400B) and a DART-QTOF to analysis 35 illicit new psychoactive substances (NPS) compounds. Most compounds matched the K_0 values previously reported with a few exceptions; the group assumes that the difference is due to the change in the ionisation source. Drift times were found between 11.741 – 19.092 ms, with K_0 values ranging from 1.5373 – 0.9466 $cm^2 V^{-1} s^{-1}$. The range of uncertainty of 27 compounds was reported as $\pm 50 \times 10^{-3}$ $cm^2 V^{-1} s^{-1}$, $\pm 30 \times 10^{-3}$ $cm^2 V^{-1} s^{-1}$ for six compounds and then $\pm 25 \times 10^{-3}$ $cm^2 V^{-1} s^{-1}$ for just two compounds. The suitability of the new library additions was assessed with the analysis of four seized illicit samples. Each sample was extracted into MeOH, diluted 1:1000 before being added to a swab and allowed to dry. IMS identification matched previous analysis from GC-MS and FT-IR but produced false positives for other compounds due to the closeness of the regions. Limits of detection were identified for the instrument through assessment of cocaine and heroin, with LoD values reported to be 1.0 and 0.2 ng respectively.

Sisco *et al.*[144] used a commercial IMS (IONSCAN 500DT) to analyse seven fentanyl and fentanyl analogues alongside five other commonly found opioids. K_0 values were obtained from analysis of reference material, spanning a range of 1.0910 – 0.9050 $cm^2 V^{-1} s^{-1}$. PTFE swabs were spiked from diluted solutions for analysis. Five pairs of fentanyl analogues were found to appear close within the manufacturers recommended drift range of $\pm 0.0020 \times 10^{-3}$ $cm^2 V^{-1} s^{-1}$. All 22 compounds analysed had no overlaps with each other or any pre-existing compounds in the spectral library and no false positives occurred during reanalysis. The LoD was assessed for these compounds reporting 1 – 5 ng dependant on the compound. Binary mixtures were assessed to see if competitive ionisation is an issue, since fentanyl is typically the minor component in heroin samples. A consistent 5 ng spike of fentanyl was used on each swab, then the adulterant was added in levels of 0, 5, 12.5, 25, 50, 250 and 500 ng. Little suppression was caused by acetaminophen and quinine. Mannitol enhanced the fentanyl signal, caffeine caused minimal suppression until 100 ng and procaine caused complete suppression at high ratios. Binary mixtures of heroin and fentanyl resolved on the previous generation of IONSCAN instrumentation (IONSCAN 400) but the slightly newer

IONSCAN 500DT used in this study has slightly lower resolution, causing the two peaks to merge to produce a single peak with a new K_0. The other fentanyl analogues, mixed with heroin, were also tested but could not be resolved. Background interferences were tested, a 10:1 heroin to fentanyl sample was used and was contaminated with several potential contaminants: artificial fingerprint mix, which caused one swab to be identified as just heroin, with a further one being identified as fentanyl. Dirt was also assessed as a contaminant by using street water from obtained from New York, USA and the amount of sample spiked was increased tenfold, producing an alarm from fentanyl, this was caused by the competitive ionisation from the contaminant. Plasticizers were also identified by swabbing trace number of samples from a plastic bag, which reported for both heroin and fentanyl.

Metternich *et al.*[145] reported on the use of IMS (IONSCAN 600) as screening tool for detecting synthetic cannabinoids within various seized prison samples. Twenty-five synthetic cannabinoids, obtained from seizures with no further purification, were assessed for their K_0 values, ranging from 1.0511 – 0.8069 $cm^2 V^{-1} s^{-1}$, with the intra-day and inter-day ranges reported as 0.0000 – 0.0321 % and 0.0082 – 0.1425 % RSD respectively. Calibration series containing five points were plotted for each reference standard between 1 – 8 ng mass loadings and the LoD values were calculated using 3x SD of the average amplitude for five blank swabs. The reported LoD values range between 0.7 – 3.6 ng mass loading. This methodology was then applied to 36 seized samples consisting of various potentially imprinted papers, herbal materials, cigarettes and typical liquid based medicines (eye drops and nasal ointment) and compared with results obtained from GC-MS. IMS identified at least one synthetic cannabinoid in all samples which contained synthetic cannabinoids but could not report on more than a single component with samples which contained a mixture of cannabinoids.

The reviewed literature has discussed the analysis of several synthetic cannabinoids, fentanyl analogues and other NPS substances. Typically, K_0 values for a compound and the spread around the K_0 values are needed for an analyst to successfully add a compound to an instrumental library. The most commonly identified substances such as cocaine, ketamine and heroin are expected to be previously assessed and included by the manufacturers. This chapter shifts the focus onto other classes of illicit substances. Cocaine and other common cocaine adulterants were analysed to improve

the instruments effectiveness for analysis of cocaine samples. The focus was then shifted to “ecstasy” tablet analysis, where there is a wider range of potential amphetamines. 76 compounds, containing a number of grouped isomers were also assessed, to identify the instrumentations’ ability to differentiate between isomers. Also included within this chapter, is an outline of the deployment of this instrument tested live at a Manchester-based festival event. Results obtained were compared to those obtained by GC-MS. Considerations surrounding the suitability of IMS for use for routine testing within the context of festival testing are also discussed.

5.2. Results and Discussion

5.2.1. Cocaine Reference Compounds

IMS has been used to screen illicit drugs in a number of different reports outlined in section 5.1.1 - Literature Examples. In this research, IMS was assessed in a number of experiments for its ability to screen cocaine samples. This is one of the main objectives for this chapter similar to the other two previous chapters, where the screening and quantification of cocaine samples are the main focus. Cocaine samples typically vary greatly from a purity perspective and often contain a wide variety of potentially toxic adulterants.[89] The identification of every component within cocaine samples is the ideal circumstance which indicates to medical staff the complete contents a sample, in the event of a user becoming unwell. Expanding the instrumentations’ library to include a number of commonly found adulterants, assessing how the instrumentation handles binary mixtures and a number of other robustness experiments, included in this chapter, aims to assess the instrumentations’ ability to analyse these more complex mixtures.

The typical cocaine components seen within Manchester were analysed by IMS and the details of where the peaks occur within obtained plasma graphs is shown in Table 38. The common cocaine adulterants have been found previously by Dixon *et al.,*[140] on samples obtained within central Manchester. K_0 ranges are reported as the total variation of the central K_0 across 20 replicate swabs.

Table 38 - Drift times and K_0 values for cocaine and common adulterants for IMS . †Aspirin eluted in negative polarity

Substance	Drift time range (ms)	K_0 range ($cm^2\ V^{-1}\ s^{-1}$)	K_0 range ($x10^{-3}\ cm^2\ V^{-1}\ s^{-1}$)
Cocaine HCl	8.896 – 8.908	1.1627 – 1.1609	18
Cocaine freebase	8.740 – 9.136	1.1620 – 1.1601	19
Phenacetin	8.716 – 8.884	1.1614 – 1.1597	17
Ketamine	7.420 – 7.660	1.3696 – 1.3684	12
Aspirin†	5.596 – 5.668	1.7570 – 1.7498	72
Tetramisole	7.000 – 7.144	1.4459 – 1.4431	28
Procaine	7.756 – 7.900	1.3053 – 1.3040	13

Cocaine hydrochloride and cocaine freebase vaporise to form the same gaseous molecules with the same drift times. Cocaine freebase has a slightly tighter range of drift times, which could be due to cocaine freebase vaporising at a lower temperature, providing higher consistency during vaporisation and thus, producing less variation in the spread of K_0 values. Phenacetin is found to overlap with cocaine hydrochloride, which causes phenacetin to be reported as cocaine. This is not ideal as seized samples containing only phenacetin, with no illegal component, may be incorrectly recorded as cocaine. Due to the inability to distinguish between these compounds, the entry for the library dataset contained both compounds, reported as cocaine / phenacetin.

Benzocaine, caffeine and paracetamol were also assessed and were found to be IMS inactive. Peaks were typically detected at 7500 ng mass loadings but at these concentrations, saturation of the ionisation source is most likely the cause of these peaks, opposed to the detection of the intended component, as saturation typically results in the formation of additional peaks.[142] Mass loadings of 250 – 500 ng should be adequate to see defined peaks for IMS analysis. Ketamine and cocaine were already included in the IMS library which matches the drift times identified from this study. Although caffeine has been identified *via* other custom-made IMS instrumentation, as reported previously by Jafari *et al.*,[146] peaks cannot be identified using the commercial instrumentation employed herein. Changes between the commercial and the custom-built instrumentation must account for the ability to identify caffeine, most likely drift tube length, ionisation source or gas flow. Further confirmation of this, is shown from work by Dussy *et al.,*[147] which concludes caffeine is IMS inactive when using a commercial IMS instrumentation. Aspirin elutes in negative polarity, whereas every other substance analysed, elutes in positive polarity.

5.2.2. Attempts to Increase Resolution

Attempts were made to increase the resolution of the instrumentation, to potentially separate overlapped peaks. Several instrumental parameters were altered, including inlet and drift tube temperatures as well as drift tube flow rate, which did not produce any useful effects and were somewhat restricted by the instrument's software. Increasing the temperature of the drift tube may potentially increase the K_0 values of the compounds, since less water molecules are present to interact with the compounds and hindering the molecules flightpath less. Similarly, decreasing temperature introduces more water which slows down compounds, reducing K_0 values.[148] For this testing, the drift tube temperature could only be increased to a maximum of 190^{o}C from the standard 170^{o}C and could not be decreased, so no useful data could be obtained. The gas flow within the drift tube could not be changed without causing pressure errors within the instrument. The flow rate was thus increased from 200 to 250 cc / min showing an overall increase in drift times but not one significant enough to increase resolution. A segment is similar to a scan during analysis, the shutter gate opens and closes a set number of times allowing ions to flow through the drift tube and reach the Faraday plate detector. Increasing the number of segments during analysis does improve the sensitivity of the instrument. Unfortunately, the system gets stuck in a "clean check" cycle because contaminants are detected at much lower amounts mass loadings. Continuing forward, would require changing the clean check tolerance for every library. This was not deemed productive as resolution was not increased significantly when an increased number of scans was employed.

A change in the inlet desorption ramp program was used, decreasing the temperature ramp and extending the overall method analysis time. This adjusted program temperature ramp is compared to the standard temperature ramp, shown in Figure 32

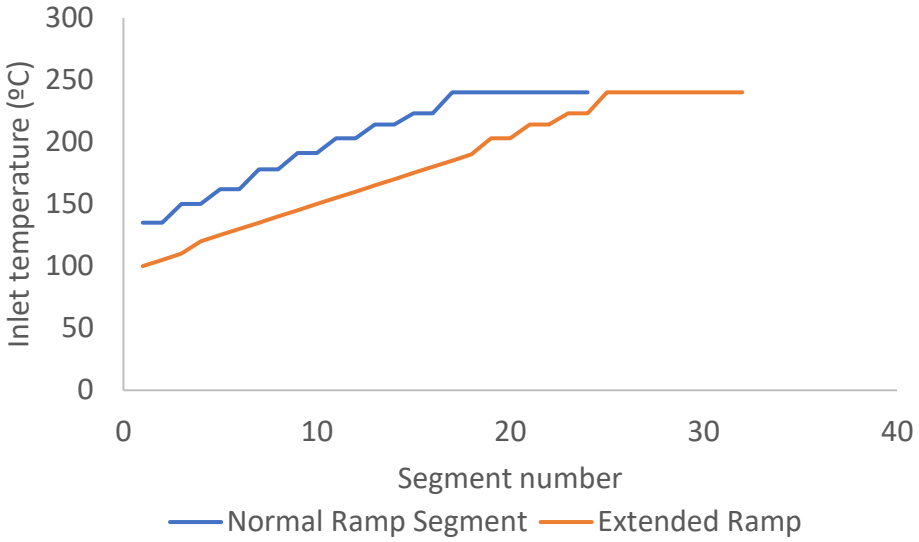


Figure 32 – Manufacturers inlet temperature ramp vs extended program

The aim of the extended method is to allow compounds which co-elute at the similar drift times to resolve individually at their respective desorption temperatures. In actuality, both components still resolve together and continue to do so across all of the extended segments, showing this cannot be used to separate two components. The rate of desorption of the components on the swab does change, causing a higher rate of desorption at the end of the temperature ramp, as expected. A comparison is shown in Figure 33, showing stacked segments which show the difference in cocaine hydrochloride desorption for each desorption ramp program.

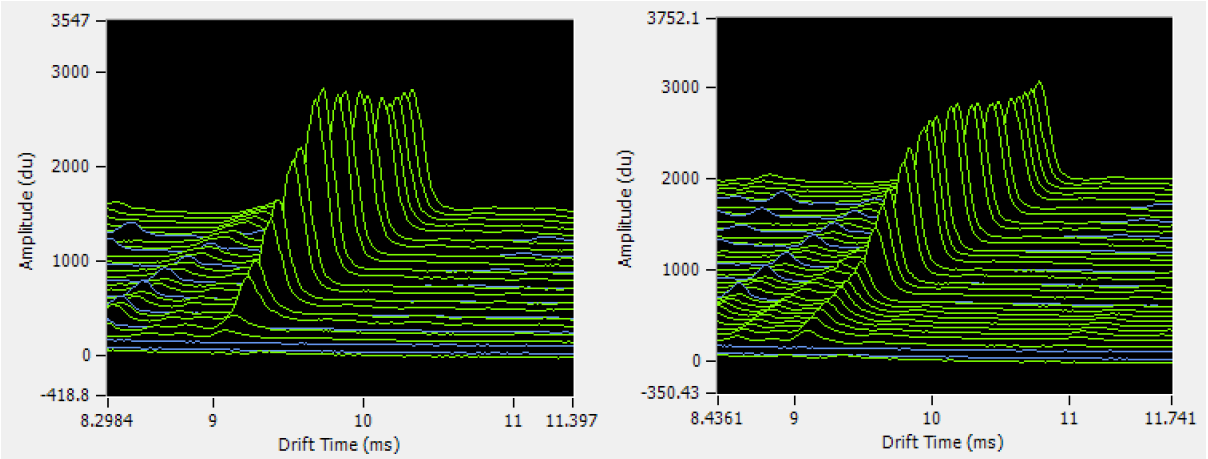


Figure 33 - Inlet desorption comparison of cocaine HCl for the manufacturer default temperature program (left) vs extended program (right)

Changing the open shutter duration is another way to potentially increase resolution. Reducing the duration of time the shutter is open, reduces the number of molecules which pass through into the drift tube and can produce narrower peaks. However, the

instrumental LoD will also decrease. Unfortunately, this setting is locked by the manufacturer, so no attempts to change the shutter speed could be made.

5.2.3. Cocaine Reference Mixture Analysis

Simulated samples containing two components (cocaine hydrochloride paired with either levamisole, procaine or phenacetin), were produced and analysed using IMS. This aimed to assess the overall effect that multiple components, within a sample, had on the overall analysis. These results are shown in Table 39.

*Table 39 - Simulated binary cocaine mixtures analysis. * = Phenacetin overlaps with cocaine so could not be identified*

Adulterant	Only cocaine identified (% (w/w) cocaine)	Both components identified (% w/w) cocaine)	Only adulterant identified (% (w/w) cocaine)
Levamisole	100 - 90	80 - 10	0
Procaine	100	90 - 10	0
Phenacetin	100 - 0 *	-	-

The LoD for this set of analysis, in terms of % (w/w), shall be referred to as the "detection threshold", as to not be confused with instrumental and method LoD. For simulated mixtures containing both cocaine hydrochloride and procaine, both peaks are identified throughout the entire tested range. For simulated cocaine hydrochloride and levamisole mixtures, the adulterant was not identified at 10% (w/w). This could be caused by competitive ionisation from the additional cocaine component, reducing the amount of levamisole molecules ionised, to an amount below the instrumental LoD, or was under this threshold originally, potentially due to a low mass loading to response factor. Phenacetin overlaps cocaine and the presence of both compounds generates a single peak that is wider than each of the two single peaks. These detection threshold limits match the results obtained by Verkouteren *et al.,*[142] who calculated detection threshold limits for a number of illicit mixtures that stopped being identifiable between 5 – 25% (w/w) dependant on the compound. Cocaine and procaine were assessed, showcasing only the adulterant component identified between 10 – 0 % (w/w) cocaine within the mixtures, similar to the cocaine and levamisole mixtures. Procaine is identified from > 90% (w/w) cocaine content, which differs from the > 80% content shown by cocaine and levamisole, showcasing the difference in the response factors between levamisole and procaine within these mixtures.

5.2.4. Cocaine Samples

The initial testing for the new method was performed on 57 cocaine samples, mainly unadulterated cocaine in both hydrochloride and freebase form, with a small number of adulterated samples. These samplesare described in Table 40.

Table 40 - Descriptions of cocaine samples used for the initial IMS testing

Adulterated Cocaine HCl	Number	Adulterated Cocaine Ffreebase	Number
Levamisole	6	Levamisole	4
Phenacetin	1	Phenacetin	7
Caffeine	1	Caffeine	0
Diphenhydramine	1	-	-
Levamisole + Benzocaine	1	-	-
Unadulterated	24	Unadulterated	12

The samples adulterated with phenacetin, only identified as cocaine / phenacetin due to peak overlap. Samples adulterated with caffeine and diphenhydramine were only identified as cocaine since caffeine is IMS inactive and diphenhydramine is not in the instrument's library. Diphenhydramine is not prevalent enough to warrant adding to the instrument's library, as it is not frequently shown in major studies.[44] The single tertiary sample, containing cocaine hydrochloride, levamisole and benzocaine, only identified as cocaine and levamisole since benzocaine is IMS inactive. There were four samples which contained cocaine freebase and levamisole, which did not identify as expected by IMS. The four samples adulterated with levamisole were quantified *via* GC-MS and three of which were found to contain between 2.54 % $\pm$ 0.38%- 6.43 % $\pm$ 0.69% (w/w) for levamisole. The remaining sample did not have enough material to accurately quantify. However, levamisole consisted of 0.74% of the total peak area within the chromatograph, aiding the assumption that levamisole was present in an extremely low % (w/w) content. Overall, IMS correctly identified the illicit component in all 57 samples but could only identify levamisole as an adulterant in 7 out of 11 samples, resulting in only 7 out of 21 adulterated samples with complete identification - no other adulteration detected. This shows that IMS is a useful technique in identifying the illicit substance in bulk samples but has a difficulty identifying cocaine adulterants as a large number of potential adulterants are IMS inactive. For complete cocaine sample analysis, another instrument should be ideally used, in tandem, to identify a wider range of potentially harmful adulterants. A more in-depth discussion has been made in Chapter 3 – Qualitative Screening and Comparison of Analytical Techniques.

5.2.5. Small Scale IMS Tablet Study

Following on from the cocaine analysis, a collection of samples (n = 112) seized from a festival, which were tested during a live event and consisted of mainly "ecstasy" tablets reported to be MDMA, were analysed using IMS. Initially, a number of library additions had to be made to allow comparative analysis. These compounds have been prevalent within the samples analysed and needed to be added to the library of the instrumentation before analysis to allow identification of these compounds. These compounds with their respective drift times and K_0 values are shown in Table 41.

Table 41 - Drift times and K_0 values compounds commonly prevalent in tablets for IMS (α-PVP = alpha-Pyrrolidinopentiophenone)

Compound	Drift time (ms)	K_0 ($cm^2 V^{-1} s^{-1}$)	K_0 range ($x10^{-3} cm^2 V^{-1} s^{-1}$)
2C-B	7.804 – 8.104	1.3070 - 1.3028	42
α-PVP	7.816 – 8.128	1.3043 – 1.3024	19
4-CMC	7.264 - 7.288	1.4451 - 1.4394	57
4-MMC	7.264 - 7.288	1.4844 - 1.4809	35
3-MMC	6.916 – 7.204	1.4726 – 1.4675	51
MDMA	7.000 – 7.264	1.4573 - 1.4516	57
Putylone	8.068 – 8.392	1.2626 – 1.2602	24

The compounds outlined in Table 41, with the exception of MDMA, are new library additions. The library parameters of MDMA, match the pre-programmed parameters included within the original library, except the K_0 range, which is 97 $x10^{-3} cm^2 V^{-1} s^{-1}$ instead of the much narrower 37 $x10^{-3} cm^2 V^{-1} s^{-1}$ from this study. The typical K_0 range differs across the original library but generally consist of wider ranges than the values obtained here. This is most likely because of the commercialisation of the instrument and method, and thus has a wider range to be robust enough to cope with the wider shifts in K_0 values due to changes in pressure, temperature, humidity etc. For a laboratory setting where the majority of the time the temperature and humidity is controlled, this large width is not needed. The prevalence shown for the 112 samples are shown in Table 42.

