Development of a method for the detection of GHB and other drugs using a handheld Raman Spectroscopy Device

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Declaration

It is hereby declared that this thesis and the research work upon which it is based were conducted by the author, Lauren O'Connor

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Acknowledgment

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Abbreviations

AEME – Anhydroecgonine Methyl Ester

BCS – British Crime Survey

CNS – Central Nervous System

DAT – Dopamine transporter

DFSA –Drug facilitated sexual assault

EMCDDA – European Monitoring Centre for Drugs and Drug Addiction

EME – Ecgonine methyl ester

FIT – Field Impairment Test

g – Grams

GBL – Gamma Butryolactone

GC/MS – Gas Chromatography/Mass Spectrometry

GHB – Gamma – Hydroxybutyric acid

IM – Intramuscular

IR – Infrared

IV – Intravenous

KGHB - Potassium Gamma- Hydroxybutyric acid

LC – MS – MS – Liquid Chromatography, tandem mass spectrometry

LOD – Limit of detection

LSD – lysergic acid diethylamide

MAO – Monamine Oxidase

MDE – methylenedioxyethylamphetamine

MDMA – 3,4 – methylenedioxymethamphetamine

mg – Milligrams

mL –Millilitre

mW - MilliWatts

NA – Noradrenalin

NaGHB – Sodium Gamma Hydroxybutyric acid

ng – Nanograms

NMDA – N – methyl – D – aspartate

OF – Oral Fluid

o.t.c – over the counter

ROSITA – Roadside Testing Assessment

RPM- Revolutions per minute

SCJS – Scottish Crime and Justice Survey

SERS – Surfaced Enhanced Raman Spectroscopy

SOP- Standard Operating Procedure

UK – United Kingdom

USA – United States of America

v/v – Volume/Volume

w/v – Weight/Volume

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Abstract

The aim of this study was to investigate the potential of a portable Raman spectroscopy device (TruScan) for the screening of illicit drugs. The study aimed to establish if the device could be used as a presumptive test on the spot in order to determine if a bulk sample, alcohol or oral fluid has an illicit substance in it. Should this device be successful in its detection and be easy to use, police would be able to utilise it in situations such as clandestine laboratories and on suspicion of drug driving. A review of the North report demonstrated a great need for the latter. It must be emphasised that this study only attempted to consider the device as a presumptive screening device and does not intrude on the confirmatory drug testing domain which includes instruments such as GC/MS.

The devices ability to detect KGHB in alcohol was investigated by spiking ethanol then analysing. This offered a brief comparison of the TruScan and DXR bench-top Raman instrument. Bulk samples of mixed powder were produced to replicate street drugs in order to establish the devices ability to detect drugs in a mixture. Blank oral fluid was spiked in order to establish the devices ability to detect drugs in saliva. SERS analysis was also attempted on spiked oral fluid samples.

It was found that the TruScan device was not effective in detecting drugs in alcohol or mixtures but it was able to detect KGHB in oral fluid down to a 30% v/v concentration. A successful SERS method was not established for the oral fluid analysis.

Contents

Chapter 1: Introduction

1.1 The identification of drugs

Many different scientific techniques have been applied to the identification of drugs from simple colour change reactions to the use of sophisticated analytical instruments. Identification techniques can be presumptive or confirmatory and can quantify the concentration or be qualitative.

An example of a simple spot test is the Marquis reagent, a mixture of formaldehyde and concentrated sulphuric acid which results in a colour change indicative of a drug when it comes into contact with a substance. This is a presumptive test which is not very specific, different drugs may produce similar colour changes. (Jickells and Nergrusz, 2008)

Nuclear magnetic resonance spectroscopy (NMR) can be used for bulk drug analysis. NMR is a technique which is based on the physical phenomenon where a magnetic field causes a nuclei to absorb and re-emit electromagnetic radiation. This technique allows the structure to be determined non-destructively which is huge advantage over mass spectroscopy however a larger amount specimen is required. Isotopes of atoms can be studied using NMR spectroscopy. NMR technology is invaluable in pharmaceutical quality control and assurance for structure identification and to check for impurities from formation processes or degradation. (Diercks et al, 2001) A paper from 2011 discussed how NMR is an effective tool in the discovery of counterfeit drugs, NMR technology was able to distinguish between genuine and counterfeit sildenifil. In this case the packets and tablets look identical looking the Pfizer pharmaceutical logo but the tablet composition was different. In a similar case, a Chinese natural sexual enhancement product which claimed to be completely natural with no sildenifil was found to contain sildenifil through NMR technology (Holzgrabe and Malet-Martino, 2011)