Table 42 - Prevalence for the small scale (n=112) "ecstasy" based seizures

Compound	Number	Prevalence (%)
MDMA	87	77.7%
2-CB	5	4.5%
3-MMC	4	3.6%
Nothing Detected	3	2.7%
4-CMC	2	1.8%
4-MMC	2	1.8%
Caffeine	2	1.8%
4-MMC + 4-CMC	2	1.8%
Amphetamine	1	0.9%
α-pvp	1	0.9%
Cocaine	1	0.9%
Ketamine	1	0.9%
Putylone	1	0.9%

For the samples containing a single component (n = 104), IMS correlated with the result provided *via* GC-MS for 102 (98.1%) samples. The two samples which did not correlate were samples for which GC-MS only identified caffeine. It is expected for IMS to produce a pass result for these samples because caffeine is IMS inactive, as described previously in the chapter.

For samples containing binary mixtures (n = 6), IMS was able to identify and match the illicit component (MDMA, 4-MMC, amphetamine and “putylone”) in all six samples but not the adulterant. This is because the adulterant for each sample was caffeine and as expected, is not IMS active.

The tertiary samples (n = 2) samples contained 4-MMC (12.7 – 15.4% (w/w)) and 4-CMC (2.7 – 4.2% (w/w)) with caffeine as an adulterant for both, which the IMS only identified 4-MMC.. This is most likely because 4-CMC is at content percentage lower than the detection threshold to be identified using this method. Since 4-MMC and 4-CMC elutes at similar K_0 values, the minor component within each sample may be undetectable due to masking from the major peak. An assessment of samples containing pairs of compounds is made later on in section 1.2.6 - “Ecstasy” Tablet Based Detection Threshold.

Unlike cocaine adulterants, there is a wide range of compounds which are still IMS active, that are typically found within “ecstasy” tablets. From this study, the detection of the illicit major component in each sample shows that the fillers typically found in illicit tablets do not cause major interference and the instrumentation can still identify

the API in the potentially lower concentrations which are typically found in tablets. Further assessment on a small number of potentially interfering illicit tablet components is made later in section 5.2.6 - “Ecstasy” Tablet Based Detection Threshold.

Putylone is a rarely identified synthetic cathinone which the characterisation, synthesis and quantification of this sample is outlined in Chapter 6 - Synthesis, Characterisation and Quantification of the new Psychoactive Substance “putylone”. It is first outlined within this chapter as it was IMS analysis, which originally alerted the writer to its presence.

5.2.6. “Ecstasy” Tablet Based Detection Threshold

Certain tablet fillers / binding agents commonly found within “ecstasy” tablets such as lactose,[98] are found to be IMS inactive. Due to the nature of the ionisation used within this technique, the presence of other compounds, irrespective of IMS activity, could potentially cause competitive ionisation, which may shift K_0 values or reduce the sensitivity of the technique. Sample mixtures containing MDMA and other IMS inactive components were co-deposited on swabs and analysed using IMS. This aimed to assess if common tablet diluents produce interference with the main analyte during analysis. These results are shown in Table 43.

Table 43 - IMS inactive MDMA tablet filler / adulterant co-swab analysis for IMS

Swab components		Alarm range (% w/w)	K_0 shift (x10^{-3} $cm^2V^{-1}s^{-1}$)
Component 1	Component 2	Component 1	
MDMA	Lactose	100.0 - 10.0	5
MDMA	Boric Acid	100.0 - 10.0	3
MDMA	Caffeine	100.0 - 10.0	5

Independent of which diluent / adulterated tested within the small range that was used, MDMA was identified within 10.0 – 100.0 % (w/w) doped on the swab. A 5% (w/w) was also tested, to identify that the minimum percentage in which this method can no longer identify an IMS active component is between 5.0 – 10.0% (w/w). This matches the previous work on cocaine adulterants earlier in the report. The shift in K_0 values for each peak were also assessed. The small change is similar to the K_0 shifts that occur during normal sample replicates, showing that these specific IMS inactive diluents / adulterants do not affect IMS analysis. An assessment extending to every

illicit tablet filler, which may interfere with IMS analysis, cannot be made due to the vast number of compounds which could be used for illicit tablet production.

Binary mixtures commonly found “ecstasy” compounds were also analysed to assess how this specific IMS instrument handles two pairs of closely eluting compounds. The results of this are shown in Table 44.

Table 44 - Near co-eluting compounds co-swab analysis using IMS

Swab components		Alarm range (% w/w component 1)			K_0 shift (x10^{-3} cm^2 V^{-1} s^{-1})	
Component 1	Component 2	Component 1	Both	Component 2	Component 1	Component 2
MDMA	4-MMC	100.0 - 60.0	50.0 - 20.0	10.0 - 0.0	22	3
4-MMC	4-CMC	100.0 - 50.0	40.0 - 30.0	20.0 - 0.0	50	32

The assumption for peaks which elute a wide range apart, similar to cocaine and levamisole assessed previously, is that each peak elutes and is identified throughout the entire range of volumes until the detection threshold of the method. For peaks which elute close together, one peak has the potential to elute underneath the larger one, essentially masking one component. This is the case with the pair of mixtures tested in these two examples. The range in which both compounds were detected were small and centred around lower amounts of component 2, indicating that typically the response factor from MDMA is greater than the response from 4-MMC, which in turn is greater than the response from 4-CMC. The K_0 shifts during the presence of both compounds are greater than normally observed shifts from replicate swabs, typically between 2 – 7 x10^{-3} cm^2 V^{-1} s^{-1}. This variation is due to the instruments capability to detect the secondary peak within inside the larger peak. Although the shifts are large, the K_0 values were consistently within the programmed range for the instrument to produce an alarm even though in some circumstances peaks were not identified. Furthermore, from inspection of the raw plasmagrams, two peaks can be identified by inspection even though they appear overlayed, showing a potential limitation with the way the instrument alarms for positive identifications.

5.2.7. Physical Limits of Detection

The physical limits of detection for this method were assessed, to identify the minimum mass loadings present on swabs, for the instrument to produce a peak with sufficient signal to noise (3.3 S:N). 33 blank swabs, only spiked with MeOH were

analysed in total and the amplitudes at the K_0 values for eight different prevalent compounds were recorded. The average amplitude for these blanks (137.3) was then multiplied by 3.3 (453.2), to find the amplitude at the statistical LoD for each compound, where the signal to noise ratio is 3.3. Amplitudes are plotted opposed to peak areas due to limitations within the instrument's software.

Calibration series were plotted for all eight compounds, between 0 – 500 ng mass loading which showed a consistent quadratic trendline. It is assumed the ionisation source and / or the detector became saturated at higher mass loadings. The equation for each trendline, was then solved for 453.2 amplitude units to identify the LoD for each individual compound. Equations and calculated LoD values for each compound are shown in Table 45.

Table 45 - Calibration series trendline equations for 0 - 500 ng mass loading of illicit material and their respective calculated LoDs

Compound	Equation	R^2	LoD (ng)
MDMA	$-0.0008x^2 + 3.1298x + 132.00$	0.9936	41.7
Levamisole	$-0.0106x^2 + 5.0718x + 180.96$	0.9735	33.4
4-MMC	$0.0014x^2 + 5.4915x + 114.18$	0.9999	20.9
4-CMC	$-0.0037x^2 + 5.5513x + 97.102$	0.9994	17.7
Cocaine HCl	$-0.0164x^2 + 9.4019x + 208.93$	0.9963	21.4
Cocaine FB	$-0.0168x^2 + 10.633x + 190.85$	0.9972	17.5
Ketamine	$-0.0069x^2 + 8.1986x + 211.05$	0.9905	25.2
NEP	$-0.0165x^2 + 13.961x + 173.99$	0.9944	12.3

Graphical plots for MDMA, levamisole, 4-MMC and 4-CMC are shown in Figure 34, whilst graphical plots for cocaine hydrochloride, cocaine freebase, ketamine and NEP are shown in Figure 35.

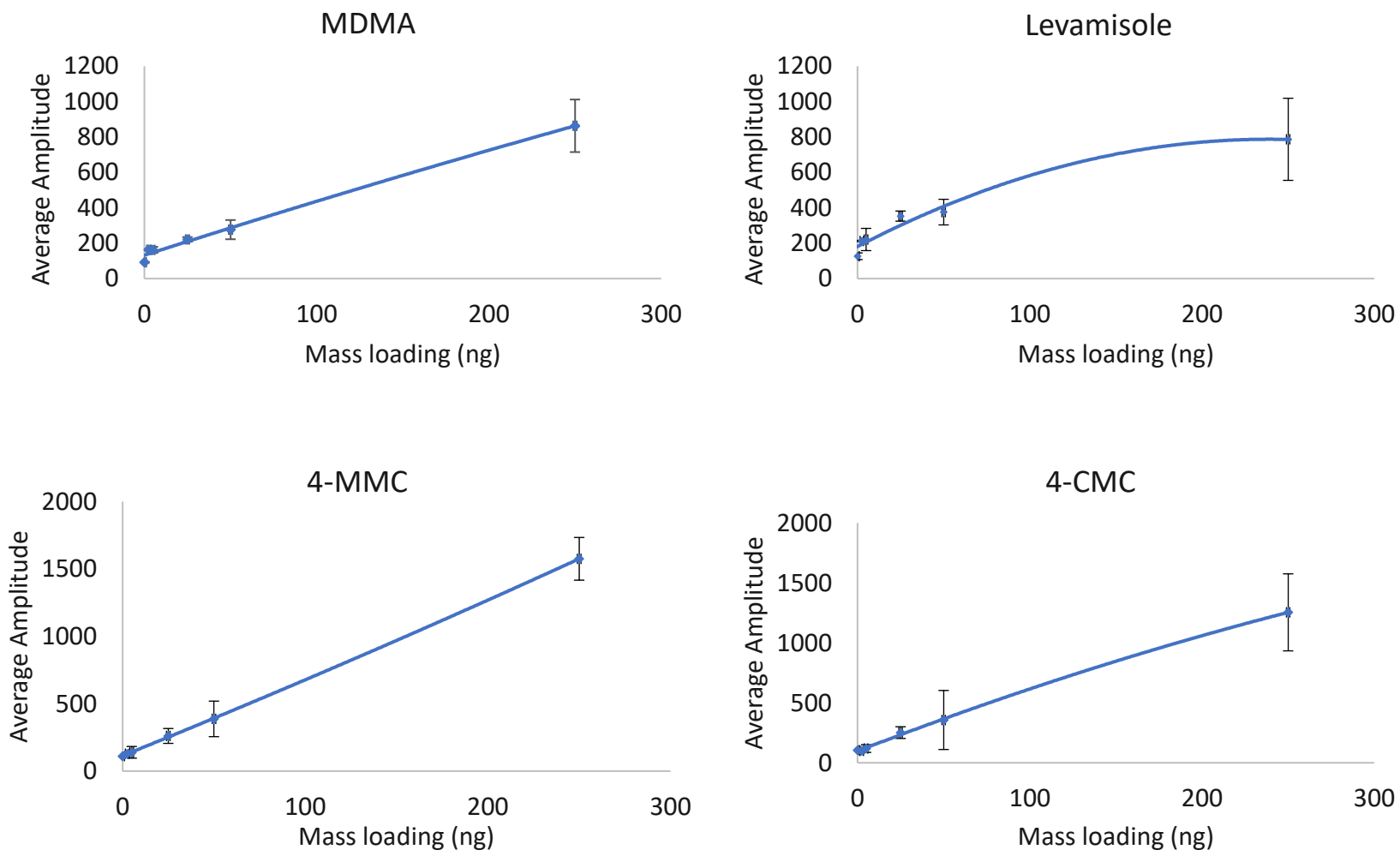


Figure 34 - Physical Limit of detection mass loading calibration plots for MDMA, levamisole, 4-MMC and 4-CMC for IMS analysis.

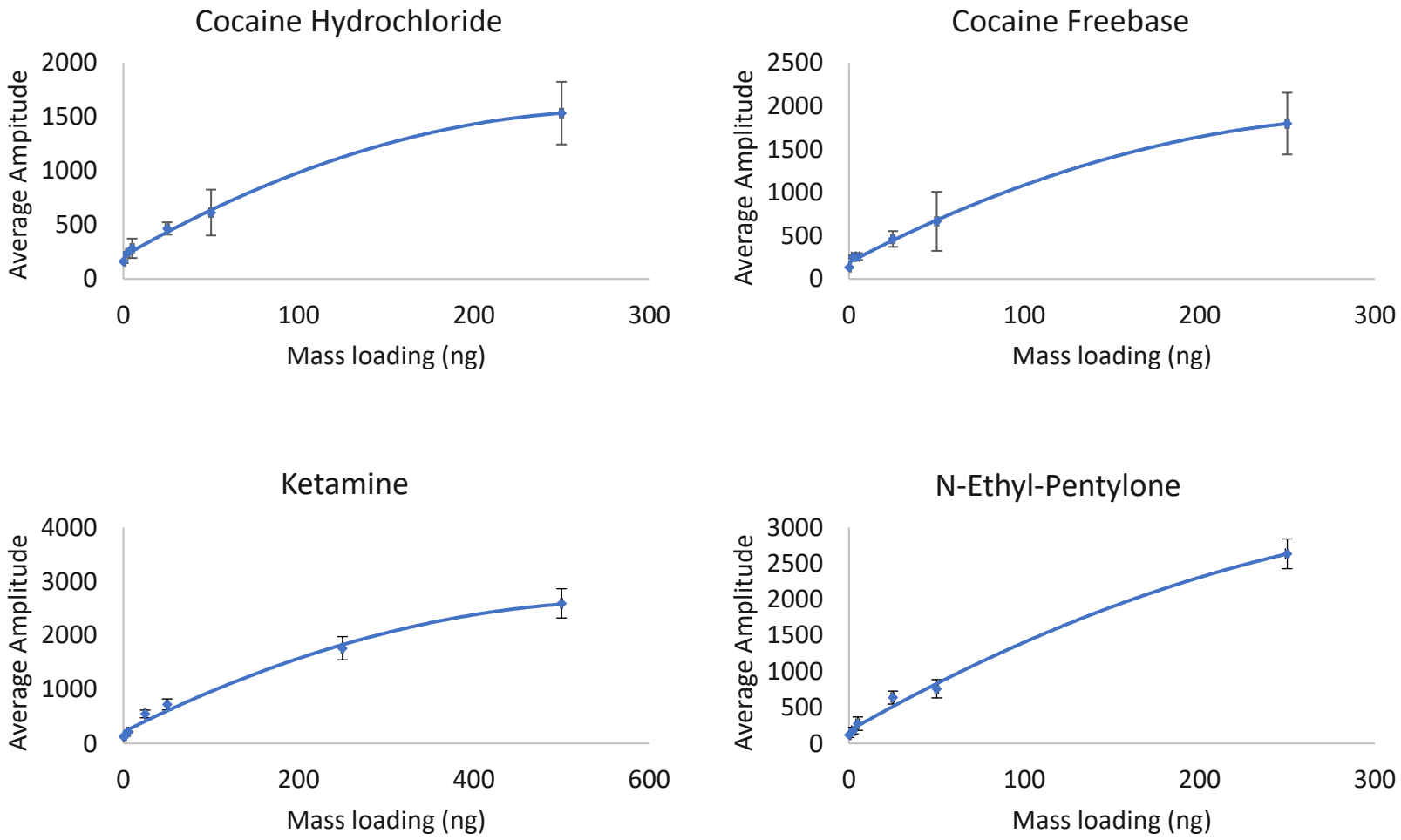


Figure 35 – Physical Limit of detection mass loading plots for cocaine hydrochloride, cocaine freebase, ketamine and NEP, for IMS analysis

The LoD varied dependant on the analyte. The LoD of these eight illicit compounds ranged between 12.3 – 41.7 ng mass loading. This is similar to the LoD range reported by Armenta *et al*.[149] This data is summarised in Table 46.

Table 46 - IMS LoD comparisons with literature

Author	Analyte group	Instrument	Source	LoD range (ng)
Armenta *et al.*[149]	Synthetic cannabinoids	IONSCAN LS	^{63}Ni	20 – 50
Dussy *et al.*[147]	New psychoactive substances	IONSCAN 400B	^{63}Ni	0.2 – 1.0
Sisco *et al.*[144]	Fentanyls and opioids	IONSCAN 500DT	^{63}Ni	1.0 – 5.0
Metternich *et al.*[145]	Synthetic cannabinoids	IONSCAN 600	CD	0.7 – 3.6
This work	Prevalent festival seized compounds	IONSCAN 600	CD	12.3 – 41.7

The differences in LoD range, between compounds, is shown to be based purely on the individual compounds analysed, since the range and scale of LoD values obtained is similar to the range reported by Armenta *et al.,*[141] despite using a different instrument with a different ionisation source. Similarly, the ranges reported by Armenta *et al.,*[141] compared against Dussy *et al.*[147] and Sisco *et al.*[144] are an order of magnitude less sensitive, despite a similar ionisation source. The single order of magnitude difference between the reported LoD values obtained between Armenta *et al.*[141] and Metternich *et al.*[145] when analysing the same class of compounds may actually be due to changes within the ionisation source and changes to the instrumentation between generations of the IONSCAN instrumentation.

5.2.8. Sample Swab Stabilities

Ideally, once the sample solution has been deposited on swabs for analysis, the swab should be inserted into the instrumentation for analysis as soon as possible without touching any surfaces. This is to minimise contamination of the swab and to ensure stability of the deposited material. If the sample swabs have to remain unanalysed for any length of time, in the case of instrumentation failure or a back log during analysis, then analysts need to be sure the swabs will remain stable for that duration of time. To assess this, a number of swabs were prepared following the normal sample analysis method and placed in an unsealed but covered clean container, which was swabbed before analysis to ensure the box was free of contamination. Separate swabs with MDMA, ketamine, cocaine hydrochloride and cocaine freebase were spiked and placed into distinct sections within the box. The order of the swab sections was MDMA,

ketamine, cocaine freebase and then cocaine hydrochloride. Each day at the same time, a swab was removed and analysed, with the results shown in Table 47.

Table 47 - IMS swab stability results

Time after deposit (hours)	MDMA	K_0 main compound ($cm^2 V^{-1} s^{-1}$)	Ketamine	K_0 main compound ($cm^2 V^{-1} s^{-1}$)	Cocaine freebase	K_0 main compound ($cm^2 V^{-1} s^{-1}$)	Cocaine hydrochloride	K_0 main compound ($cm^2 V^{-1} s^{-1}$)
0	MDMA	1.4571	Ketamine	1.3701	Cocaine	1.1629	Cocaine	1.1614
1	MDMA + Cocaine	1.4568	Ketamine + Cocaine	1.3691	Cocaine	1.1606	Cocaine	1.1606
4	MDMA + Cocaine	1.4567	Ketamine + Cocaine	1.3694	Cocaine	1.1621	Cocaine	1.1606
7	MDMA + Cocaine	1.4562	Ketamine + Cocaine	1.3691	Cocaine	1.1617	Cocaine	1.1602
24	MDMA + Cocaine	1.4557	Ketamine + Cocaine	1.3703	Cocaine	1.1611	Cocaine	1.1611
48	MDMA + Cocaine	1.4551	Ketamine + Cocaine	1.3696	Cocaine	1.1612	Cocaine	1.1612
72	MDMA + Ketamine	1.4568	Ketamine	1.3689	Cocaine	1.1621	Cocaine	1.1619
96	MDMA + Cocaine	1.4561	Ketamine + Cocaine	1.3708	Cocaine	1.1607	Cocaine	1.1620
168	MDMA + Ketamine	1.4544	Ketamine	1.3689	Cocaine	1.1610	Cocaine	1.1610
192	MDMA + Ketamine	1.4549	Ketamine	1.3691	Cocaine	1.1602	Cocaine	1.1602
216	MDMA + Ketamine	1.4545	Ketamine	1.3701	Cocaine + Ketamine	1.1607	Cocaine	1.1607
240	MDMA + Ketamine	1.4567	Cocaine + Ketamine	1.3698	Cocaine + Ketamine	1.1612	Cocaine	1.1612
264	MDMA + Ketamine + Cocaine	1.4548	Ketamine	1.3682	Cocaine	1.1618	Cocaine	1.1621

Initial swab analysis (0 hours) returned expected results, with each swab possessing only the deposited component. All analysis after the initial point contained contamination from the other compounds present within the box, with the exception of cocaine hydrochloride. Contamination is just as likely to occur on the cocaine hydrochloride swabs, however, the adjoined section contained cocaine freebase, which the IMS cannot distinguish from cocaine hydrochloride. For MDMA, ketamine and cocaine freebase, the contamination is present mainly from the neighbouring sections, despite the box remaining still, flat and covered for the duration of the experiment, indicating that the deposited dried compounds may become airborne with slight disturbances. If contamination is occurring in this manner, then storage of swabs for analysis together would be inadvisable due to the risk of contamination.

For each set of sample swabs, the deposited compound was the most prevalent peak on their respective plasmagrams, implying that without contamination the swabs would remain stable enough for analysis for two weeks or potentially more. Compound degradation is assumed not to be present as no other peaks were identified, with the exception of known contamination. However, if degradants elute near the main component, then small amounts may not be detected. Without identifying potential degradants *via* forced degradation first, no further assumptions can be made.