Drugs in biological matrices are typically analysed using Gas Chromatography/Mass Spectroscopy (GC/MS) or Liquid Chromatography/Mass Spectroscopy. (LC/MS) These instruments allow the compound to be separated

out by chromatography using gas or liquid as a carrier through a column. The time it takes for a substance to elute from the column is the retention time and this gives an identifying feature to the substance. The mass spectrometry (MS) part fragments the effluent ions in a reproducible pattern allowing the drug to be identified. The MS detector can specifically scan for pre-selected masses which are characteristic for the substance in question, this is called selected ion monitoring (SIM) or it can scan in full scan mode which gathers all the ions in the mass range given. Both LC/MS and GC/MS require the drug to be extracted from the matrix which is time consuming and adds to the expense of the analysis. Some drugs may need to be derivatised to be made more suitable for GC/MS analysis. This requires a specific chemical to be added to the extracted sample under heated conditions, this also adds time and money onto the analysis. The gold standard in drug detection in biological matrices was considered to be GC/MS, (Jickells and Nergrusz, 2008) however LC-MS has become increasingly favourable in recent years due to its ability to detect drugs in biological matrices at low concentrations. A study by Gallardo *et al* discusses the how LC-MS can be very advantageous for work place drug testing where hair, oral fluid or sweat could be used. LC-MS has the sensitivity to detect the low concentrations of drugs present in this matrices. Another advantage of LC-MS is there is no need to derivatize samples which reduces sample preparation time. However LC-MS can be susceptible to matrix effects and this can vary between specimens, this is a factor which must be considered during the validation of a method as this can affect the accuracy of the quantitation. (Gallardo *et al*, 2009)

Using GC-MS, GHB can be detected to the nano gram level however an effective screening device with minimal sample preparation is desirable, not just for biological samples but for bulk drug analysis and spiked alcohol analysis. A screening device which could achieve this would save time and money.

1.2 Prevalence

1.2.1 Prevalence of drug use

The prevalence of drug use in the general population can only be estimated through the use of surveys, this also applies to the prevalence of motorists driving under the influence of drugs. The Scottish Crime and Justice Survey (SCJS) found that 8.4% of under 60 year olds have used cannabis in the year 2008-09, 3.7% had taken cocaine, 2.5% ecstasy and 1.4% amphetamine. (Wishart, 2010) Since 2006 there appears to be a decrease in illicit drugs in Scotland, the use of cocaine and the benzodiazepine temazepam however has remained unchanged. (Wishart, 2010)

A review by Jackson and Hilditch considers the British Crime Survey (BCS) as the most extensive drug use survey of England and Wales. The survey's respondents lived in a household and were between the ages of 16 and 59. According to Hoare the survey is an underestimate as it is restricted to people who live in a household and does not include groups which have the potential to have high rates of drug abuse such as the homeless or prisoners. Hoare also notes that opiate and cocaine addicts (including crack cocaine addicts) may lead such a chaotic lifestyle that they may also be missed by the survey. (Hoare, 2009)The survey found that the most common drug used in 2009 was Cannabis with 7.9% of respondents admitting using the drug, powder cocaine is the second most common (3%) then ecstasy (1.8%) amyl nitrate (1.4%) and amphetamines. (1.2%)

The Scottish Crime and Justice Survey (SCJS) and BCS have shown similarly figures in the prevalence of drugs. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) also report similar findings with Cannabis being

the most common drug followed by cocaine.

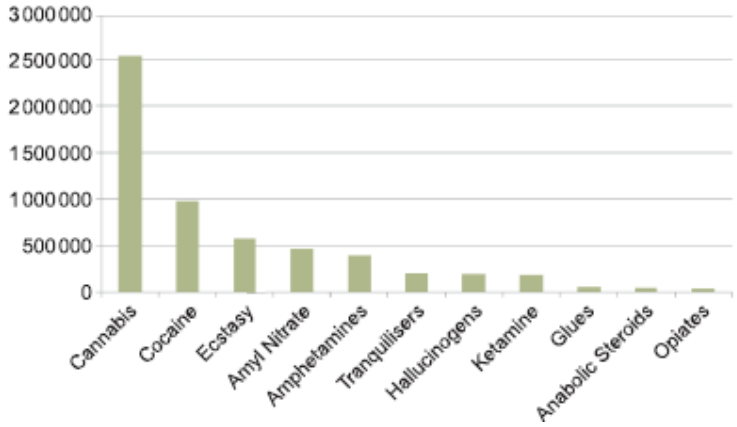

Figure1.1: Estimated number of drug users for each drug in 2009 (Hoare, 2009) (cocaine includes crack cocaine, amphetamine includes methamphetamine, hallucinogens include magic mushrooms and LSD and opiates include methadone and heroin.)