5.2.9. Manchester Based Festival Live Event Testing

IMS was used for routine illicit drug sample screening (n = 195) across a live Manchester-based festival event (Aug 2022). A number of library additions had to be made to accommodate the change in prevalence, which are shown in Table 48. The overall prevalence of materials identified from the study is described in Table 49.

Table 48 - Manchester based festival reference library additions (MDPHP = 3',4'-methylenedioxy-α-pyrrolidinohexiophenone DMP = Dimethylpentylone)

Compound	Drift time (ms)	K_0 ($cm^2\ V^{-1}\ s^{-1}$)	K_0 range ($x10^{-3}\ cm^2\ V^{-1}\ s^{-1}$)
MDPHP	8.836 – 9.184	1.1536 – 1.1515	21
Pregabalin	6.832 – 6.976	1.5361 - 1.5313	48
NEP	7.984 - 8.212	1.2670 - 1.2649	21
DMP	8.248 – 8.356	1.2750 – 1.2728	22

Table 49 - Manchester based festival sample prevalence (August 2022), analysed via IMS

Compound	Number	Prevalence
Cocaine	120	61.5%
Ketamine	31	15.9%
MDMA	26	13.3%
MDPHP	6	3.1%
‘Clean'	4	2.1%
Amphetamine	2	1.0%
Pregabalin	1	0.5%
Caffeine	1	0.5%
Cocaine + Ketamine	2	1.0%
Cocaine + MDMA	1	0.5%
MDMA + Ketamine	1	0.5%

As shown in Table 49, the most prevalent drugs encountered were cocaine, ketamine and MDMA samples (90.7% of samples surveyed). Four samples (2.0%) contained different combinations of cocaine, ketamine or MDMA which cannot be placed into a single prevalence category. This data was compared with the results obtained *via* GC-MS. For this reason, it was expected that samples containing only GC inactive material would identify as a ‘clean’ chromatograph containing only methyl stearate (used as an internal standard), during GC-MS analysis. Typically, compounds that are GC inactive are assumed IMS inactive, due to the vaporisation step required in both techniques. This is typical shown with good agreement between GC-MS and IMS results identified during this work. No other instrumentation was used for this sample set, as a much more in-depth comparison on instrumental techniques has been made previously in Chapter 3 – Qualitative Screening and Comparison of Analytical Techniques.

The prevalence for the single component samples (n = 163) are outlined in Table 50. Five samples were not analysed using IMS, due to insufficient material for multiple analysis.

Table 50 - Single component identification comparison of samples obtained from a Manchester- based festival (August 2022), analysed via GC-MS and IMS

GC-MS			IMS		
Compound	Number	Prevalence	Compound	Number	Prevalence
Cocaine	97	59.5%	Cocaine	97	59.5%
Ketamine	30	18.4%	Ketamine	30	18.4%
MDMA	24	14.7%	MDMA	22	13.5%
MDPHP	6	3.7%	MDPHP	6	3.7%
'Clean'	4	2.5%	'Clean'	4	2.5%
Pregabalin	1	0.6%	Pregabalin	1	0.6%
Caffeine	1	0.6%	Pass	1	0.6%
			MDMA + Cocaine / Phenacetin	1	0.6%
			MDMA + Levamisole	1	0.6%

As shown in Table 50, all but three samples (98.2%) correlated with the identification obtained from GC-MS. One sample contained only caffeine, which as discussed previously, cannot be detected using this instrumentation. For one MDMA sample, contamination within the sample was most likely to have occurred since cocaine / phenacetin was also identified. For another MDMA sample which was identified as MDMA and levamisole, the dual identification is due to overlap within the expected K_0 values which causes the software to report two components for a single peak. If these adulterants were present within the sample, they would have been detected by GC-MS. In normal cases, re-analysis would occur to confirm if the presence of the additional component but there was insufficient sample remaining to perform repeat analysis. The remaining 160 single component samples matched the analysis performed using GC-MS resulting in a 98.2% correlation rate.

26 samples were identified as binary mixtures from GC-MS analysis. These samples were also analysed by IMS and the results of this investigation are shown in Table 51.

Table 51 - Manchester festival based binary mixtures. GC-MS vs IMS analysis

Sample	GCMS Identification	IMS Component 1	IMS Component 2	Correlation
Q22123	Cocaine + Phenacetin	Cocaine / Phenacetin		Components overlapped
Q22146	Amphetamine + Caffeine	Amphetamine		Adulterant IMS inactive
Q22093	Amphetamine + Caffeine	Amphetamine		
Q22100	Cocaine + Benzocaine	Cocaine / Phenacetin		
Q22132	Cocaine + Benzocaine	Cocaine / Phenacetin		
Q22134	Cocaine + Benzocaine	Cocaine / Phenacetin		
Q22135	Cocaine + Benzocaine	Cocaine / Phenacetin		
Q22143	Cocaine + Benzocaine	Cocaine / Phenacetin		
Q22175	Cocaine + Caffeine	Cocaine / Phenacetin		
Q22190	Cocaine + Caffeine	Cocaine / Phenacetin		
Q22002	MDMA + Caffeine	MDMA		
Q22015	Cocaine + Procaine	Cocaine / Phenacetin		Adulterant low % (w/w)
Q22036	Cocaine + Levamisole	Cocaine / Phenacetin		
Q22110	Cocaine + Levamisole	Cocaine / Phenacetin		
Q22054	Cocaine + Levamisole	Cocaine / Phenacetin		
Q22044	Cocaine + Levamisole	Cocaine / Phenacetin	Levamisole	Complete correlation
Q22083	Cocaine + Levamisole	Cocaine / Phenacetin	Levamisole	
Q22142	Cocaine + Levamisole	Cocaine / Phenacetin	Levamisole	
Q22030	Cocaine + Levamisole	Cocaine / Phenacetin	Levamisole	
Q22089	Cocaine + Levamisole	Cocaine / Phenacetin	Levamisole	
Q22139	Cocaine + Levamisole	Cocaine / Phenacetin	Levamisole	
Q22180	Cocaine + Levamisole	Cocaine / Phenacetin	Levamisole	
Q22006	Cocaine and MDMA	Cocaine / Phenacetin	MDMA	
Q22027	Cocaine + Ketamine	Cocaine / Phenacetin	Ketamine	
Q22102	Cocaine + Ketamine	Cocaine / Phenacetin	Ketamine	
Q22147	MDMA + Ketamine	Ketamine	MDMA	

For 11 samples (42.3% of binary samples), IMS identified both components present resulting in a complete identification compared to results obtained from GC-MS. For ten samples (38.5% of binary samples), IMS enabled the identification of the illicit component within the mixture but not the adulterant. With respect to caffeine and benzocaine adulterants, since these compounds are IMS inactive, this result was expected. For one sample containing both cocaine and phenacetin, IMS facilitated the potential identification of both and cocaine / phenacetin was returned.. This is because cocaine and phenacetin eluted at extremely close K_0 values, being indistinguishable to IMS, due to the inherent nature of the technique. For the remaining four samples, the adulterant component was not identified; this is due to a low content (% (w/w)) within each sample, which was outside the LoD of this method. Only one of the samples was quantified, Q22054, which possessed 3.4% $\pm$ 5.2% (w/w) levamisole content. The other three did not have sufficient material remaining, so the peak area percentage of levamisole / procaine of the total peak area on the chromatograph was used, showing a range between 1.8% – 6.0%. This shows agreement with the assessed detection threshold assessment outlined previously in the chapter, where fabricated samples between 0.0 - 20.0% (w/w) levamisole and 0.0% – 10.0% (w/w) procaine were not detected.

One sample was identified as a tertiary mixture containing cocaine, benzocaine and levamisole. IMS identified the illicit component cocaine with one adulterant levamisole. However, IMS analysis did not identify benzocaine, which is expected since this compound is IMS inactive as previously discussed.

5.2.10. Qualitative Instrumental and Method Assessment

During the Manchester-based live festival (August 2022), multiple analysts were given the opportunity to use IMS on a small selection of pre-prepared illicit drug samples and provide feedback on the technique and the method in the form of a short survey.

A pool of nine samples, plus one control sample, were analysed across ten analysts. Two analysts were asked to repeat their assigned samples to check repeatability and one analyst was asked to repeat analysis but on a different assignment of samples resulting in 13 total tests. The sample analysis has outlined a number of potential drawbacks from the method, which has resulted in changes to the control parameters (CP) and the standard operating procedure (SOP) for IMS analysis. The CP consists of a

file in which both the operating parameters (temperatures, flow rate, shutter speed etc.) and the compound library are stored. The analysts' results for each sample are outlined in Table 52.

Table 52 - IMS live event survey sample analysis results (Cl-P = Chloro-phenidine)

Sample Number											
Analyst	Control	Control K_0	1	2	3	4	5	6	7	8	9
1	4-Cl-P	1.1523	Levamisole			MDMA			4-Cl-P		
1 - Repeat	-	-	MDMA			MDMA			4-Cl-P		
2	4-Cl-P	1.1520		Butylone /Eutylone			Cocaine			2-Cl-P	
2 - Repeat	MDPHP	1.1523	Levamisole			MDMA, Levamisole			4-Cl-P		
3	MDPHP	1.1526			Cocaine			Heroin, Levamisole			Heroin, NEP, 3-Cl-P
4	MDPHP + 4-Cl-P	1.1524		Butylone /Eutylone				Levamisole, 4-MMC, 4-CMC		2-Cl-P	
4 – Repeat	MDPHP	1.1523		Ketamine, Butylone /Eutylone				Levamisole		2-Cl-P	
5	MDPHP + 4-Cl-P	1.1522			Cocaine	MDMA, Levamisole, Cocaine					Heroin, 3-Cl-P
6	MDPHP	1.1530	Pass				Cocaine		Pass		
7	Ketamine, Butylone /Eutylone, MDPHP	1.1523			Cocaine		Cocaine				3-Cl-P
8	MDPHP	1.1525	MDMA, Levamisole					Levamisole, Cocaine, 4-MMC, 4-CMC	Cocaine, MDPHP, 4-Cl-P		
9	MDPHP	1.1526		Ketamine, Butylone /Eutylone		Pass				2-Cl-P	
10	4-Cl-P	1.1518		Ketamine		MDMA, Levamisole				2-Cl-P	
Actual	**MDPHP**		**MDMA**	**Ketamine**	**Cocaine**	**MDMA (Tablet)**	**Cocaine + Levamisole**	**4-CMC**	**4-Cl-P**	**2-Cl-P**	**3-Cl-P**

The results from the sample analysis showcase a number of issues with the CP at the time of analysis. MDPHP was used as the control to ensure each analysts' analysis was consistent. When analysing the average plasmagram obtained from each sample, each MDPHP peak is present and in a very tight range in all of the control samples, as shown by the reported K_0 values for the control peaks. The reason for the difference in the substance reported is due to how the CP was programmed. When obtaining the spread of data for MDPHP, only a small number of replicates were used, opposed to the multiple replicates spread across multiple days as required. The range of K_0 values were thus not accurate, causing unreliable identification for MDPHP. A similar issue occurred for ketamine, showcased with sample 2. For analysts 4 and 5, MDPHP and 4-Cl-P were both reported. This is due to the way in which the instrument identifies peaks. If the tip of the peak falls within multiple substance ranges, all of the alarms should be shown. For 4-Cl-P and MDPHP, K_0 ranges overlap slightly resulting in both substances being reported. For analyst 7, ketamine could have contaminated the swab, assumingly the analyst placed the swab down on an unclean surface during analysis, causing contamination of the swab. This is likely to have occurred since bulk sample analysis was performed using the instrument in tandem with these experiments. The issue with sample peaks eluting between multiple alarm ranges is an issue throughout the testing, also shown in samples 1, 2, 4, 6, 7 and 9. The average plasmagrams have been manually analysed and confirmed that each peak elutes within each expected regions. Contamination has also occurred for analysts 3, 5 and 8 with 4-CMC, MDMA (tablet) and 4-CMC / 4-Cl-P showing contamination respectively. Certain samples were also under the required threshold needed for the instrument to alarm; both occasions with analyst 6 for MDMA (tablet) and 4-Cl-P. It is possible that the analyst did not transfer enough sample solution, combined with the low concentration of the pre-made samples, causing each sample to be on the borderline of the detection threshold. This was backed up after manual analysis of the plasmagrams, the samples that only showed a "pass" did have their respective MDMA / 4-Cl-P peaks present but were under the threshold needed to be reported.

Samples 3 and 8 gave expected results. Levamisole was not identified in sample 5, most likely due to low percentage content (% (w/w)) of levamisole in the sample. Sample 6 alarmed for three different substances which all can be expected to be present within the sample. Swab contamination is the most likely cause of the unexpected

identifications. This lack of selectivity is a drawback of the technique, showcasing the need to use more than one technique when performing analysis of this nature.

Replicate analysis was performed by three analysts, all performed on different days of the event. For analysts 1 and 4, the same samples were analysed to assess inter-day and inter-analyst repeatability, which provided consistent results for 2- / 4-Cl-P, compounds of which were programmed into the library using the described larger number of reference runs. Results were different for the references with reduced number of replicates, MDMA, ketamine and 4-CMC. This showcases the shift in K_0 values for sample peaks across different days, due to changes in temperature, pressure, humidity etc. and the requirement for the addition reference replicates. Analyst 2 performed replicate analysis on a different set of samples; although all consisted of positive identifications, no other comparisons can be made.

Following on from this event, several changes were made to the SOP. Firstly, reference analysis was repeated for MDPHP and ketamine to include the larger number of intra- / inter-day replicates. The SOP was updated to increase the initial concentration of the samples, to allow lower percentage content (% (w/w)) API in samples to be detected.. Also, the inclusion of a warning about swab contamination to the SOP, either by not placing the swab down at all or placing on a clean surface which is swabbed to test cleanliness every few samples, was added.

5.2.11. Isomeric Discrimination

Since IMS separates and identifies molecules primarily by their size and shape, as explained within section 1.1.5,[49, 50, 53] this technique has the potential to separate between different isomers within groups of pharmaceutically active compounds. 22 different groups of isomers were analysed for their respective K_0 values and respective ranges, encompassing substituted cathinones, ephenidines, diphenidines and (di-)substituted fentanyls. Isomers such as these are useful compounds for analysing the separation power of IMS. The majority of these isomers are grouped together in triplets where only the position of the substituent changes, affecting the shape of the molecule but not the weight, highlighting that any changes in K_0 values should be due to only the change in shape in space and cross-sectional area. Groups of isomers are separated by their class and then ordered by their estimated size. The K_0 values reported are the spread of K_0 values obtained from five replicate swabs, doped with

reference material, replicated over four days. Chemical details of isomers of methcathinone are shown in Table 53, with their K_0 spread shown in Figure 36. To aid in the identification of trends, isomers with ortho substituents are coloured red, meta substituents are coded green and para substituents are blue. This will be consistent throughout this chapter.

Table 53 – Chemical details of the methcathinone Isomers analysed by IMS

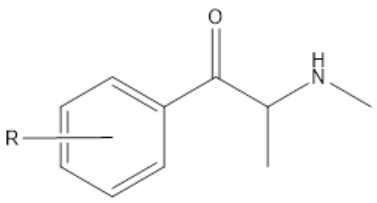
Compound No.	Compound Name	Abbreviation	R - Group	Structure
1a	Methcathinone	MC	-	
2a	2-Fluoromethcathinone	2-FMC	2-F	
2b	3-Fluoromethcathinone	3-FMC	3-F	
2c	4-Fluoromethcathinone	4-FMC	4-F	
3a	2-Methylmethcathinone	2-MMC	2-Me	
3b	3-Methylmethcathinone	3-MMC	3-Me	
3c	4-Methylmethcathinone	4-MMC	4-Me	
4a	2-Chloromethcathinone	2-CMC	2-Cl	
4b	3-Chloromethcathinone	3-CMC	3-Cl	
4c	4-Chloromethcathinone	4-CMC	4-Cl	

Figure 36 - K_0 spread for a selection of methcathinone isomers. Substituent position: Blue = Para, Green = Meta and Red = Ortho.

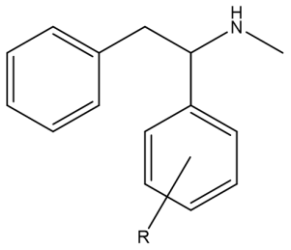
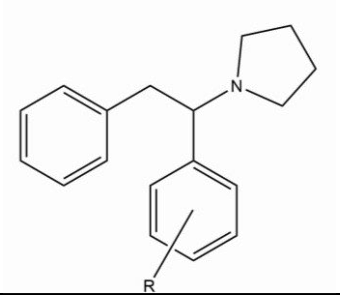
Within the groups of cathinone isomers, the combination of both cross-sectional area and molecular weight both contribute to the drift time elucidation of the compound. This is shown by two key observations; the molecular mass of the MMC (177.24 g/mol)

isomers is slightly less than the mass of the FMC (fluoromethcathinone) (181.21 g/mol) isomers but still has an increased drift time, the opposite as expected. This could be attributed to the increased cross-sectional area from the substitution of the fluoro group to a bulkier methyl group.[150] Between MMC and CMC isomers, the CMC isomers have the higher drift time. This is most likely due to the increase in mass between the MMC (177.24 g/mol) and the CMC (197.66 g/mol) isomers, causing more of a decrease in molecular mobility then the substitution to the less bulky chloro group. Since both mass and cross-sectional area contribute to the molecular mobility of these isomers, an in-depth discussion will not be provided for each set of isomers.

Between the triplicates, the order of elution starts with the ortho substituted derivative eluting the earliest followed by the para substituted derivative followed by the meta substituted derivative. This could be due to the cross-sectional area increasing following the trend.

The isomers of 1-(fluorophenyl)-N-methyl-2-phenylethanamine, abbreviated to fluoromephenidine (FMP), and fluorolintane (FL) are two groups of isomers which are similar in structure but differ by the chain on the amine present. The results for these are shown in Table 54, with the respective K_0 values plotted in Figure 37 and Figure 38 respectively.

Table 54 – Chemical details of the 1-(fluorophenyl)-N-methyl-2-phenylethanamine and FL isomers analysed by IMS

Compound No.	Compound Name	Abbreviation	R-Group	Structure
5a	2-Fluoromephenidine	2-FMP	2-F	
5b	3-Fluoromephenidine	3-FMP	3-F	
5c	4-Fluoromephenidine	4-FMP	4-F	
6a	2-Fluorolintane	2-FL	2-F	
6b	3-Fluorolintane	3-FL	3-F	
6c	4-Fluorolintane	4-FL	4-F	

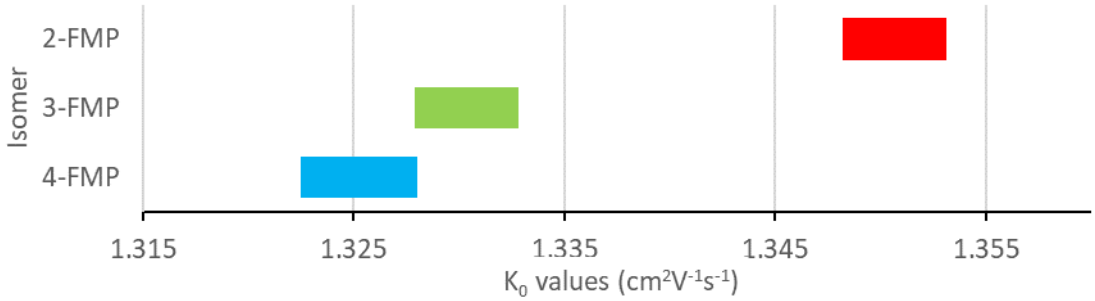


Figure 37 – K_0 spread for 2- / 3- / 4 – Fluoromethphenidine (FMP)

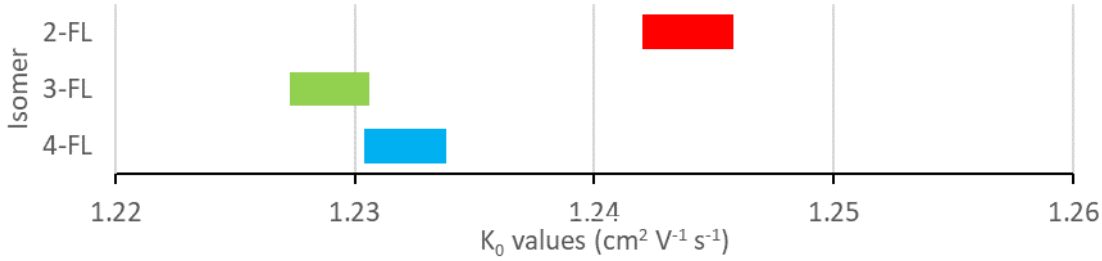


Figure 38 – K_0 spread for 2- / 3- / 4 – Fluorolintane (FL)

The FMP isomers are the first of the isomer sets in which the ortho group elutes first, following the meta and then para groups, a contrast to the methcathinone isomers which elute in the order ortho, para and then meta. The simple conclusion must be due to influences within the cross-sectional area of the isomers but without molecular modelling projection software of these isomers, no further explanation can be given. It is consistent that the ortho group always elutes first, which is as expected since the position of the substituent on the phenyl ring, pointing into the centre of the molecule

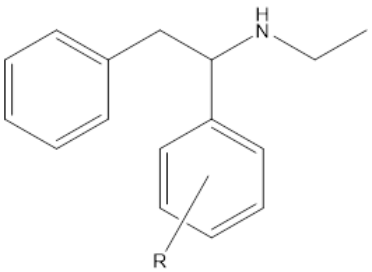
will influence the overall cross-sectional area less, opposed to substituents pointing away from the centre. The overlap is small between **5b** and **5c**, showing a slight chance for both the meta and the para position to be reported during sample analysis.

The addition of the larger five-membered ring on FL compared to the small methylamine present on FMP, causes a huge shift in K_0 values from roughly 1.50 to 1.23, as the larger isomers elute later from the drift tube, as expected. The positional change of the fluoro substituent causes less of a shift in K_0 values compared to the smaller FMP isomers, this is understandable as the "bulk" of the molecule is less distorted by the change in positional isomer.

Ephenidines are similar in structure to FMP and FL, however, they possess an ethyl chain on the amine. The substituents vary on the closest phenyl ring to this amine. Details of the isomers are shown in

Table 55 with the respective K_0 spread shown in Figure 39.

Table 55 – Chemical details of the ephenidine isomers analysed by IMS

Compound No.	Compound Name	Abbreviation	R Group	Structure
7	Ephenidine	EP	-	
8a	2-Fluoroephenidine	2-FEP	2-F	
8b	3-Fluoroephenidine	3-FEP	3-F	
8c	4-Fluoroephenidine	4-FEP	4-F	
9a	2,3-Difluoroephenidine	2,3-DFEP	2,3-F	
9b	2,4-Difluoroephenidine	2,4-DFEP	2,4-F	
9c	2,5-Difluoroephenidine	2,5-DFEP	2,5-F	
9d	2,6-Difluoroephenidine	2,6-DFEP	2,6-F	
9e	3,4-Difluoroephenidine	3,4-DFEP	3,4-F	
9f	3,5-Difluoroephenidine	3,5-DFEP	3,5-F	
10	2,3,4-Trifluoroephidine	Tri-FEP	2,3,4-F	
11	2,3,4,5-Tetrafluoroephenidine	Te-FEP	2,3,4,5-F	

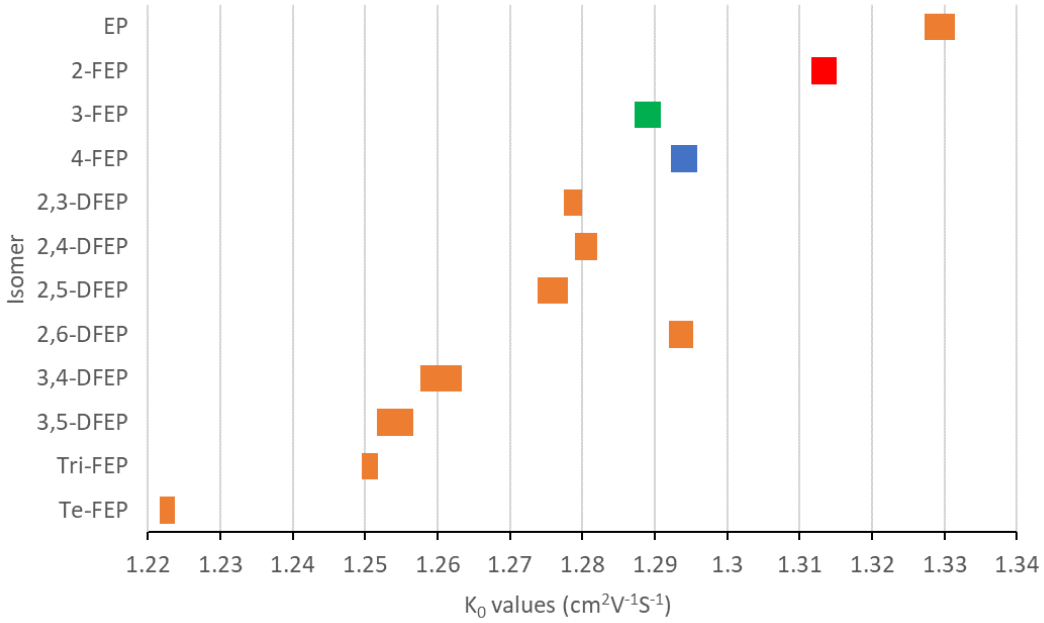
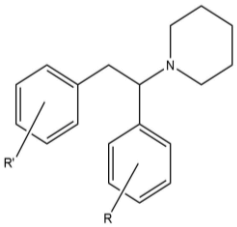


Figure 39 - Details of the K_0 ranges for the ephenidine group of isomers

For the ephenidine isomers, the general trend shows the more fluoro-substituents that are present, the longer the drift time of the compound, most likely due to the increase in mass and cross-sectional area. Compounds **8a-c** elute in the order ortho > para > meta following the methcathinone trend. The double-fluoro-substituted isomers **9a-f**, elute with the general trend of double ortho substituent positioned isomer **9d** elutes the quickest followed the single ortho substituted isomers **9a, 9b, 9c**, followed by the single meta substituted isomers (**9e, 9f**). In general, these follow the ortho > para > meta elution order, however, the inclusion of two substituents prevents any further conclusions due to the complexity.

Diphenidines are similar to the ephenidine structure but with a six-membered amine. Phenidines and diphenidines have the same molecular structure but substitution refers to substituents present on a different ring. Phenidines have substituents present on the closest ring to the amine, whereas diphenidines refer to the ring further away. The isomers assessed are shown in Table 56 with information on the spread of K_0 values shown in Figure 40.

Table 56 - Details of the diphenidine isomers, analysed by IMS

Compound No.	Compound Name	Abbreviation	R Group	R' Group	Structure
12	Diphenidine	DP	-	-	
13a	2-Fluorophenidine	2-FP	2-F		
13b	3-Fluorophenidine	3-FP	3-F		
13c	4-Fluorophenidine	4-FP	4-F		
14a	2-Fluorodiphenidine	2-FDP		2-F	
14b	3-Fluorodiphenidine	3-FDP		3-F	
14c	4-Fluorodiphenidine	4-FDP		4-F	
15a	1-(2-methyl-bibenzyl-α-yl)-piperidine	2-TP	2-Me		
15b	1-(3-methyl-bibenzyl-α-yl)-piperidine	3-TP	3-Me		
15c	1-(4-methyl-bibenzyl-α-yl)-piperidine	4-TP	4-Me		
16a	2-Methyldiphenidine	2-MDP		2-Me	
16b	3-Methyldiphenidine	3-MDP		3-Me	
16c	4-Methyldiphenidine	4-MDP		4-Me	
17a	2-Methoxyphenidine	2-MXP	2-O-Me		
17b	3-Methoxyphenidine	3-MXP	3-O-Me		
17c	4-Methoxyphenidine	4-MXP	4-O-Me		
18a	2-Trifluoromethoxyphenidine	2-TFMXP	2-O-CF_3		
18b	3-Trifluoromethoxyphenidine	3-TFMXP	3-O-CF_3		
18c	4-Trifluoromethoxyphenidine	4-TFMXP	4-O-CF_3		

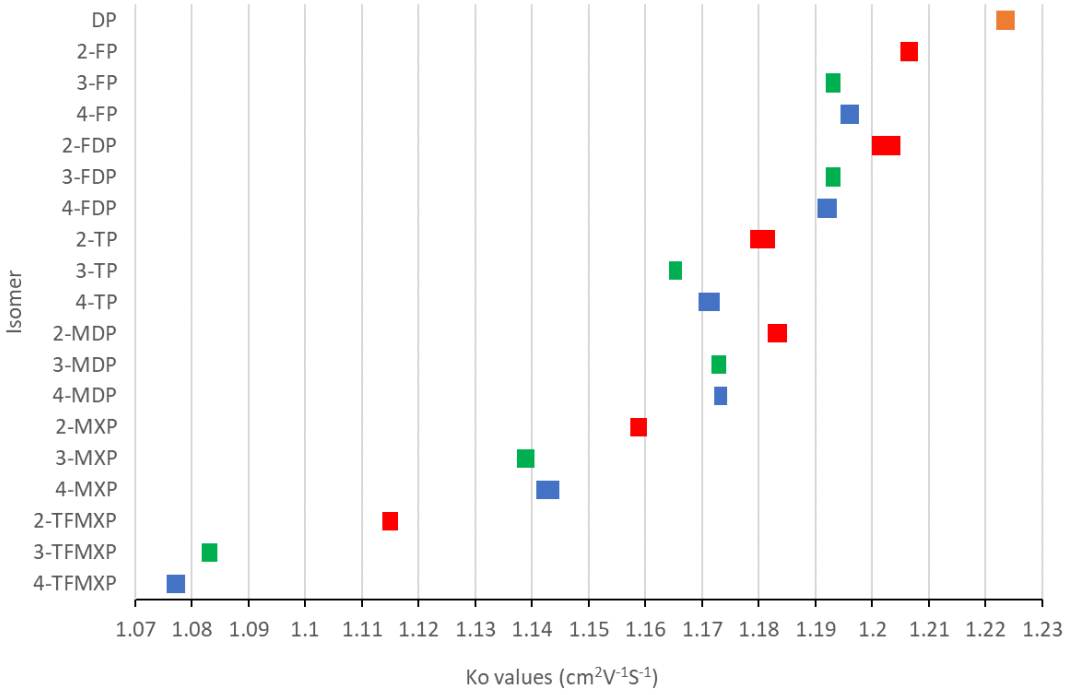
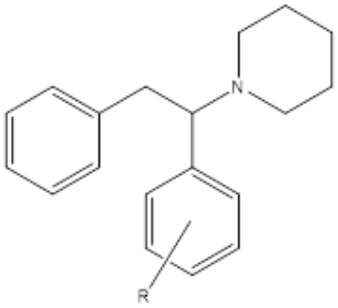


Figure 40 – K_0 spread of the diphenidine isomers

For the diphenidine isomers, they typically elute in the order ortho > para > meta, with the exception of **14a-c**, **16a-c** and **18a-c** which elute in the order of ortho > meta > para. Both **14b/c** and **16b/c** overlap almost entirely, showing little ability to distinguish between these isomers using IMS alone.

Halo-substituted phendines differ only by the position and presence of a chloro, bromo or iodo group on the phenidine ring. The isomers were tested, with the resulting K_0 ranges shown in Table 57.

Table 57 – Chemical details for halo subsituted phenidines tested by IMS

Compound No.	Compound name	Abbreviation	R Group	Structure
19a	1-[1-(2-chlorophenyl)-2-phenylethyl]piperidine	2-CP	2-Cl	
19b	1-[1-(3-chlorophenyl)-2-phenylethyl]piperidine	3-CP	3-Cl	
19c	1-[1-(4-chlorophenyl)-2-phenylethyl]piperidine	4-CP	4-Cl	
20a	1-[1-(2-bromophenyl)-2-phenylethyl]piperidine	2-BP	2-Br	
20b	1-[1-(3-bromophenyl)-2-phenylethyl]piperidine	3-BP	3-Br	
20c	1-[1-(4-bromophenyl)-2-phenylethyl]piperidine	4-BP	4-Br	
21a	1-[1-(2-iodophenyl)-2-phenylethyl]piperidine	2-IP	2-I	
21b	1-[1-(3-iodophenyl)-2-phenylethyl]piperidine	3-IP	3-I	
21c	1-[1-(4-iodophenyl)-2-phenylethyl]piperidine	4-IP	4-I	

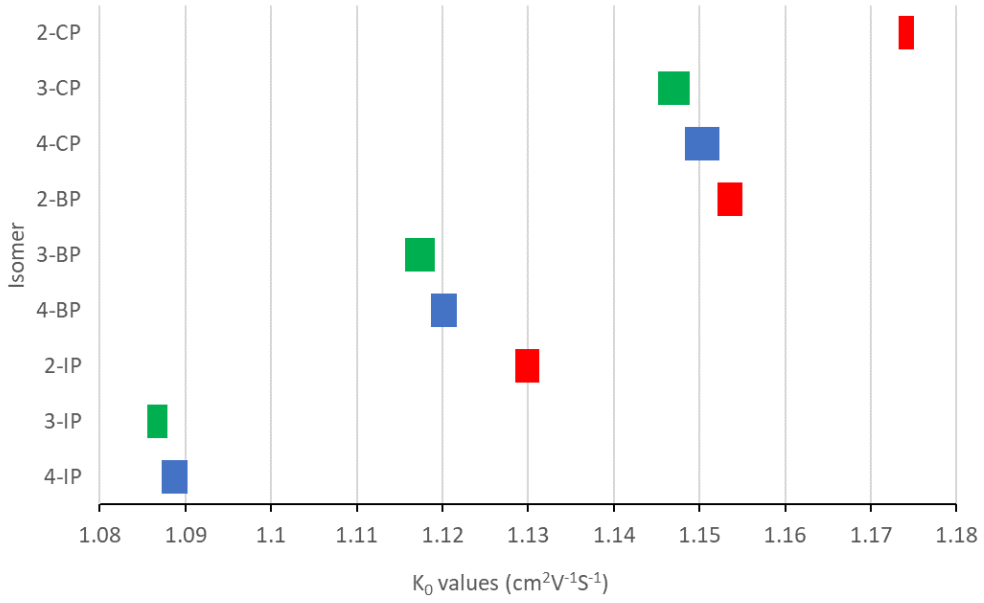
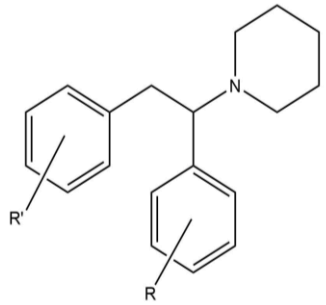


Figure 41 – K_0 spread for the halo-substituted phenidines

The halo substituted phenidines **19a-c, 20a-c and 21a-c**, show a similar trend throughout where the positional isomers elute in the order ortho > para > meta. The drift times increase as expected, as the heavier substituent increases in mass moving down the halogens. There is also consistent overlap present within the individual 3- and 4-positional isomers, where roughly 50% of the overall range overlaps, resulting in possible dual identification for these isomers.

This section contains exclusively a number of double substituted diphenidines isomers. Details of the compounds assessed are shown in Table 58 and their respective K_0 ranges are shown in Figure 42.

Table 58 - Compound information for the double substituted diphenidines

Compound No.	Compound name	Abbreviation	R Group	R' Group	Structure
22a	2,3-difluorodiphenidine	2,3-DFDP		2,3-F	
22b	2,4-difluorodiphenidine	2,4-DFDP		2,4-F	
22c	2,5-difluorodiphenidine	2,5-DFDP		2,5-F	
22d	2,6-difluorodiphenidine	2,6-DFDP		2,6-F	
22e	3,4-difluorodiphenidine	3,4-DFDP		3,4-F	
22f	3,5-difluorodiphenidine	3,5-DFDP		3,5-F	
23a	2,3-methylenedioxyphenidine	2,3-MDDP	2-O-Me-O-3		
23b	3,4-methylenedioxyphenidine	3,4-MDDP	3-O-Me-O-4		
24a	2,3-dimethoxyphenidine	2,3-DMXP	2,3-O-Me		
24b	2,4-dimethoxyphenidine	2,4-DMXP	2,4-O-Me		
24c	2,5-dimethoxyphenidine	2,5-DMXP	2,5-O-Me		
24d	2,6-dimethoxyphenidine	2,6-DMXP	2,6-O-Me		
24e	3,4-dimethoxyphenidine	3,4-DMXP	3,4-O-Me		
24f	3,5-dimethoxyphenidine	3,5-DMXP	3,5-O-Me		
25a	2,3-methylenedioxyphenidine (difluoro)	2,3-MDDP (F)	2-O-CF_2-O-3		
25b	3,4-methylenedioxyphenidine (difluoro)	3,4-MDDP (F)	3-O-CF_2-O-4		

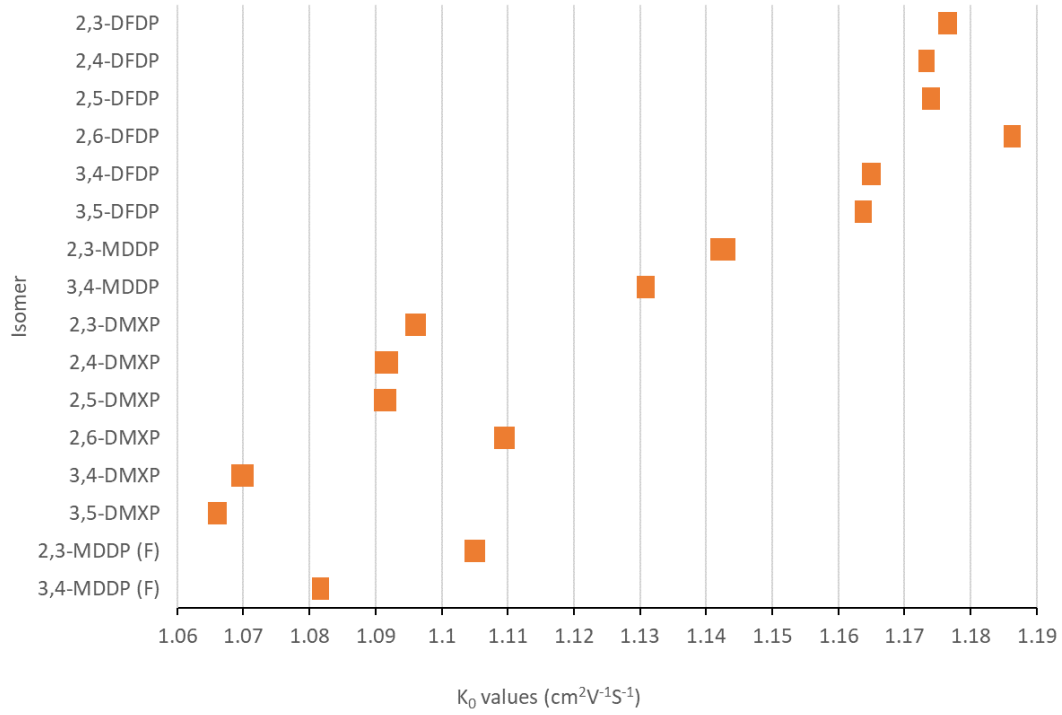


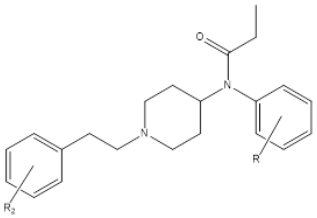
Figure 42 – K_0 spread for the double substituted diphenidines isomers

As expected, these isomers follow the general trend, as the heavier molecules take longer to elute. For the two sets of double substituted isomers **22a-f** and **24a-f**, these repeat the same elution order as the DFEP isomers **9a-f**, with a small change between the ortho-meta isomers, **22c** and **24c** eluting before the ortho-para isomers, **22b** and **24b**.

For the methylenedioxy- substituted isomers, **23a-b** and **25a-b**, the elution order is as expected, where the substituent within the ortho-meta (R ring) position elutes first, before the isomer containing the substituent in the meta-para (R ring) position.

Lastly, a small selection of fluorinated fentanyl isomers wasassessed with a single double substituted isomer. Additional fluorinated isomers were not available and the synthesis of such were outside the scope of this project when an adequate assessment was made using the diphenidine isomers. The fentanyl isomers assessed are shown in Table 59 and their K_0 respective shifts are shown in Figure 43.