According to the BCS, cocaine use among young people aged 16-24 has seen a huge increase in use, in one year it jumped from 5.1% of respondents admitting use to 29%. The use of ketamine also increased. (Hoare, 2009)

1.2.2 Prevalence of drug use and driving

The report “Illicit Drugs and Driving” produced by the Scottish Executive published in 2006 highlighted that 6% of the 17-39 year old drivers surveyed had drug driven. It was found the most common drug used before driving under the influence was cannabis. Ecstasy was the second most common drug, followed by cocaine and then amphetamines. (Myant *et al*, 2006)

1.3 Legislation

1.3.1 Misuse of Drugs Act 1971

The main piece of legislation which addresses illicit drugs in the UK is the Misuse of Drugs Act 1971. The Act was designed to prevent the use of drugs which are 'capable of having harmful effects sufficient to constitute a social problem'. (North, 2010)

Drugs are divided into three categories, Class A, B or C. Class A drugs are considered to be the most dangerous and carry the highest penalties. In the UK it is an offence to:

- Possess a controlled drug
- Possess a controlled drug with the intent to supply
- Offer to supply a controlled drug
- Produce, manufacture or cultivate a controlled drug
- Import or export controlled drugs
- Allow premises to be used for use, supply or production of controlled drugs

Class A drugs include heroin, cocaine, crack cocaine and ecstasy. Possession of a Class A drug can result in a prison sentence of up to seven years and an unlimited fine. Life imprisonment and an unlimited fine is the maximum penalty for the supply of Class A drugs. (Misuse of Drugs Act 1971)

Class B drugs include amphetamine and cannabis. If a Class B drug is prepared for injection it becomes a Class A drug, this is common with amphetamine. Possession of a Class B drug can lead to a prison sentence of up to five years and an unlimited fine. Supply of a Class B drug can result in a prison sentence of up to 14 years and an unlimited fine.

Drugs controlled under Class C include Benzodiazepines, Ketamine and GHB. Possession of a Class C drug can result in the maximum of two years imprisonment and an unlimited fine. Supply of a Class C drug can result in a maximum of 14 years imprisonment and unlimited fine. (The Misuse of Drugs Act, 1971) Any company who needs exemption from these laws such has a university carrying out drug research can apply for a Home Office domestic licence. This study was conducted under this licence. (Home Office, 2012)

1.3.2 Misuse of Drug Regulations 2001

The Misuse of Drug Regulations 2001 divides illicit drugs into five schedules which reflect their medicinal use and their potential for misuse.

Schedule 1 includes the drugs cannabis, ecstasy, LSD and raw opium. Schedule 1 drugs are not authorised for any medicinal use and should only be possessed, supplied and administered under a Home Office licence. Schedule 1 drugs have a high potential for abuse.

Schedule 2 drugs have a medicinal use but a possession is only legal with a prescription. Strict storage requirements and recordkeeping must be employed with these drugs in circumstances such as a hospital setting. Schedule 2 drugs include morphine, cocaine, amphetamines and dihydrocodeine. The potential for abuse is still high.

Schedule 3 drugs are not required to be kept under strict storage and a register is not required. Schedule 3 drugs include barbiturates and temazepam.

Schedule 4 is divided into two parts; part 1 includes most of the benzodiazepines. Possession is legal under prescription but supply is illegal. Part 2 includes anabolic steroids which can be possessed for medicinal purposes without a prescription however supply to others is illegal.

Schedule 5 includes over the counter drugs such as cough medicines and mild painkillers. The risk of misuse is reduced with these drugs as any controlled substance used in their preparation is at a low level. (Misuse of Drugs Regulations 2001)

1.3.3 The Medicines Act 1968

The Medicines Act 1968 controls the distribution of medicines. Medicines can be prescription only and therefore supplied by a pharmacist only when in receipt of a doctor's prescription. Pharmacy medicines can be bought from a pharmacist without a prescription and general sales medicines can be bought from a variety of shops with no need for prescription or pharmacist. These are called over the counter medicines. (o.t.c) (The Medicines Act 1968)

1.3.4 Drug Harm and the law

In 2010 a study carried out by Nutt *et al* was published on drug harm in the UK. This study was carried out by an interactive workshop attended by the members of the Independent Scientific Committee. The participants were asked to score 20 drugs on 16 harm related criteria. Nine criteria related to how the drug affects the user and seven on the harm it causes to others. (Nutt *et al*. 2010) It was found that heroin, crack cocaine and methamphetamine are the most harmful drugs to the user. Alcohol, heroin and crack cocaine were the most harmful drugs to others and alcohol followed by heroin and crack cocaine were the most harmful drugs overall. (Nutt *et al*, 2010) The study calculated their findings correlations with the Misuse of Drugs Act 1971 as 0.04 and therefore no relation. They concluded that current drug laws are not indicative of the level of harm a drug may cause. (Nutt *et al*, 2010)