Table 59 - Compound information for the fluoro substituted fentanyls analysed by IMS

Compound No	Compound Name	Abbreviation	R Group	R_2 Group	Structure
26a	ortho-fluorofentanyl	O-FF	Ortho-F		
26b	meta-fluorofentanyl	M-FF	Meta-F		
26c	para-fluorofentanyl	P-FF	Para-F		
27a	2'-fluorofentanyl	2'-FF		2-F	
27b	3'-fluorofentanyl	3'-FF		3-F	
27c	4'-fluorofentanyl	4'-FF		4-F	
28	2'-fluoro-ortho-fluorofentanyl	2'-O-FF	Ortho-F	2-F	

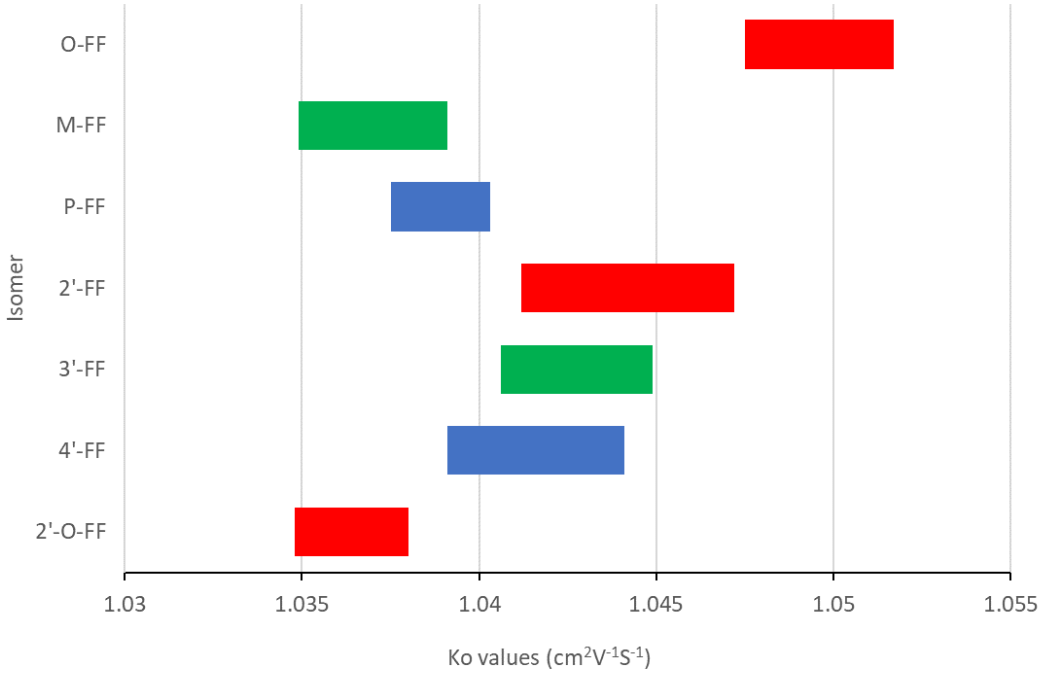


Figure 43 – K_0 spread for the substituted fentanyls

The substituted fluoro fentanyl isomers, **26a-c** and **27a-c**, showcase the difference in elution order between when the position of the substituent on the phenyl rings changes. The difference in R group position shows an ortho > para > meta elution order, whereas the difference in R' group position the alternate ortho > meta > para elution order. The double substituted isomer **28**, has the longest drift time out of all the fluoro fentanyl isomers, due to the increased mass.

Unfortunately, due to the small changes between the different isomers, the closeness of the K_0 values shows overlap for the majority of this isomers. The overlap and thus the inability to distinguish between these isomers, is almost expected, as other literature has shown the discrimination of these techniques difficult using a number of

techniques: with similar mass spectral data obtained *via* GC-MS, similar colour test results and similar R_f values using TLC.[112]

5.3. Conclusions

IMS has been assessed for its ability to analyse illicit bulk samples, tested on three groups of samples, one consisting of only cocaine samples (n = 57), one in which the majority was “ecstasy” tablets (n = 112) and one consisting of a number of samples obtained from a live Manchester-based festival (n = 195).

The K_0 shifts for four typical cocaine adulterants, phenacetin, aspirin, tetramisole and procaine, were added to the instrument’s library. Caffeine, benzocaine and paracetamol were also assessed but found to be IMS inactive. A method in which 250 ng of sample / reference material was deposited onto a swab for analysis, was identified and then used throughout. Cocaine and phenacetin, a common composition identified in cocaine samples, eluted at very similar K_0 shifts, so a number of parameters were altered, as suggested by previous literature, to attempt to increase the resolution of the instrument, which was unsuccessful due to the manufacturer locking certain settings.

Simulated samples were then produced and analysed to identify the detection threshold of a component within a sample, required for detection by the instrument. Cocaine was identified between 100 – 10% (w/w) content, levamisole and procaine identified between 80 – 10% and 90 – 10% (w/w) respectively, whereas phenacetin, was masked by cocaine due to peak overlap.

Cocaine samples, consisting of cocaine in both hydrochloride (n = 34) and freebase (n = 23) form, were then analysed and cocaine was identified in all of the samples. Only seven out of 21 adulterated samples had matches of both cocaine and the adulterant, due to IMS inactive adulterant caffeine being present in one sample, diphenhydramine which is not within the library, overlapping adulterant phenacetin present in seven samples and a low percentage of levamisole in the remaining binary mixtures, under the detection threshold needed for identification.

The focus then shifted onto samples consisting of a high “ecstasy” tablet prevalence (n = 112), starting with ten new library additions assessed for their K_0 values. The prevalence across the overall sample set found that MDMA was the most prevalent (77.7%) followed by other cathinones and then amphetamines typically found in “ecstasy” tablets (15.3%). The remaining samples (7%) consisted of adulterants,

samples with no API and two single samples of cocaine and ketamine. IMS was able to identify the illicit component in all six binary mixtures but no adulterant, due to caffeine being IMS inactive. For two tertiary samples, IMS identified only one component, due to overlap from the closely eluting components and low adulterant composition (2.7 – 4.2% (w/w) 4-CMC content).

The effect of components, which were typically used as illicit tablet fillers, were analysed, to assess potential effects on the ionisation of samples. The inclusion of IMS inactive compounds, lactose, boric acid and caffeine did not affect both thedetection threshold and K_0 values, in instances where MDMA was identified. A pair of two closely eluting compounds was assessed using simulated samples, to find that on average both components were identifiable between 45 – 25% (w/w) content but overall, it relies on a number of factors including the separation of the peaks and the response factor of the analytes.

The minimum mass loadings for detection, of a set of eight compounds, were calculated after plotting calibration plots between 0 – 500 ng mass loadings. The instrumental LoD for these compounds ranged between 12.3 – 41.7 ng, which is similar to other results identified within literature.

The stabilities of sample material on doped swabs, were assessed to determine how long before analysis a swab could be stored. Four different compounds, MDMA, ketamine, cocaine hydrochloride and cocaine freebase were spiked onto a set of swabs and analysed across 14 days. The main component was identifiable across the whole 14 days, however, contamination from other compounds was present, regardless of the care taken to separate each swab. These results indicate that swabs can be left for a short time after the initial doping but contamination is more likely to occur the longer the swabs remain untested.

IMS was then employed for illicit drug screening at a live event. The prevalence across the samples (n = 195) consisted of cocaine (61.5%), ketamine (15.9%), MDMA (13.3%) with the remaining 9.3% containing MDPHP, amphetamine, caffeine, pregabalin and samples with no active API. Single component samples (n = 160) had a correlation rate of 98.2% with GC-MS. Binary mixtures (n = 26) contained 11 complete identifications (42.3%) of both the illicit component and the adulterant with the remaining 15 samples

(57.3%) only the illicit component was identified due to IMS inactive adulterants, components eluting at the same K_0 values and component content (% (w/w)) lower than the required detection threshold. One tertiary sample only identified the illicit component and one adulterant, due to the low content (% (w/w)) of the other adulterant present. This showed that IMS is a useful tool for screening samples of this nature but inherently suffers for samples with multiple components.

At the live festival testing event, volunteer analysts were given an opportunity to use the IMS for bulk sample screening. This identified a small number of issues with the way in which the libraries were programmed, mostly due to the spread of the K_0 values for replicate analysis across intra- and inter-day analysis, which has since been amended. All of the samples which were tested on the day have been processed manually and all unexpected results can be explained. Within the samples analysed at the live festival event, alarms for peaks which elute at similar K_0 values were identified instead, or multiple components were detected due to multiple alarm regions being triggered. Similarly, contamination was a small issue during analysis and amendments have been made to the SOP to minimise this occurrence during future analysis.

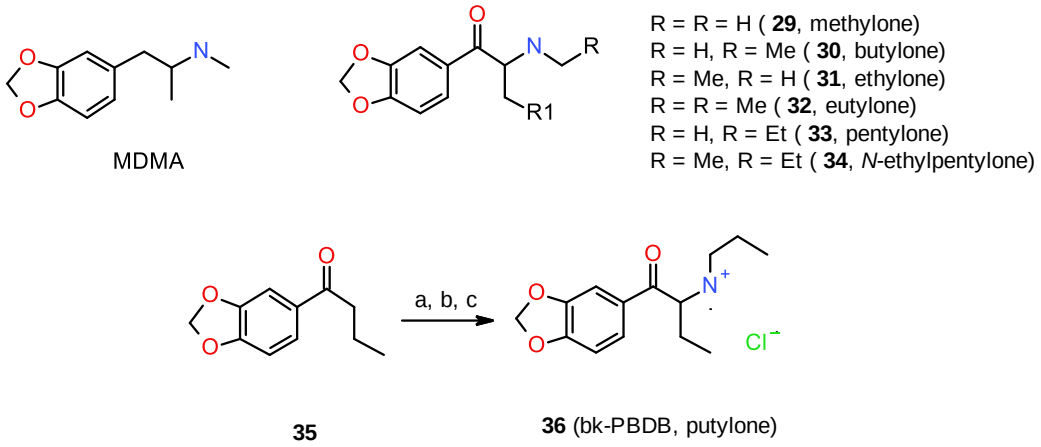
Isomeric discrimination of number different groups of methcathinones, ephenidines, phenidines, diphenidines and fentanyls showcased clearly that mass and cross-sectional area affect the drift time of a compound during IMS analysis. Two distinct patterns of positional isomers were identified, nine isomer groups identified with ortho > para > meta elution order vs five ortho > meta > para substituted isomer groups, with no obvious trend or pattern to explain the differences between the groups. Without further potential molecular modelling to calculate theoretical cross-sectional areas for these molecules, no further discussion can be given. Discrimination between different isomers within groups typically occurred with a few exceptions. The majority of the isomers that overlapped were the larger, double substituted phenidines and diphendines, the halo phenidines and the fluoro-fentanyl isomers. The smaller methcathinones and the single substituted phenidines typically separated. Even overlapping isomers have the chance to separate during analysis, causing only one potential identification, since the overlap shown is typically small. This is particularly useful since methcathinone isomers are commonly found the routine “ecstasy” tablet analysis, as shown in section 1.2.5 - Small Scale IMS Tablet Study.

The assessments performed on IMS, showcase the instrumentation and the method of analysis as a useful tool for bulk illicit sample analysis. The disadvantages of the method, including a lack of specificity, lack of quantification and potential overlap / co-elution of compounds, can be heavily outweighed by the ease of use, fast analysis time and simple to interpret results, as well as the benefit to use as a detector for trace analysis.

6. Chapter 6 - Synthesis, Characterisation and Quantification of the new Psychoactive Substance "putylone"

6.1. Introduction

The history, chemistry, and pharmacological action of synthetic cathinones have been the subject of several reviews.[151-154] Synthetic cathinones represent the second largest group of NPS that are monitored by the UNODC and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).[89, 155] Specifically, 3,4-methylenedioxy-*N*-alkylcathinones (Scheme 2), which mimic the psychostimulant effects of 3,4-methylenedioxymethamphetamine (MDMA),[151, 152, 156, 157] are characterized by the addition of a 3,4-methylenedioxy- moiety to the aromatic ring and alkyl-substitutions in both the amino group and α-carbon of the side chain (e.g., methylone, **29**; butylone, **30**; ethylone, **31**; eutylone, **32**; pentylone, **33** and *N*-ethylpentylone, **34**).[89] This sub-family constitutes approximately 18% of the total number of synthetic cathinones recorded within the United Nations Early Warning Advisory on NPS database and many are frequently encountered products sold under a variety of guises including *"research chemicals"*, *"plant food*", *"bath salts"* or *"glass cleaner"*.[155] These psychostimulants are usually available in several solid-dosage forms including powders or crystals (in colours ranging from white, off-white, beige to brown), tablets (resembling *"Ecstasy"* pills) or capsules. Synthetic cathinones typically have a chiral centre, so they may exist as two stereoisomers and the pharmacological, pharmacokinetic or metabolic behaviour of their enantiomers are expected to differ.[158] However, these NPS are often manufactured and sold as a racemic mixture as stereoselective synthesis is both expensive and difficult to perform.[159]



Scheme 2 - Reagents and Conditions: (a) Br_2 / HBr (48% in water) / CH_2Cl_2 / rt / 1h; (b) n-propylamine / benzene / Δ / 12h; (c) HCl (3 M) in CMPE (23% yield from 35).

Once absorbed, 3,4-methylenedioxy-*N*-alkylcathinones produce a variety of behavioural effects and can affect locomotor activity, thermoregulation, learning and memory, primarily mediated by interactions with the dopamine, noradrenaline and / or serotonin monoamine transporters.[151, 160] Short-term adverse effects reported, following use, are variable and may include loss of appetite, blurred vision, anxiety, post-use depression, confusion, hallucinations, short-term psychosis, and mania.[161] Individuals who have been intoxicated have displayed a variety of symptoms common to sympathomimetic toxicity including palpitations, tachycardia, agitation, aggression, hallucinations, coma and, in some cases, death.[162] Habitual users have also reported the development of tolerance, dependence, or withdrawal symptoms with prolonged use.[163] Between 2008 – 2021, as a result of increasing use of generic scheduling to prohibit the possession, supply, and production of many synthetic cathinone-based stimulants and due to their inherent adverse effects associated with fatal intoxications, the Commission on Narcotic Drugs specifically placed several 3,4-methylenedioxy-*N*-alkylcathinones under international control, within schedule II (methylone, 2015;[164] ethylone, 2017;[165] *N*-ethylpentylone, 2019 and eutylone, 2022)[166, 167] of the United Nations Convention on Psychotropic Substances (1971). Despite these control measures, synthetic cathinones continue to be encountered prominently in casework with one review, from early-2019 to mid-2022, reporting 29 novel structures detected for the first time.[168] One recently detected substance is 1-(1,3-benzodioxol-5-yl)-2-(propylamino)butan-1-one (**36**, bk-PBDB, putylone), the *N*-propyl substituted synthetic cathinone analogue of butylone, which was first reported in Czechia (19th January

2015), then subsequently in the USA (21st July 2022) and Canada (30th August 2022).[155] The published analytical data for this specific compound is limited (GC-EI-MS and LC-qTOF-MS only)[169] and no validated methods for its quantification have been reported. Bk-PBDB has been co-detected with *N, N*-dimethylpentylone (dipentylone) in four toxicological samples, however its pharmacology, pharmacokinetics and / or toxicological profile has not been fully elucidated.[169] Due to the potential health risk associated with putylone there is an increased need for new reliable methods for its detection to reduce potential drug-related harms should it become established within the market. This chapter presents the synthesis, full structural characterization and development of a validated GC-EI-MS approach for quantification of putylone found in solid dosage forms, mis-sold as MDMA *("Ecstasy"*) tablets, obtained in Manchester, UK (20th December 2022) (Figure 44). Analytical features of putylone were characterized by ^{1}H-NMR and ^{13}C(^{1}H)-NMR GC-EI-MS and FT-IR to provide both a comprehensive analytical profiling and validated chromatographic method for this substance. To the best of our knowledge, this is the first study detailing the synthesis, comprehensive structural characterization of putylone and provision of a validated GC-EI-MS method for the routine quantification of the synthetic cathinone within bulk samples, which will be valuable as a reference point for future forensic analysis of this and related compounds.

Figure 44 - Photograph of front and reverse sides of blue, "Donald Trump" embossed "Ecstasy" tablets (GM443) suspected to contain MDMA (mean tablet weight = 686.7 mg) obtained in Manchester, UK (20th December 2022).

6.2. Results and Discussion

6.2.1. Nuclear Magnetic Resonance (NMR) Spectroscopy

The reference standard of putylone) was synthesized, purified and obtained as a stable, colourless powder (Scheme 2, 23% overall yield from **35**) using an adaptation of previously reported protocols.[81, 82] The synthesised putylone standard was analysed by NMR to facilitate its characterisation. ^{1}H and $^{13}C(^{1}H)$ NMR spectra of putylone is shown in Figure 45 and Figure 47, respectively. The full assignment of ^{1}H and $^{13}C(^{1}H)$ NMR signals of putylone are presented in Table 60. All spectra including the 2D correlation NMR spectra are available within the Electronic Supplementary Information (**Fig. S1** – **S6**). The assignment relied on correlation spectroscopy (COSY; **Fig. S3**) to observe ^{1}H-^{1}H couplings, heteronuclear multiple quantum coherence (HMQC; **Fig. S4**) for one-bond ^{1}H-^{13}C couplings, heteronuclear multiple bond correlation (HMBC; **Fig. S5**) for two- or three-bond ^{1}H-^{13}C couplings (acquired using an evolution period equivalent to $^{2}J_{HC}$ = 8 Hz) and Distortionless Enhancement by Polarization Transfer (DEPT, **Fig. S6**) to determine the multiplicity of carbon atoms.

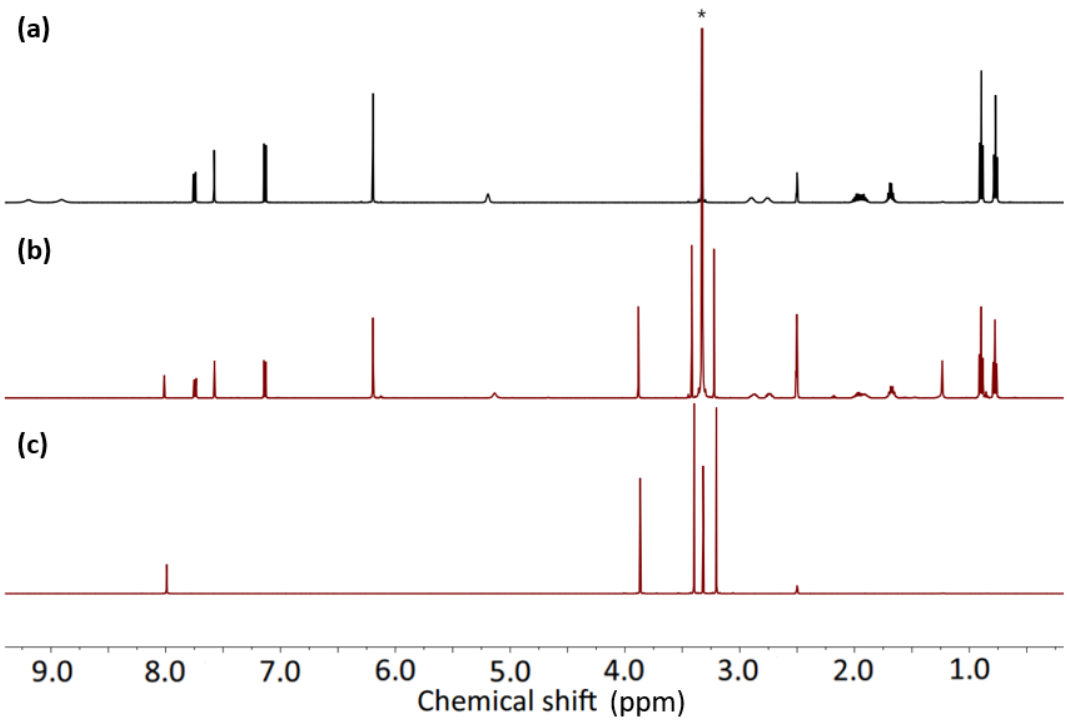
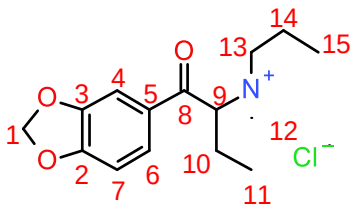


*Figure 45 - Representative ^{1}H NMR spectra of (a) putylone (8) reference standard, (b) seized blue, "Donald Trump" embossed "Ecstasy" tablets (GM443) suspected to contain MDMA and (c) caffeine reference standard acquired in DMSO-d_6 (δ 2.50) at 500 MHz. Note: * = residual water (δ 3.30)*

The high-field ^{1}H NMR spectra of putylone in DMSO-d_6 are shown in Figure 45 and **Fig. S1**. The 3,4-methylenedioxy ring aromatic protons are evidenced by the signals at δ 7.75 (doublet of doublets), 7.57 (doublet) and 7.14 (doublet). The signal at δ 7.75 possesses *J*-coupling of 1.8 ($^{4}J_{HH}$) and 8.3 ($^{3}J_{HH}$) Hz. The former is reflected in the signal δ 7.57, whereas the latter is also possessed by the signal at δ 7.14. These couplings are reflective of the 1,3,4-tri-substituted nature of the ring system. Cross-peaks for these interactions are observed in the respective ^{1}H-^{1}H COSY spectrum (**Fig. S3**). The 3,4-methylenedioxy protons are present as a singlet at δ 6.19. The proton attached to the chiral centre is observed at δ 5.19. This signal shows a cross-peak in the ^{1}H-^{1}H COSY NMR spectrum to a signal at δ 1.95, which presents as a multiplet. This multiplet shows a further cross-peak to a triplet at 0.77 ($^{3}J_{HH}$ = 7.6 Hz). This terminates the butyl chain. The *N*-propyl chain consists of signals at δ 2.90, 2.76, 1.69 and 0.90. The latter is a triplet ($^{3}J_{HH}$ = 7.5 Hz) whereas the other two signals are best described as multiplets. The signals at δ 2.90 and 2.76 are due to diastereotopic protons and as such they give rise to two unique signals. The two NH protons are observed as two singlets at δ 9.20 and 8.92. Both signals are broad singlets. Therefore, all signals in the reference standard are accounted for in the ^{1}H NMR spectrum.