1.4 Common Illicit Drugs

1.4.1 Cocaine

Cocaine is extracted from the leaves of *Erythroxylon coca* and is produced either as a hydrochloride salt or a base (crack) 0.7% is the average concentration of cocaine produced per leaf. Each coca shrub has a life expectancy of around 50 years and is harvested three or four times a year. (Karch, 2006) The isolation and extraction process is fairly easy with no great technical knowledge or scientific equipment required. The technique is mostly passed down through the generations. (Karch, 2006) The purity of street cocaine is highly variable and can be as low as 1% Common cutting are sugars and other drugs such as amphetamine, caffeine and codeine and procaine which has a similar anaesthetic property but is not a CNS stimulant. However, at the latter end of 2010 the Forensic Science Service noted that cocaine purity was increasing from around 17% to 26% on average. It is thought that this increase is a reaction to the popularity of legal highs. Mephedrone appeared on the legal high market in 2009 as a cheap substitute to cocaine, as it was not cut with inert substances like benzocaine. Mephedrone is still widely available despite being banned in April 2010.Police have also targeted the cutting agents market which could also have influenced the increased purity**.** (Daly, 2010)

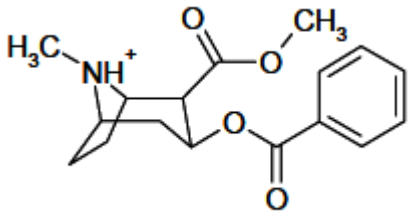

Figure 1.2: Cocaine Chemical Structure (Ravina *et al* 2006)

Cocaine is classed as a stimulant. Stimulants are used recreationally for their euphoric effects, these effects create a distraction and this is not an idle state for a driver. Acute effects can be feelings of elation, powerful and superiority, users can become agitated, impatient and sometimes even violent. An individual may take more risks whilst driving if under the influence of cocaine.

The most common way cocaine hydrochloride is administered is by nasal insufflation or snorting, the user will often rub the remaining powder into their gums. It is not very effective to smoke the hydrochloride form of cocaine as it has a high boiling point (197°C) therefore the majority of the drug is wasted and a high amount would be required for effect which would be very expensive. “Crack” cocaine is smoked as it has a much lower boiling (98°C). It can be smoked in a pipe, inhaled from heated foil or putting into a cigarette with tobacco. Injecting cocaine is less common, the hydrochloride form is generally used as it is more soluble than crack. (Wills, 2005) Cocaine is not commonly taken orally as the onset of effects is slow and produces a low blood concentration opposed to other routes of administration, this may be due to first pass metabolism to ecgonine methyl ester (EME) (Drummer, 2001) Smoking and injecting produces similar effects, the onset time is quick but peaks within minutes. Oral and insufflation take longer to peak, oral may take up to an hour and insufflation, 15-30 minutes. (Wills, 2005) By inhibiting sodium influx into the cells, cocaine acts as a local aesthetic this is likely responsible for the vasodilatory action seen with cocaine use. Cocaethylene is formed when cocaine and alcohol are consumed at the same time, it is estimated that around half of users consume both together. Cocaethylene is a more potent sodium

channel blocker than cocaine and the effects of this may cause sudden death. (Karch, 2008)

The reuptake of dopamine, noradrenaline and serotonin is inhibited by cocaine at the nerve synapses thereby prolonging their effects. The behavioural effects of cocaine are more associated with the accumulation of dopamine then the other two neurotransmitters. Cocaine blocks the dopamine transporter (DAT) this is located along the presynaptic nerve terminal walls. When the DAT is blocked the rapid termination of the effects of dopamine are prevented and the release of dopamine is increased in the synaptic terminal. The mesolimbic dopamine system, area responsible for memory and emotion, is the part of the brain cocaine effects in particular. This is what causes the feeling of euphoria. (Wills, 2005, Winger *et al*, 2004) When cocaine is administered locally the conduction of the axon potential is prevented as sodium channels on the neuron membrane are blocked by the drug. The vasoconstriction effect is due to the increasing levels of norepinephrine, which acts on the alpha-adrenergic receptors on the blood vessels. (Winger *et al*, 2004)

Thickening of the heart muscle or myocardial hypertrophy is associated with stimulant drug use. Coronary artery reserves decline as ventricular mass increases leading to impaired myocardial contractility, this means the heart is under strain and this may also be responsible for sudden cocaine related death. (Karch, 2008)

The left ventricles are larger in laboratory rats and rabbits that are treated with cocaine and the hearts of cocaine users are heavier than those of controls by around 10%. Cocaine use can also cause coronary atherosclerosis and thrombosis. (Karch, 2008)