36 (bk-PBDB, putylone)

Figure 46 - Structure of putylone – ^{1}H environments are assigned in Table 59.

Table 60 - ^{1}H and ^{13}C NMR data (in DMSO-d_6) for the bk-PBDB reference standard (36). Analogous ^{1}H NMR data for blue, "Donald Trump" embossed "Ecstasy" tablets (GM443) are also included for comparison purposes. †Additional signals for caffeine observed at δ 8.01 (s, aromatic H) 3.88 (s, CH_3), 3.42 (s, CH_3) and 3.22 (s, CH_3).

Assignment	Reference Standard of putylone (**36**, bk-PBDB)			Sample **GM443**†
	^{1}H	$^{13}C(^{1}H)$	DEPT-135	^{1}H
1	6.19 (s, 2H)	102.6	-ve	6.19 (s, 2H)
2		152.9	+ve	
3		148.2	+ve	
4	7.57 (d, 1H, $^{4}J_{HH}$ = 1.8 Hz)	107.8	+ve	7.57 (d, 1H, $^{4}J_{HH}$ = 1.7 Hz)
5		128.5	+ve	
6	7.75 (dd, 1H, $^{3}J_{HH}$ = 8.3 Hz, $^{4}J_{HH}$ = 1.8 Hz)	125.9	+ve	7.74 (dd, 1H, $^{3}J_{HH}$ = 8.3 Hz, $^{4}J_{HH}$ = 1.8 Hz)
7	7.14 (d, 1H, $^{3}J_{HH}$ = 8.3 Hz)	108.6	+ve	7.13 (d, 1H, $^{3}J_{HH}$ = 8.2 Hz)
8		194.0	N/A	
9	5.19 (app. quin., 1H, $^{3}J_{HH}$ = 5.1 Hz)	61.3	+ve	5.14 (s, 1H)
10	1.87-2.03 (m, 2H)	23.2	-ve	1.86-2.02 (m, 2H)
11	0.77 (t, 3H, $^{3}J_{HH}$ = 7.6 Hz)	8.4	+ve	0.78 (t, 3H, $^{3}J_{HH}$ = 7.6 Hz)
12	9.20 (s, 1H) and 8.92 (s, 1H)	N/A	N/A	9.23 (s, 1H) and 8.92 (s, 1H)
13	2.90 (app. q, 1H, $^{3}J_{HH}$ = 9.1) and 2.76 (app. q, 1H, $^{3}J_{HH}$ = 9.2)	47.5	-ve	2.85-2.92 (m, 1H) and 2.71-2.77 (m, 1H)
14	1.63-1.74 (m, 2H)	19.1	-ve	1.62-1.73 (m, 2H)
15	0.90 (t, 3H, $^{3}J_{HH}$ = 7.5 Hz)	10.9	+ve	0.90 (t, 3H, $^{3}J_{HH}$ = 7.4 Hz)

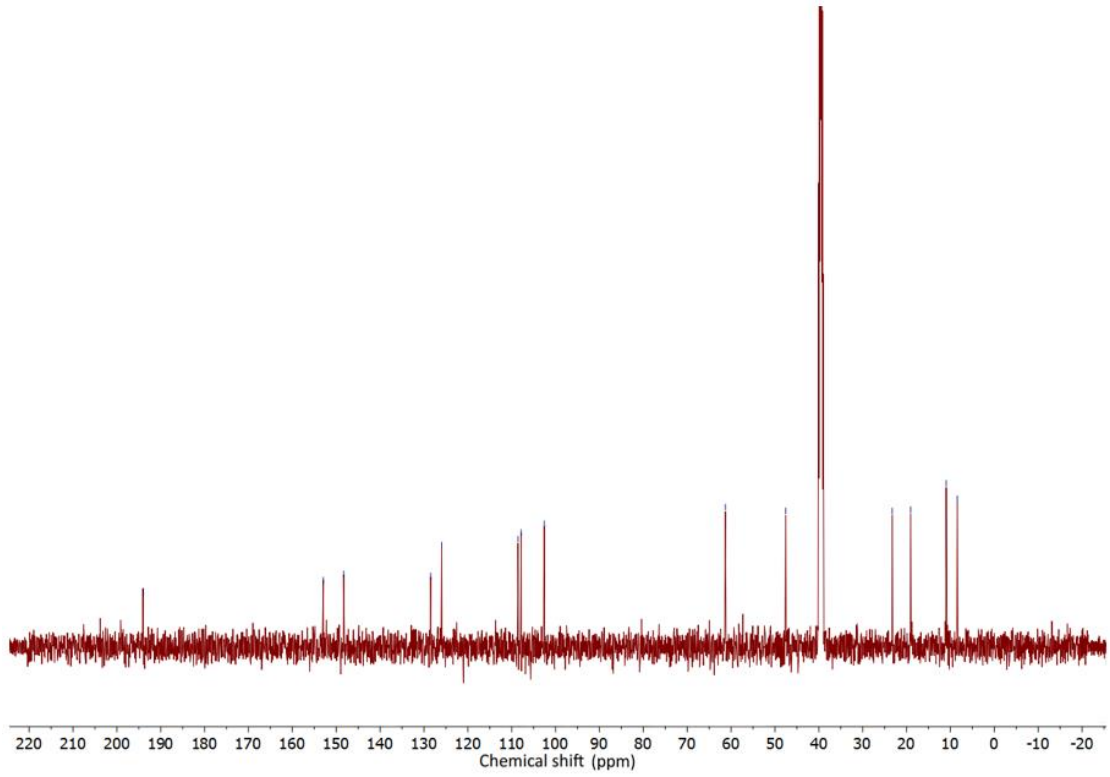


Figure 47 - $^{13}C(^{1}H)$ NMR spectrum of putylone reference standard acquired in DMSO-d_6 at 125 MHz.

The reference material of putylone was further characterised by ^{13}C NMR spectroscopy. The $^{13}C(^{1}H)$ NMR spectrum (Figure 47), possesses 14 signals. The most deshielded signal is that of the carbonyl at δ 194.0. The quaternary nature of this signal was confirmed by DEPT (**Fig. S6**). There are seven signals in the aromatic region and six in the aliphatic. Of the seven signals in the aromatic region, three of them are quaternary (δ 152.9, 148.2 and 128.5) as shown by their absence in the DEPT-135 spectrum. The remainder are CH environments (δ 125.9, 108.6 and 107.8), with the exception of the signal at 102.6 which is emissive in the DEPT-135 spectrum, and therefore belongs to the methylene carbon that is bonded to two oxygens. The most de-shielded aliphatic signal is located δ 61.3 and is absorptive in the DEPT-135 NMR spectrum; this signal is attributed to the chiral carbon. This was confirmed through the use of ^{1}H-^{13}C HMQC. The remaining five signals are methylene carbons (δ 47.5, 23.2 and 19.1) or methyl signals (δ 10.9 and 8.4); the most de-shielded methylene environment belongs to the methylene directly bonded to nitrogen.

Figure 45b shows a representative ^{1}H NMR spectrum of the blue, *"Donald Trump"* embossed *"Ecstasy"* tablets (**GM443**), also collected in DMSO-d_6. The correlation

between this spectrum and that of the reference sample is very significant. There are four additional signals at δ 8.01, 3.88, 3.42 and 3.22, which are in a ratio of 1:3:3:3; these all belong to caffeine, which is present as an adulterant in the sample. As well as the GC-MS analysis (section 6.2.4 - Gas Chromatography Electron Ionisation Mass Spectrometry (GC-EI-MS) analysis), from which caffeine was confirmed, these signals in the ^{1}H NMR spectrum of the seized material correspond very well with Figure 45c, which is a sample of caffeine dissolved in DMSO-d_6. The tabulated data is shown in Table 60. The biggest change between sample GM443 and the reference standard is that of the chiral proton, which shows a 0.05 ppm difference. This environment could be sensitive to changes in the matrix caused by the presence of caffeine.

6.2.2. Analysis by Benchtop NMR in-conjunction with a Reference Database

Sample GM443 when subjected to analysis by an automated benchtop (60 MHz) ^{1}H NMR approach, which has been reported previously for the analysis of similar 3,4-methylenedioxy moiety containing compounds,[41] returned butylone (**30**) and oxandrolone as the highest rank hit. The hit score was 0.863, indicative of some uncertainty in the returned hit (threshold 0.82). The second highest ranked hit was butylone (hit score = 0.862). However, the structural similarity of putylone and that of the structure of butylone (same 3,4-methylenedioxy moiety as well as butyl chain) showcases that the class of compound could be identified in the seized sample, despite the fact that putylone was not present in the database.

The reference sample putylone was then added to the database and the seized sample re-analysed. The highest hit was that of putylone with a hit score of 0.920. The following hits was those of mixtures, with putylone being the dominant API in each case. The tenth ranked hit was that of putylone and caffeine, with a hit score of 0.894. The second to ninth ranked matches had hit scores of 0.900 to 0.894; the hit scores of the ninth (putylone and harmaline) and tenth ranked matches have identical scores to four decimal places.

6.2.3. Fourier-Transform Infrared spectroscopy (FTIR) Analysis

The infrared spectrum of putylone (**Fig. 4a**) collected on an FTIR spectrometer shows the characteristic bands associated with the hydrochloride salts of 3,4-

methylenedioxycathinones such as butylone (**Fig. 4c**).[170] The spectrum shows strong, broad bands between 2490 – 2965 cm^{-1} corresponding to a combination of C-H stretches and absorption bands from the ammonium salt, an absorbance at 1672 cm^{-1} for the carbonyl group in conjugation with the 3,4-methylenedioxyphenyl moiety, aromatic C=C ring vibration bands at 1603 cm^{-1}, a strong C-O stretching vibration from the 3,4-methylenedioxy group at 1261 cm^{-1} and a medium C-N stretching band at 1030 cm^{-1}.

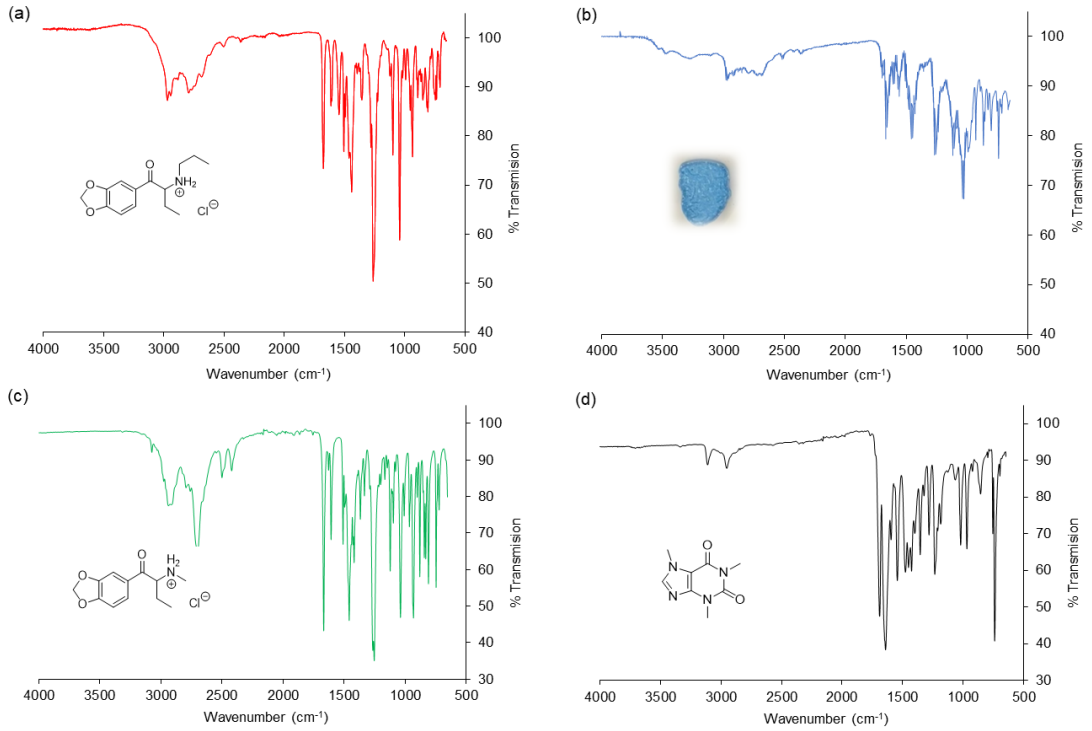


*Figure 48 - (a) FTIR spectrum of synthesised putylone hydrochloride (8) (b) Representative FTIR spectrum of blue, "Donald Trump" embossed "Ecstasy" tablets (**GM443**) suspected to contain MDMA; (c) FTIR spectrum of butylone hydrochloride (2); (d) FTIR spectrum of caffeine.*

The infrared spectra of the seized tablets (Figure 48b) were acquired, under identical conditions, and automatically compared to the OMNIC (Thermo Scientific, Rochester, USA) against defined libraries (Scientific database (version 10.5.3.738) and SWGDRUG IR Library (version 2.1)). The closest database match was determined based on spectral similarity (highest match score). Visually these tablets were similar to *"Ecstasy"* (MDMA) tablets with each batch being colored and stamped with a particular motif or logo.[42] In each case, infrared analysis confirmed that none of the seized samples described herein contained MDMA and the synthetic cathinone, butylone (match score: 51 – 53%), was detected. A high level of adulteration can mask active

ingredient signals and affect the identification of seized drug samples by infrared analysis.[41, 171]

6.2.4. Gas Chromatography Electron Ionisation Mass Spectrometry (GC-EI-MS) analysis

The qualitative GC-EI-MS method (*ca.* 10 min) used required an extremely straightforward solvation of the samples in methanol (10.0 µg/mL containing 20.0 µg/mL methyl stearate as internal standard) followed by direct injection into the instrument. No derivatization step was required. An exemplar total ion chromatogram demonstrating the separation of butylone (**30,** t_R = 4.94 min), caffeine (t_R = 5.90 min), *N*-ethylpentylone (**34,** t_R = 7.12 min) and putylone (**36**, bk-PBDB, t_R = 7.35 min) is presented in Figure 49a. The use of GC-EI-MS facilitated the visualization of the mass spectral data for putylone and this is presented in Figure 49c. The GC-EI-MS total ion chromatogram of a methanolic extract (containing 20.0 µg/mL methyl stearate as internal standard) of the seized tablets (**GM443**) and the corresponding EI-MS are presented in Figure 49b, Figure 49d and Figure 49e respectively. The data indicates that the seized sample contains two components: caffeine (minor component, t_R = 5.89 min, Figure 49e) and putylone (major component, t_R = 7.34 min) and the EI spectrum (Figure 49d) is in good agreement with both the synthesised reference standard of putylone (Figure 49b) and the information reported by NPS Discovery.[168]

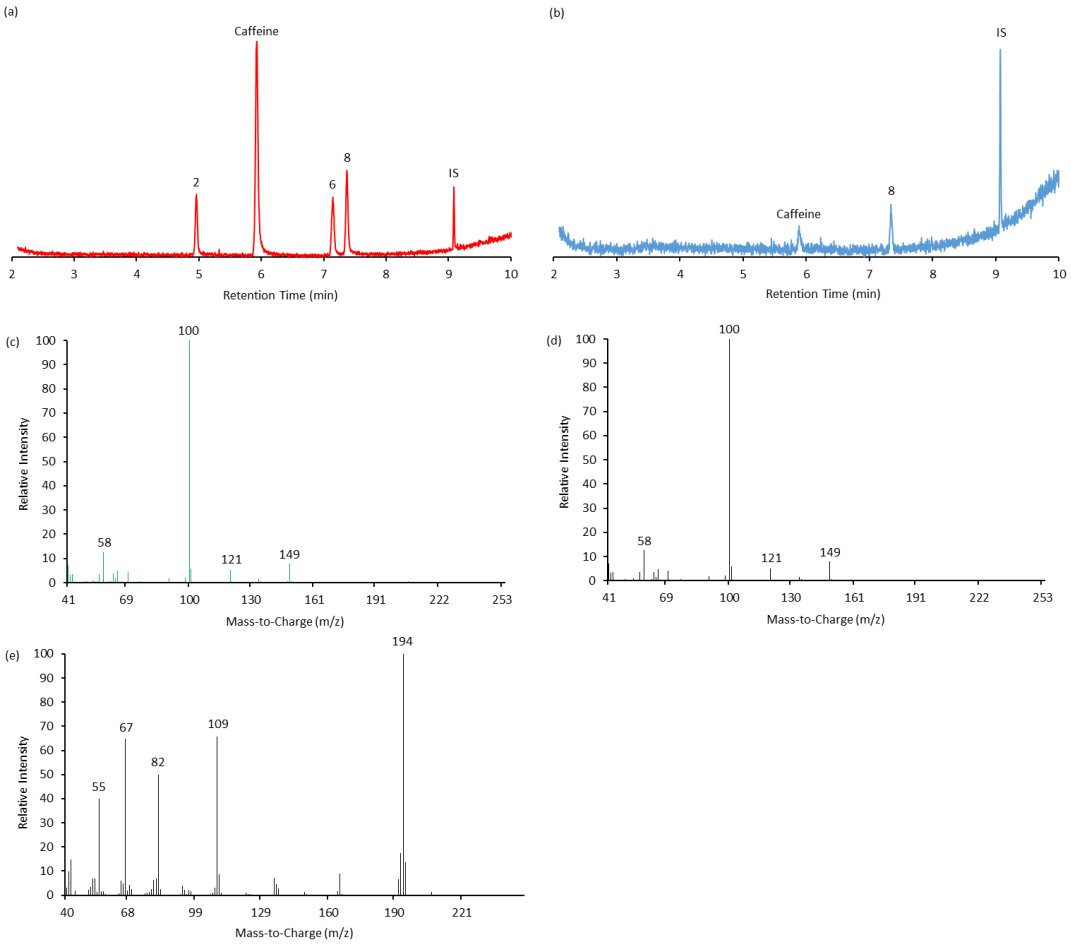
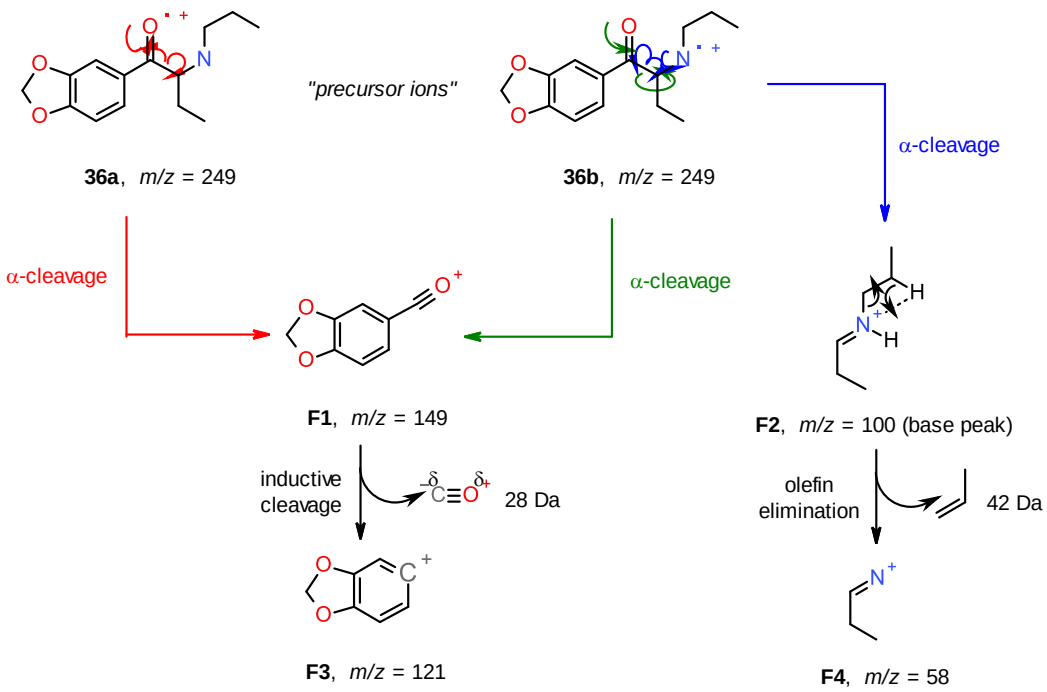


*Figure 49 - (a) Exemplar total ion chromatogram demonstrating separation of butylone, caffeine, NEP, putylone and methyl stearate (internal standard, IS). (b) Representative total ion chromatogram of blue, "Donald Trump" embossed "Ecstasy" tablets (**GM443**); (c) EI-MS spectrum (+ve ion mode) of the putylone reference standard (t_R = 7.35 min); (d) EI-MS spectrum (+ve ion mode) of putylone (t_R = 7.34 min) in seized tablets (**GM443**); (e) EI-MS spectrum (+ve ion mode) of caffeine (t_R = 5.89 min) in seized tablets (**GM443**). Note: t_R (methyl stearate) = 9.08 min.*



Scheme 3 - Proposed GC-EI-MS fragmentation pathway for putylone (36) adapted from Davidson et al.[172]

The structures of the molecular ion (*m/z* = 249) and diagnostic fragment ions are presented in Scheme 3. The proposed fragmentation pathway for putylone is like those reported for similar synthetic 3,4-methylenedioxycathinones.[172] The 3,4-methylenedioxyphenylacylium (**F1**, *m/z* = 149, red pathway) and iminium (**F2**, *m/z* = 100, blue pathway) ions are the dominant fragmentation pathways for synthetic cathinones. Subsequent inductive cleavage of carbon monoxide (28 Da) from the (**F1**) produces the 3,4-methylenedioxyphenylium ion (**F3**) at *m/z* = 121. The 3,4-methylenedioxyphenylacylium ion (**F1**) can also form through α-cleavage of the bond between the carbonyl carbon and the α-carbon, which is initiated or stabilized by a lone pair of electrons on the oxygen atom (green pathway). The iminium ion (**F2**) is the most intense ion (base peak) in the spectrum and is produced *via* an α-cleavage of the bond between the carbonyl carbon and the α-carbon adjacent to the aminopropyl moiety. The iminium ion pathway subsequently leads to the secondary fragment (**F4**, *m/z* = 58) through a 4-center olefin elimination along the propyl chain.

The quantitative GC–MS method using three ions specific to each analyte, was developed, and validated in accordance with the ICH guidelines.[80] Calibration standards were prepared, and all four analytes (butylone, NEP, putylone and caffeine)

demonstrated a linear response (r^2 = 0.999) over a 2.0 – 12.0 µg/mL range (containing 20.0 µg/mL methyl stearate) with satisfactory repeatability (RSD = 0.61–4.6%, n = 6). Due to the level of sensitivity required for the detection of the analytes within bulk samples the LoD and LoQ values were determined in SIM mode. The LoD and LoQ for the analytes (in bulk samples) were determined, based on the standard deviation of the response and slope of the calibration curve, as being 0.08 – 0.13 and 0.26 – 0.39 µg/mL, respectively.

Table 61 - GC-EI-MS validation data (SIM) for the quantification of butylone, caffeine, NEP and putylone.. Note: Methyl stearate: tR = 9.08 min; SIM ions (base peak indicated in bold) = 74.0, 87.0 and 143.0.

	Analyte			
Parameter	Butylone (30)	Caffeine	*N*-ethylpentylone (34)	Putylone (36)
SIM ions (for quantification)[a]	**72.1**, 121.0, 148.9	**67.0**, 109.0, 194.0	**58.0**, 100.0, 149.0	**58.0**, 100.0, 149.0
t_R (min)	4.94	5.90	7.12	7.35
RRT[b]	0.67	0.80	0.97	1.00
R_s[c]	-	14.2	16.0	3.4
A_s[d]	1.2	0.7	1.0	1.2
N (plates)[e]	110,651	96,122	140,006	233,140
H ($x10^{-4}$ mm)[f]	2.71	3.12	21.4	1.29
Linearity (r^2)	0.999[g]	0.999[h]	0.999[i]	0.999[j]
LoD (µg/mL)[k]	0.09	0.13	0.09	0.09
LoQ (µg/mL)[l]	0.27	0.39	0.28	0.26
Precision (%RSD, n = 6)				
2.0 µg/mL	1.76	4.61	1.13	0.62
4.0 µg/mL	0.66	3.90	0.86	0.99
6.0 µg/mL	1.38	1.87	1.39	0.89
8.0 µg/mL	0.82	0.74	0.81	1.11
10.0 µg/mL	1.27	1.33	1.30	0.97
12.0 µg/mL	1.27	0.93	1.33	0.81
Assay Recovery (%, n = 3)				
8.0 µg/mL (80%)	97.0	89.7	98.5	99.4
10.0 µg/mL (100%)	96.5	89.5	96.4	99.5
12.0 µg/mL (120%)	98.5	91.0	105.5	100.8
Average recovery (%)	97.3	90.1	100.1	99.9
%RSD	1.64	1.47	4.31	0.95
Relative Error (%)[m]	2.67	9.92	0.14	0.11

Key: [a]Base peak indicated in bold; [b]Relative retention time (with respect to putylone, **36**); [c]Resolution; [d]Asymmetry (or tailing) factor; [e]Number of theoretical plates; [f]Height of a theoretical plate; [g]y = 0.0629x – 0.056; [h]y = 0.0582x – 0.0064; [i]y = 0.0610x – 0.0055; [j]y = 0.0541x – 0.0057; [k]LoD; [l]LoQ; [m]Deviation between the average experimental recovery and a 100% recovery.

The percentage recovery study of the assay was determined from spiked samples, prepared in triplicate, at three levels over a range of 80 – 120% of the target

concentration (10 µg/mL). Experimental concentration was determined using the developed calibration and compared with the theoretical concentration (assay recovery). The relative error shows how the mean assay recovery diverges from an expected 100%. The precision (RSD %) of the method and the percentage recovery for each of the three replicate samples demonstrated good recoveries (butylone, 97.3 ± 1.64%; caffeine, 90.1 ± 1.47%; NEP, 100.0 ± 4.31% and putylone, 99.9 ± 0.95%) within the desired concentration range.

Table 62 - Qualitative and quantitative analysis of seized blue, "Donald Trump" embossed "Ecstasy" tablets (GM443, n = 3) obtained from Greater Manchester Police (Manchester, UK, 20th December 2022).

Sample	Weight (mg)	Qualitative Analysis	Quantitative Analysis	
		GC-EI-MS (scan)	GC-EI-MS (SIM)	qNMR
GM443A	692.8	caffeine (minor, t_R = 5.89 min) and putylone (major, t_R = 7.34 min)	caffeine (minor, 43.0±1.89 mg/tablet) and putylone (major, 135.5±1.16 mg/tablet)	caffeine (minor, 39.1±0.36 mg/tablet) and putylone (major, 133.7±1.63 mg/tablet)
GM443B	681.2	caffeine (minor, t_R = 5.89 min) and putylone (major, t_R = 7.34 min)	caffeine (minor, 40.2±1.63 mg/tablet) and putylone (major, 130.6±0.15 mg/tablet)	caffeine (minor, 36.6±1.14 mg/tablet) and putylone (major, 128.9±1.51 mg/tablet)
GM443C	686.1	caffeine (minor, t_R = 5.89 min) and putylone (major, t_R = 7.34 min)	caffeine (minor, 43.4±0.84 mg/tablet) and putylone (major, 132.5±1.40 mg/tablet)	caffeine (minor, 47.7±3.33 mg/tablet) and putylone (major, 130.8±1.51 mg/tablet)

The GC-EI-MS approach was deemed suitable for the analysis of the street sample (**GM443**). The three tablets individually reanalysed (in triplicate) and quantification of putylone and caffeine were performed in SIM mode. The quantitative GC-EI-MS results confirmed that all three tablets contained putylone (t_R = 7.34 min, **36**) at levels ranging between 130.6 – 135.5 mg/tablet, with low levels of caffeine (t_R = 5.89 min, 40.2 – 43.4 mg/tablet) also present (Figure 49). To verify the GC-EI-MS quantitative results, quantitative nuclear magnetic resonance (qNMR) data was also contemporaneously collected for comparison purposes. For the qNMR approach, reference samples consisting of 10 mg/mL of putylone and caffeine in D_2O were separately acquired, and the spectra of the samples were normalised to the reference spectrum, in order for quantification to be performed.[173] The qNMR results confirmed that the three surveyed tablets contained putylone and caffeine at levels ranging between 128.9 – 133.7 mg/tablet and 36.6 – 47.7 mg/tablet respectively corroborating the quantitative GC-EI-MS results. It is important to note that due to the small sample size (n = 3), the results presented herein may not truly reflect the typical prevalence or concentrations of samples that contain putylone nationally, however, these results demonstrate that

the 10-minute GC-EI-MS method, employing SIM mode, described herein is potentially suitable for the routine screening of suspect samples, which may contain this novel cathinone.

6.3. Conclusion

This study reports the first synthesis and comprehensive analytical profiling (^{1}H, $^{13}C(^{1}H)$ NMR, FT-IR and GC-EI-MS) of the novel synthetic cathinone: “putylone” confirmed within a seized bulk sample. In addition to the synthetic methods and spectral data this study presents the development of a rapid, validated GC-EI-MS method (employing SIM) for the routine detection (within 10 mins) and quantitative analysis, (LoD: 0.09 µg/mL, LoQ: 0.26 µg/mL, respectively) for “putylone”, suitable for processing of bulk samples encountered in casework. The seized tablets were determined to contain a mixture of putylone (130.6 – 135.5 mg/tablet) and caffeine (40.2 – 43.4 mg/tablet) respectively. It is envisaged that the data presented herein will be valuable as a reference point for future analysis of this novel 3,4-methylenedioxy-*N*-alkyl cathinone and any structurally related compounds as they emerge on the illicit drug market. This work provides the means for the identification and quantification of “putylone” within routine sample analysis, allowing alerts to be made to welfare staff and local authorities.

7. Chapter 7 – Conclusions and Future Work

7.1. Conclusions

Across four separate data chapters a series of investigations have been outlined with an overall theme to aid and improve current literature for the screening and quantification of illicit drugs with predominant focus on specifically cocaine. This is due to the wide variety of sample purity levels which are regularly identified and dynamic range of adulteration.

In Chapter 3 – Qualitative Screening and Comparison of Analytical Techniques, a qualitative comparison of GC-MS, FT-IR and BT-NMR was examined on a set of 318 samples (259 single components, 47 binary mixtures and 4 tertiary mixtures) finding cocaine as the most prevalent sample across the single (61%), binary (96%) and tertiary (100%). Single components showed good agreement between the three techniques except for of non-volatiles which GC-MS could not identify. For binary mixtures, NMR identified both components in 53% of samples and FT-IR could not identify more than one component unless spectral subtraction was employed. The use of spectral subtraction allowed both components to be identified in 66.7% of samples and the illicit component identified in the remaining 33.3%. LoD studies showed NMR could identify cocaine and the adulterant in mixtures of 70 – 40 % w/w and FT-IR identified both components throughout the tested mixtures once spectral subtraction was employed. This showcases that one single technique is not enough for complete elucidation of illicit drugs and adulterants present within samples. Furthermore, FT-IR should be used with spectral subtraction. This instrumental comparison allows analysts who perform frequent on/off-site illicit sample testing to make informed decisions about the advantages and disadvantages of each technique. For FT-IR users, spectral subtraction is showcased to be a useful techniques, removing one of the main disadvantages of FT-IR, allowing more reliable analysis of compounds during testing.

In Chapter 4 – Quantification of Cocaine Samples using Nuclear Magnetic Spectroscopy, a qNMR method for the quantification of cocaine hydrochloride (D_2O) and freebase ($CDCl_3$) within illicit cocaine samples was discussed for low-field NMR, with a total spectral acquisition time of ~4.5 minutes. A method was described showcasing quantification using both an external reference standard and against TMS / TMSP internal standard with good linearity (R^2 = 0.9967 – 0.9995), adequate LoD (0.289 –

0.724 mg/mL) and LoQ (0.875 – 2.193 mg/mL) values for bulk sample analysis, good accuracy (98.66 – 99.52%), good repeatability (1.53 – 3.14 % RSD) with acceptable sample recoveries (87.30 – 119.76%). Comparisons were made with analysis performed using GC-MS (R^2 = 0.9399) and high-field NMR (R^2 = 0.9837) showing good correlation. A method was also adapted for no-D solvents, showing good agreement of cocaine content between deuterated and no-D solvents. Use of BT-NMR allows an alternative quantification method to be performed off-site with applications towards on-site analysis. This is an alternative to both to some of the less accurate quantification techniques that are used for on-site analysis (TLC, colour tests and FT-IR) and the more readily used techniques, which are limited to off-site analysis (GC-MS, HPLC and NMR).

In Chapter 5 – Detection and Discrimination of Controlled Drugs by Ion Mobility Spectroscopy, a method for the use of a commercial IMS instrument as an illicit bulk sample drugs detector has been outlined with the addition of 16 commonly identified compounds to the IMS library. A cocaine-based dataset (n = 57) was analysed and cocaine was identified in all samples. For seven out of 21 binary mixtures, all components were fully identified; the other 14 consisted of adulterants that were IMS inactive adulterants, which prevented their detection. An “ecstasy” tablet-based dataset (n = 112) was also assessed and this data correlated with GC-MS identification for 98.1% (n =110) of single component samples; the remaining two samples possessed caffeine as adulterant, which is IMS inactive. Only the major illicit component was identified in the binary samples (n = 6), composing of MDMA, 4-MMC, amphetamine and “putylone”. With only the major illicit component identified (4-MMC) within and tertiary samples (n = 2) containing 4-MMC, 4-CMC and caffeine. Contamination with boric acid, caffeine and lactose was shown to not cause any issues with analysis. The LoD for eight commonly identified substances was found to be 19.0 – 109.1 ng mass loading. Sample testing at a live event (n = 163) showed a correlation with GC-MS analysis for 98.2% of single component samples, the remaining samples (n = 3) did not match due to contamination and IMS inactive components. For 42.3% (n = 11) of binary samples both components were identified, whilst for 38.5% (n = 10) of the samples, only the illicit component due to IMS inactive adulteration and peak overlap was identified. The remaining samples 19.2% (n = 4) could not be identified due to low % w/w content. Individuals who had not used IMS before, were briefly introduced to the

technique during a short study in which contamination was identified as a major issue. A number of positional diphenidine and fluorofentanyl isomers were assessed to showcase the separational potential of the technique. Ortho-isomers typically eluted first followed by *para*- and then *meta*- isomers for nine sets of isomers. Alternatively, the elution order, ortho > meta > para was observed for five isomer sets, with no indication of what caused these differences. Higher molecule weight isomers typically overlapped with minimal discrimination potential whereas smaller more commonly identified isomers, such as CMC and MMC, showed good separation. The adaptation of a widely used trace analysis detector to perform as a bulk sample illicit drugs detector can further broaden the number of groups / analysts which can perform routine sample testing. Furthering this, mixture analysis and LoD assessment can outline the drawbacks of the technique, to educate analysts on the limitations of the newly adapted instrumentation.

In Chapter 6 - Synthesis, Characterisation and Quantification of the new Psychoactive Substance "putylone", as part of routine drug testing work, a newly identified cathinone "putylone" (or bk-PBDB) was identified. Previously, putylone had only been detected rarely in Czechia (Jan 2015), USA (July 2022) and Canada (August 2023)[155] with minimal characterisation data (GC-EI-MS and LC-qTOF) showcased by Krotulski *et al.*[169] The full characterisation and quantification was not previously detailed. Chapter 6 outlines the characterisation of this cathinone by NMR (both 1D and 2D techniques) and mass spectrometry, as well as methodology for its quantification by GC-MS. The GC-MS quantification showed good linearity (>0.999), sufficient LoD (0.09 – 0.13 μg/mL) and LoQ (0.26 – 0.39 μg/mL), with good precision (0.66 – 4.61 % RSD) and acceptable recoveries (89.5 – 105.5 %). NMR spectral data and EI-MS fragmentation patterns have been outlined so future identification is simple. The showcase of this method and data allows welfare staff to identify this newly emerging synthetic cathinone and allow medical staff to promptly administer the correct medical treatment.

Across the last four chapters of this thesis, the main aims outlined earlier in the thesis have been achieved. These aims although do not directly aid "harm reduction", do aid the potential analysis in which groups who are directly involved with "harm reduction".

Showcasing new techniques and methods of analysis, allow alternative options of analysis as allow harm reduction testing to be performed easier and more efficiently.

7.2. Future Work

A number of additional objectives can be added to the work detailed within this thesis in order to build upon and improve the harm reduction potential of the overall work.

Further illicit drug testing at future live events, could allow a wider variety of samples to be identified, in various different matrices, including within herbal mixtures, tabs and in various liquid forms. This may showcase how each analytical technique deals with samples in a more complex matrix and allow further comparisons to made. Additional samples should allow a wider range and variety of binary and tertiary mixtures. The wider range of mixtures could identify potential limitations of the NMR drugs detection algorithm and spectral subtraction for FT-IR.

The BT-NMR quantification of cocaine samples could be further developed to allow untrained analysts to perform routine analysis during on-site live testing events. More robustness experiments assessing the influence of external temperatures could be performed to assess suitability for on-site analysis. Use of a solvent suppression pulse sequence for no-D solvent analysis, should negate the use of harsh spectral baseline correction, saving analyst time and improving quantification accuracy. The use of an automated script will then improve reliability, reduce potential operator bias during spectral processing and allow analysis to be performed by analysts who are untrained on NMR spectral processing software, widening the range possible potential analysts. Furthermore, application of this new method into an off-site live testing event should assess the advantages and disadvantages on NMR analysis, for on-site harm reduction testing of cocaine samples.

Further work for the use of IMS for bulk sample analysis would focus around expanding the sample range to include a wider range of samples in different matrixes, such as herbal material and e-liquids to assess the reliability of IMS for samples outside of typically seized tablets and powders. This also includes potential library additions of cannabinoids outside of THC (tetrahydrocannabinol) and potentially synthetic cannabinoids, as well adaption of the method to include extraction from herbal and vape liquid material. Repetition of the study performed by individual analyst could be

beneficial, to confirm that the changes to the instruments CP library are robust and function as anticipated. Further LoD determination for substances which are potentially more harmful than seen in standard prevalence, such as fentanyl, as this data could allow further adaption of the method for routine fentanyl screening. For the positional isomer discrimination, computer modelling software could be used for each set of isomers to calculate the cross-sectional area of each isomer and show potential correlation between the elution order using IMS. This could provide further insight into the differences in two elution orders, shown between the isomer groups. Potential software has been outlined previously in work by Shrivastav *et al.*[174]

Clearly, the "war on drugs" is showing no sign of relenting in the near future and the challenge facing law enforcement agencies is to be "one-step-ahead" of the clandestine drug manufacturers. By working collectively, analytical chemists, policy makers, law enforcement and forensic practitioners can identify potential classes of molecules that may become the next generation of NPSs. Advanced methods for the detection and quantification of both these substances and established illicit drugs can be produced, thereby developing on-site harm reduction approaches. This allows to inform professionals and user groups and can aid legislation against potentially dangerous compounds before they pose a serious threat to human health.

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9. Supplementary information

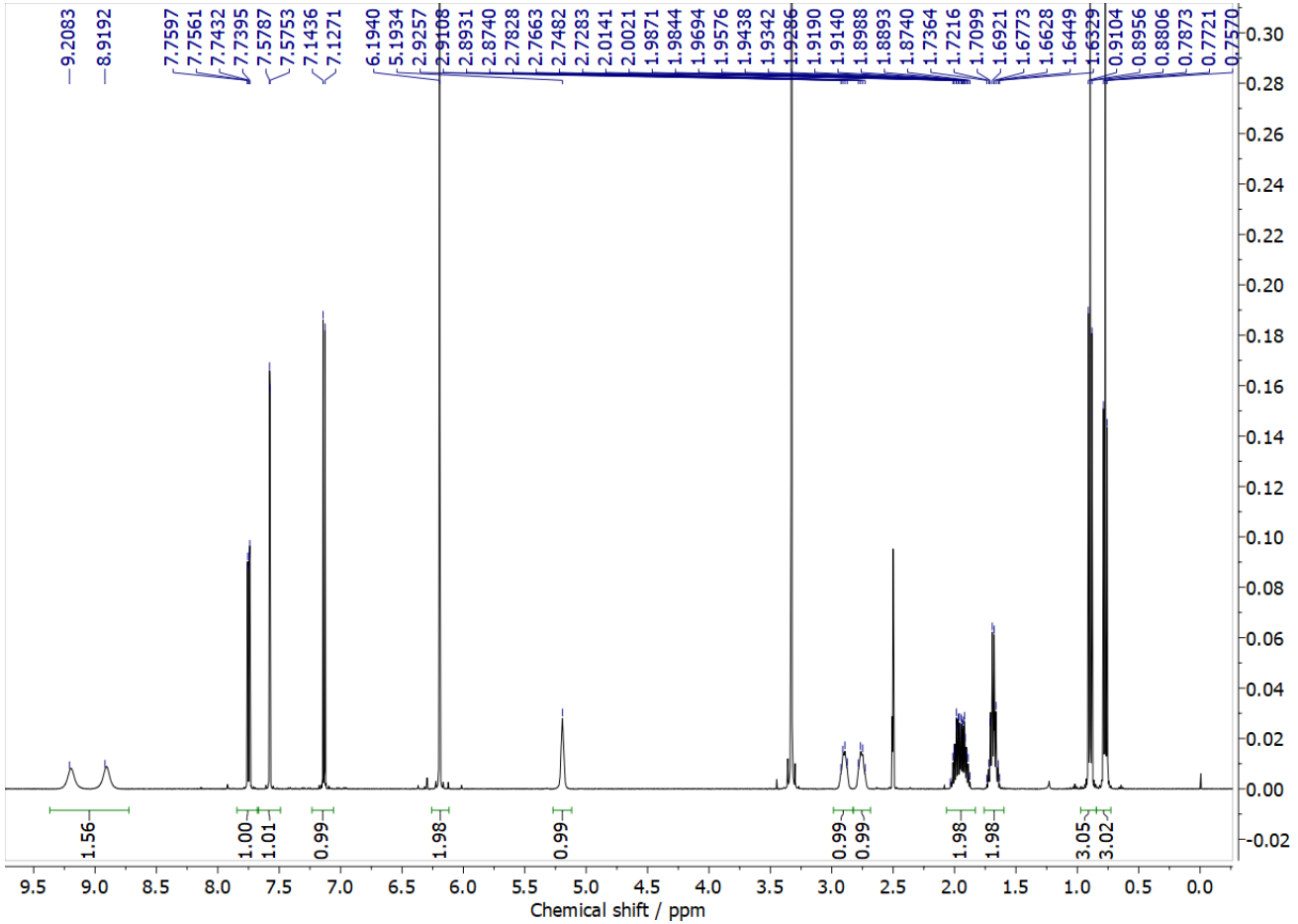

Fig. S1: ^{1}H NMR spectrum of (**36**) collected in DMSO-d_6 on a 500 MHz spectrometer.