1.4.1.1 Metabolism

The methyl ester of cocaine is hydrolysed chemically and by enzymes to produce the primary enzyme detected in blood and urine after use, benzoylecgonine. In 2005 the River Po in Italy was tested for benzolylecgonine, in order to determine if any had polluted the river by getting into the sewage system via users urine. The river has found to be carrying nearly 4kg of benzoylecgonine daily. This is the equivalent of 40 000 doses daily in the

region, which puts the street value of cocaine consumed in the area at around £84 million per year. (Italian river “full of cocaine,” 2005) Other cocaine metabolites are produced such as ecgonine methyl ester (EME), ecgonine and nor cocaine. The enzyme butyrlcholinesterase in the blood converts cocaine to ecgonine methyl ester and benzoylecgonine, these are relatively inactive metabolites. (Winger *et al*, 2004) Norcocaine is further metabolised to N-Hydroxynorcocaine and this may be responsible for cocaine’s toxicity of the liver. (Drummer, 2001) Anhydroecgonine methyl ester (AEME) is only produced when cocaine is smoked as it is not produced from metabolism but from pyrolysis. This makes it a helpful and unique marker. (See Fig 1.3) Another marker is cocaethylene which is only formed when the user is consuming both cocaine and alcohol. Greater intoxication has been reported by users when cocaine is combined with alcohol. This is thought to be due to cocaethylene binding to dopamine receptors as well as the cocaine molecule; therefore dopamine reuptake is further blocked leading to a greater build-up of dopamine heightening the euphoric effects. (Wills, 2005)

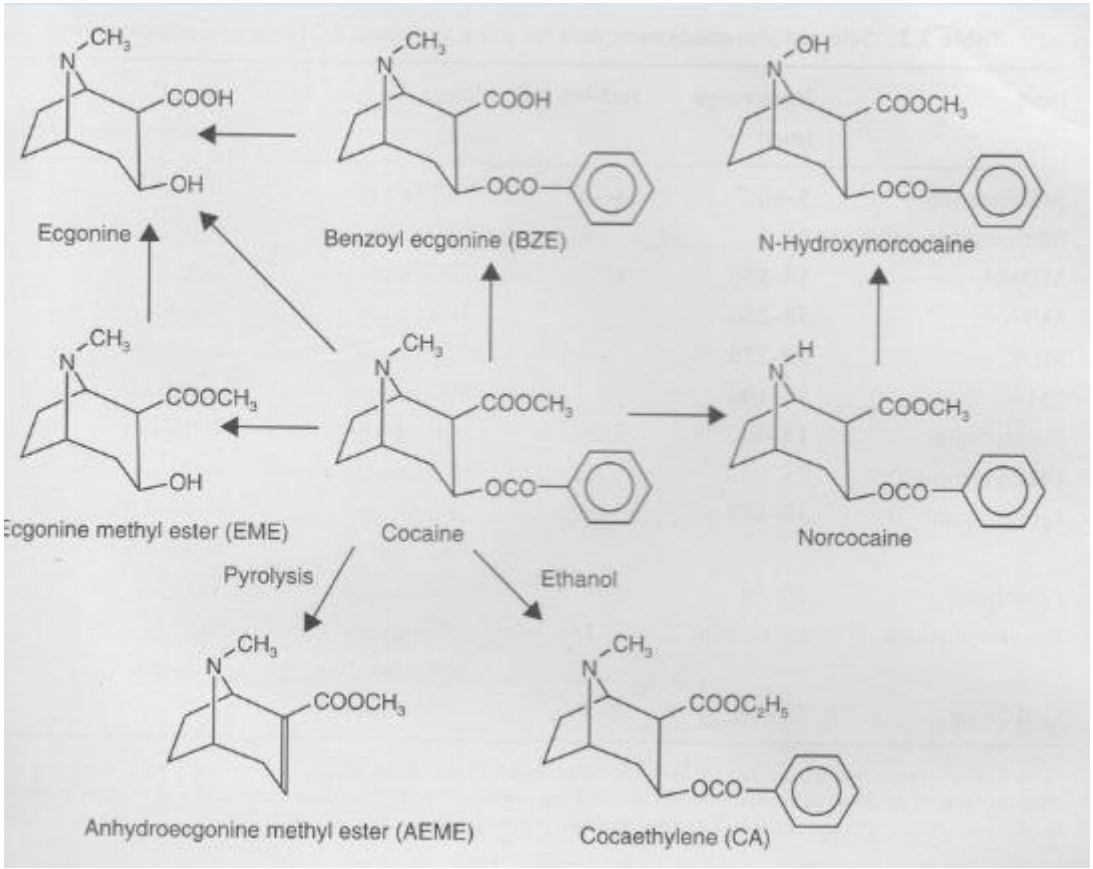

Figure 1.3: Pathways of Cocaine metabolism (Drummer, 2001)

1.4.2 Amphetamines.

Amphetamines are illegally synthesised, usually by a process known as the Leuckart reaction. This involves the condensation of phenyl -2-propanone with formamide then a hydrolysis of N-formylamphetamine and finally purified by steam distillation. The Leuckart reaction does not produce any hazardous chemicals and produces a good yield which may explain its popularity. (Jickells and Negrusz, 2008)

Street amphetamines are bought in small “wraps” and have the appearance of an off-white powder, they are cut with adulterants such as caffeine to provide a stimulant effect and mask the low level of drug, Sugars are another common diluent.

Methamphetamine is more popular in the USA and Japan than the UK and is usually sold as methamphetamine hydrochloride. (Jickells and Negrusz, 2008)

If amphetamine is injected, smoked or vaporised the effects can be quick as a few seconds, giving a rush of euphoria. Snorting does not give the same heighten effect of euphoria and takes minutes for the effect to take hold, it may take up to 20 minutes for any effects to kick in if it is taken orally. (Wills, 2005)

Common effects users experience includes alertness, self confidence, very talkative, impulsive and increased stamina. The psychoactive effects of amphetamine usually last for around 4 hours, the effects of methamphetamine can last for around 12 hours if snorted or taken orally. (Wills, 2005)

Smoking the crystals of methamphetamine is the purest form of the drug. The same crystals can be reheated several times and will still produce the same high due to their high melting point. (Wills, 2005) A brownish yellow powder with a waxy appearance is more common form of methamphetamine. This appearance is caused by impurities. (Wills, 2005)

Amphetamines main mechanism of action is to stimulate the release of certain neurotransmitters heightening their natural effect. (See Fig.1.4) Amphetamines act as indirect sympathomimetics. Amphetamine enters the nerve by the noradrenaline (NA) transporter and then into the synaptic vesicles by the vesicular monoamine transporter in exchange for Noradrenaline which gathers in the cytosol. Monoamine oxidase (MAO) degrades some of the NA within the cell and some is released from the cell via the NA transporter in exchange for amphetamine. The realised NA acts on the postsynaptic receptors, the action of realised Na is enhanced as amphetamine also reduces NA reuptake. (Rang and Dale, 2000)

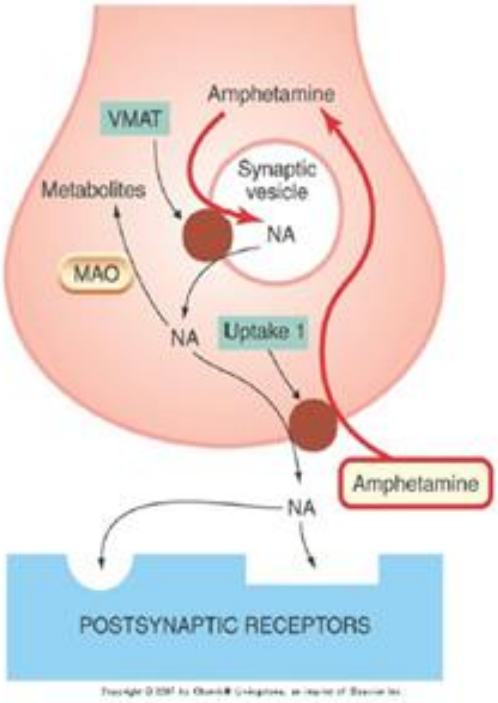

Figure.1.4: Mechanism of action for Amphetamine.(Rang and Dale, 2003)

Tolerance to amphetamine can develop with repeated doses as it is thought the stores of noradrenaline deplete. (Rang and Dale, 2003)

Amphetamine is also responsible for increase the levels of Dopamine and Serotonin in the synaptic cleft therefore heightening the effect of the post synaptic receptor in select areas in the brain which results in a temporary feel good effect. (Rang and Dale, 2003) Amphetamine enters the presynaptic nerve terminal through the dopamine receptor, the dopamine active transporter or DAT. It then encourages dopamine to be released through DAT, dopamine is usually released from synaptic vesicles and not through a receptor. As with cocaine, it is the high levels of dopamine which causes a behavioural effect (Winger *et al*, 2004)

Amphetamine use can cause rhabdomyolysis, pulmonary odema, and acute myocardial infarction. Rhabdomyolysis, which is the breakdown of muscle fibres contents called myoglobin into the bloodstream, this is toxic to the kidneys. (Karch, 2008)

1.4.2.1 Metabolism

The half live of amphetamine is around 7 hours. Around 30% of amphetamine is excreted from the body unchanged by the kidney however it does go through Phase I and Phase II metabolism. (Winger *et al*, 2004) Two enzyme systems are involved in Phase I, these are cytochrome P450 and flavin monooxygenase. In Phase II the metabolites are conjugated and eliminated from the body. (Foye *et al*, 2008) Methamphetamine is metabolised to amphetamine which is then metabolised to benzoic acid. (Winger *et al*, 2004)