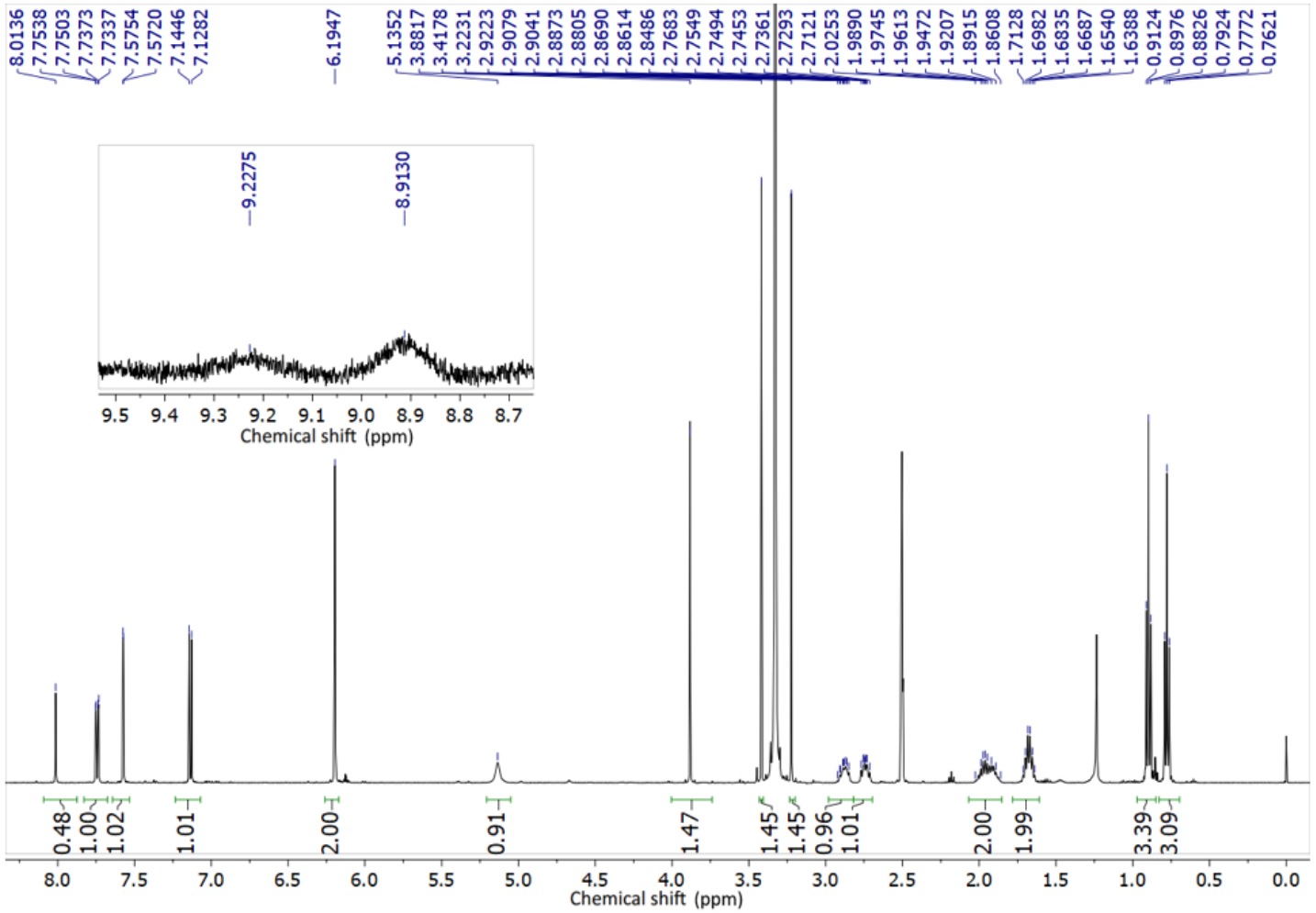

Fig. S2: ^{1}H NMR spectrum of **GM443** collected in DMSO-d_6 on a 500 MHz spectrometer.

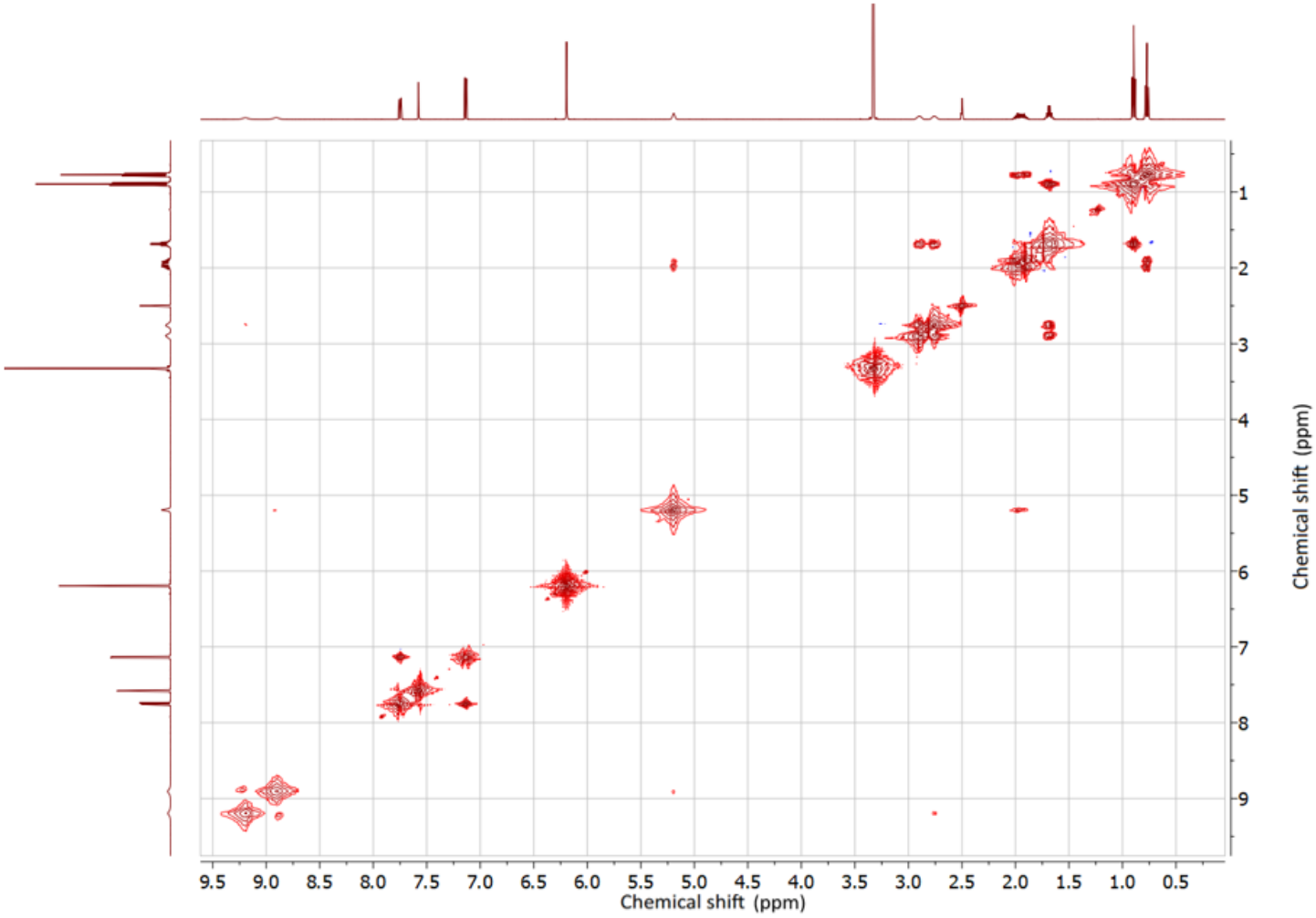

Fig. S3: ^{1}H-^{1}H COSY NMR spectrum of (**36**) collected in DMSO-d_6 on a 500 MHz spectrometer.

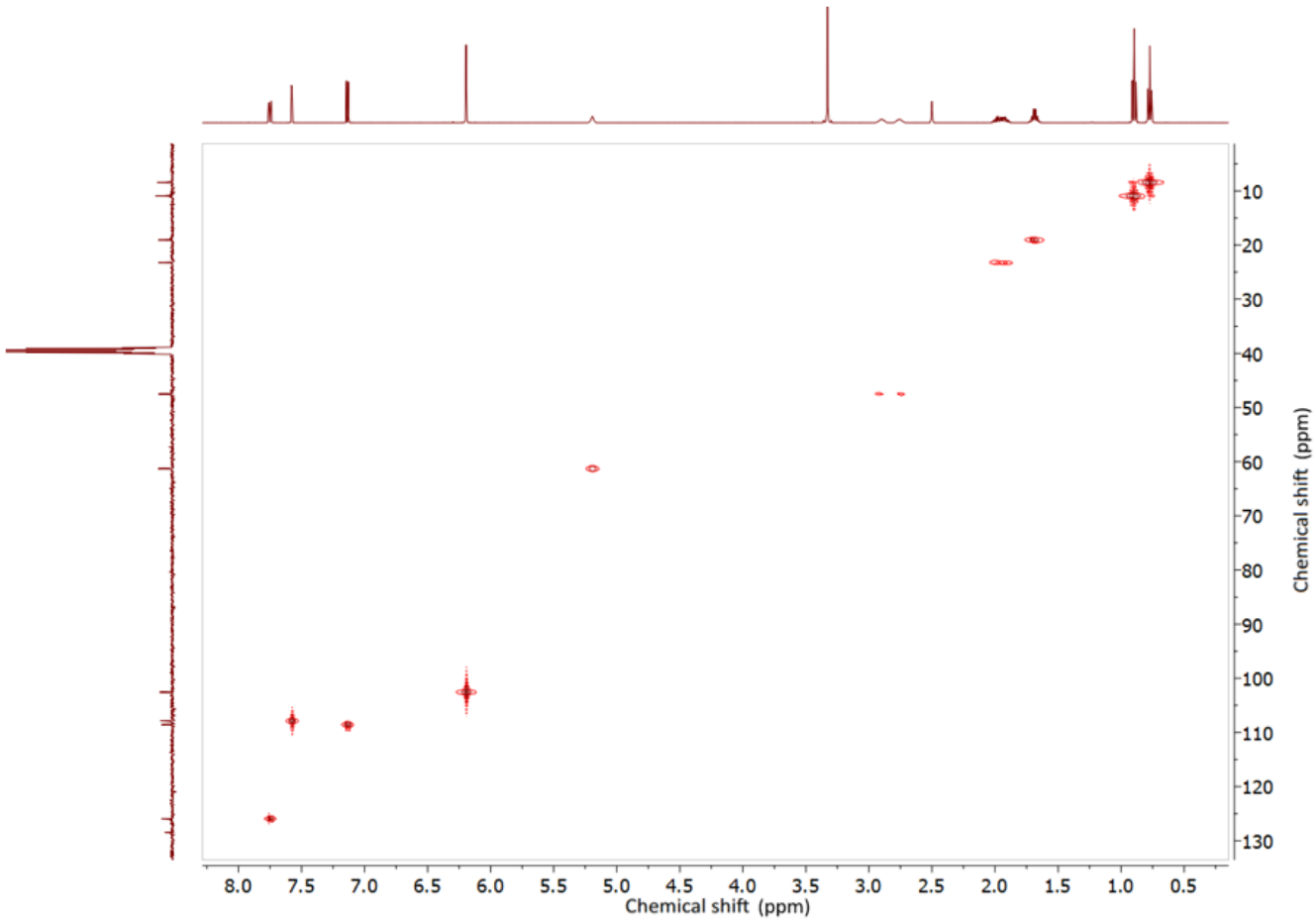

Fig. S4: ^{13}C-^{1}H HMQC NMR spectrum of (**36**) collected in DMSO-d_6 on a 500 MHz spectrometer.

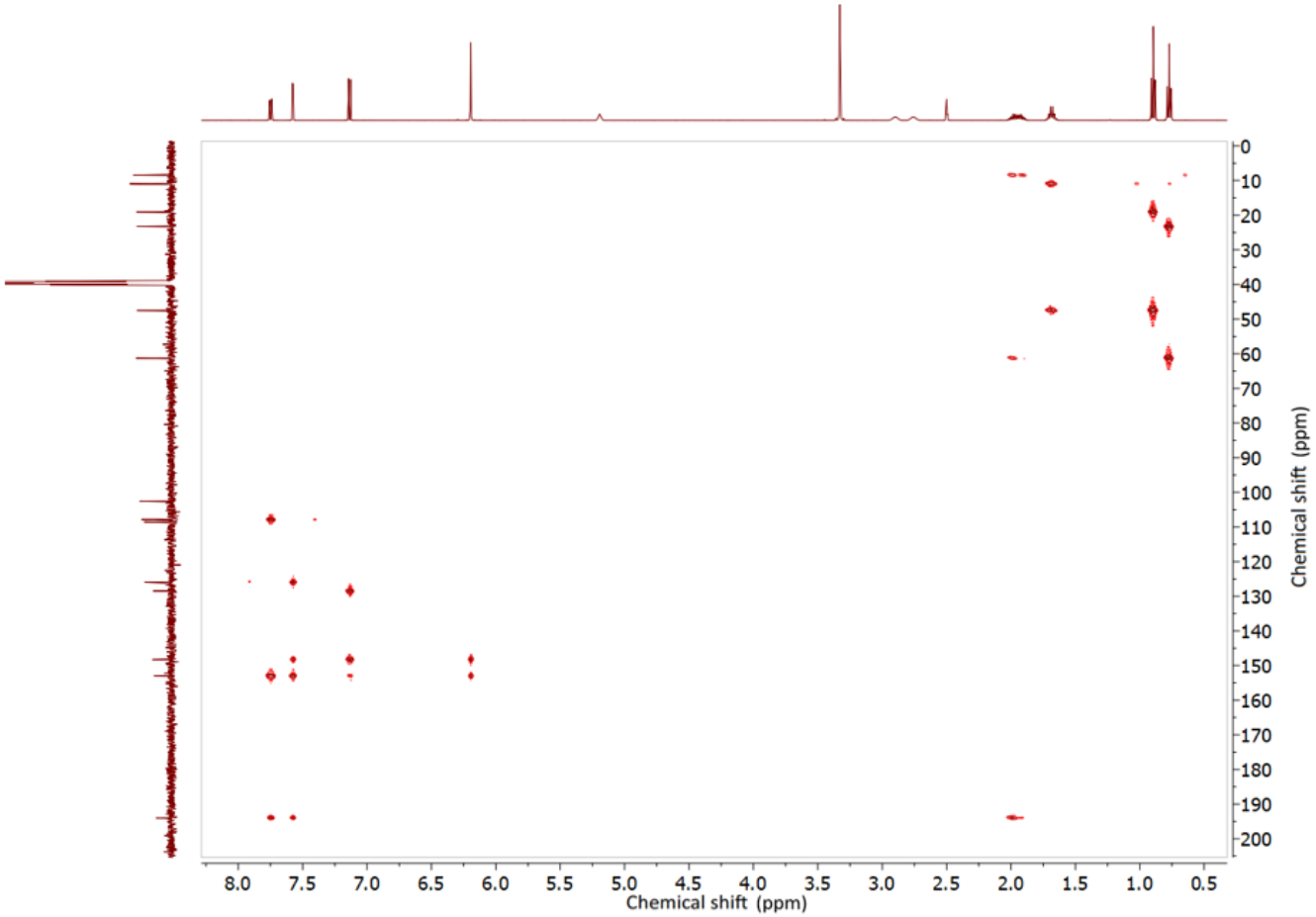

Fig. S5: ^{13}C-^{1}H HMBC NMR spectrum of (**36**) collected in DMSO-d_6 on a 500 MHz spectrometer.

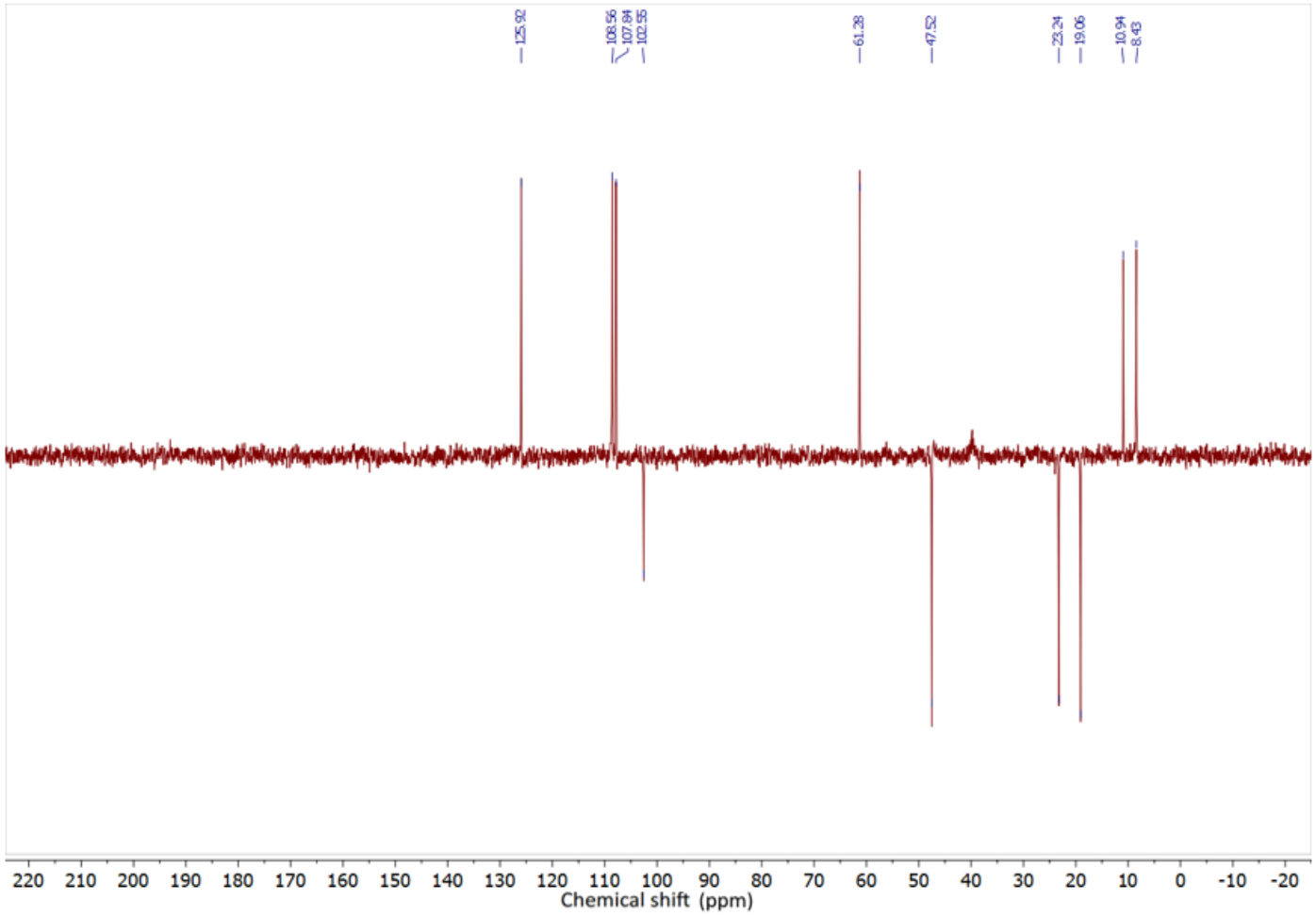

Fig. S6: $^{13}C(^{1}H)$ DEPT-135 NMR spectrum of (**36**) collected in DMSO-d_6 on a 500 MHz spectrometer.