The metabolism of amphetamine is dependent on urine pH, if the pH is unregulated around 15% of the drug is excreted in urine unchanged. In acidic urine the drug is trapped as it is a basic compound, its ability to be reabsorbed into the blood is reduced and clearance is more rapid than usual. Alkaline urine therefore delays clearance from the body. Users often take a substance that alkalises urine such as sodium bicarbonate to extend the effects of the drug. The cytochrome P450 enzyme CYP2D6 is involved in the metabolism. (Wills, 2005)

1.4.3 MDMA

3,4-methylenedioxymethamphetamine (MDMA) was first synthesised by a German company known as Merck in 1913. Users may not always receive MDMA when they buy "ecstasy" it may be another psychedelic amphetamine such as MDEA or MDA. These similar chemicals are reported to give very similar stimulant effects but it's MDMA which is associated with the feeling of empathy and warmth. Repeated use of one of these chemicals results in the user becoming tolerant to their effects. However there does not appear to be any cross tolerance between the chemicals therefore if a user is tolerant to MDMA, they will still get a high if they take MDEA. (Saunders, 1993)

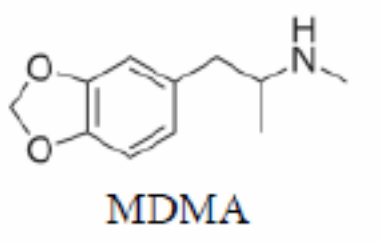

Figure 1.5: Chemical Structure of MDMA (Farquharson *et al* 2011)

Ecstasy is almost always taken orally, injecting the drug has been reported but is rare. Ecstasy is supplied in tablet form, which are often embossed with logos. (Wills, 2005)

MDMA increases the secretion and reuptake of the serotonin, dopamine and nor-adrenaline in the brain causing feelings of euphoria and increased energy. MDMA can cause the users judgement to be impaired which results in dangerous behaviour. Dehydration, hypertension and hyperthermia are short term health risks associated with using MDMA, in the long term permanent disruption of serotonin in the CNS can lead to depression. (Jickells and Negrusz, 2008)

A placebo controlled study using human volunteers showed that MDMA indirectly stimulates the hormone Oxytocin through agonising the $5HT_{1A}$ receptor. Oxytocin is the hormone naturally produced after hugging and childbirth, it facilitates bonding and trust. This is the reason users feel connected and warmth towards others. (Dumont *et al* 2009)

1.4.3.1 Metabolism

MDMA is absorbed from the gut and reaches peak plasma concentration around 2 hours after oral administration. It is mainly metabolised by the liver mainly using the enzyme CYP2D6. Several other enzymes are involved in the metabolism however these appear to be saturated at fairly low concentrations, the higher the dose results in the higher affinity becoming saturated. This means that the risk of toxicity increases greatly as the dose is increased just slightly. (Kalant, 2001)

Around 5-10% of the Caucasian population are deficient in the CYP2D6 enzymes and it is thought that this may mean they are at greater risk of an ecstasy related death should they take the drug. (Gilhooly and Daly, 2001)

The half live of MDMA is around 8 hours so elimination from the blood is relatively slow, as it takes 5 half lives for a drug to be 95% elimination from the body, therefore it takes around 40 hours for the drug to be eliminated. Users report some effects the day after which may be due to active metabolites such as MDA. (Kalant, 2001)

1.4.4 Ketamine

Ketamine is structurally related to phencyclidine and gives the same anaesthetic and analgesic effects without causing cardiac or respiratory depression. Ketamine is used in human and veterinary medicine, when sold illicitly it has usually come from a diverted legitimate supply or theft of a legitimate supply. Commercial ketamine is a racemic mixture composed of both R(-) and S(+) isomers. S(+) ketamine has four times the affinity for the NDMA receptor than R(-) ketamine. It also binds to the opioid receptors Mu and Kappa. (*Weiner et al*, 2000)

Ketamine is *N*-Methyl-D-aspartate (NMDA) receptor antagonist. The NMDA receptor allows the transfer of electrical signals from the brain and the spinal cord. Glutamate and Glycine are the substrates required by the the receptor to open the channel to allow transfer. Ketamine is a non competitive antagonist which binds to allosteric sites blocking the ion channel.

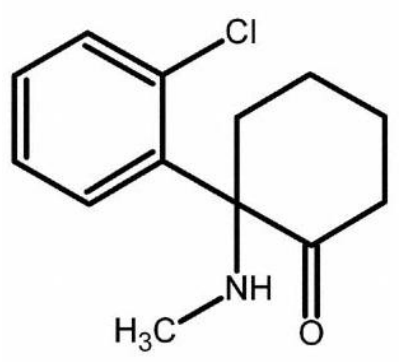

Figure 1.6: Chemical Structure of Ketamine (Stafford, 1992)

Ketamine is a short acting drug and the hallucinatory experience sought after will only last around 2 hours when ingested and around one hour if snorted or injected. The bioavailability of IV, IM, nasal and oral administration are 90%,90%,50% and 20% respectively. (Stafford, 1992)

At low doses the user may feel dissociative effects such as outer body experiences, at higher doses (60-125 mg IM, 100-250 mg insufflated) users can experience vivid hallucinations, memory loss and mimic the symptoms of schizophrenia. These effects are known as the "K-Hole" (Curran and Monaghan, 2001)

It has been suggested that there are six broad categories of experiences caused by ketamine abuse based on reports from users. This are 1. Dissociation such as out of body or near death experiences. 2. Entry into information networks. 3. The ability to enter alternative realities. 4. The ability to communicate with aliens. 5. Enhancement of sexual experiences. 6. Creative and personal problem solving skills increased. (Stafford, 1992)

1.4.4.1 Metabolism

Ketamine is mostly metabolised through N-demethylation to norketamine which is an active metabolite. CYP2B6 in the liver is major route of clearance although other routes are involved. 90% of the dose is cleared from urine within 5 days. Methadone and diclofenac, inhibit the conversation of Ketamine to norketamine

as they are CYP2B6 and CYP2C9 substrates. 90% of Ketamine is excreted in the urine. (Curran and Monaghan, 2001)

1.4.5 Gamma-Hydroxybutyric Acid

Gamma-Hydroxybutyric acid or GHB ($C_4H_8O_3$) is a short chain carboxylic acid which occurs naturally in the mammalian body (See Figure1.7) It is soluble in water and is formed from gamma butryolactone (GBL). It is also known as "liquid ecstasy".GHB has no medicinal use except when found in Xyrem which is a treatment for narcolepsy. Doses of 6 to 9 g are recommended nightly for the treatment of narcolepsy. This dose is not taken all at once however. (Winger, *et al* 2004) The lack of hangover effects is a property which makes GHB an attractive recreational drug. GHB is the precursor to the gamma-aminobutyric acid (GABA) neurotransmitter which is an inhibitory neurotransmitter which promotes sleep and relaxation. (Bennet and Steiner, 2009)

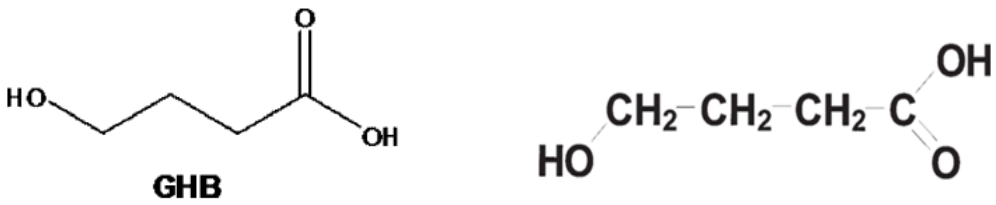

Figure 1.7: The chemical structure of gamma-hydroxybutyric acid. (Brewster *et al* 2008)

GHB can cause mild euphoria, increased sensuality, lower inhibitions, cause memory loss and lack of consciousness. (Bennet and Steiner, 2009) These effects are dose dependent. A low dose which is defined as between 0.5 and 1 gram gives these desirable effects and has led to the drug being taken recreationally. Higher doses between 2.5 and 4 grams give the sedative effect desired for its use in DFSA. (Brewster *et al* 2008)

GHB was initially sold in health food stores for the purpose of burning fat, increasing muscle and improving physical performance.

Illicit GHB is usually sold in small containers such as eye dropper bottles or vitamin bottles. Mixing the colourless liquid with another drink, often water or orange juice is a typical ingestion method. GHB is sold in a variety of different concentrations and its chemical composition varies highly in its illicit form, this makes it dangerous and difficult to dose correctly.

GHB and GBL could be purchased with ease on the internet before classification. Some suppliers provided a pipette for accurate dosing for recreational use. GBL is sold as a chemical cleaner, marketed as a 99.99% solvent cleaner which cleans car wheels, removes graffiti and glue. The Misuse of drugs act 1971 was amended in 2009 to include GBL as a dangerous drug. (The Misuse of Drugs Act 1971 (Amendment) Order 2009)

Since the amendment to the legislation in December 2009 some websites selling GBL under the pretence that they are selling a car cleaning product, such as www.alloycleaner.com ceased selling the product in compliance with the law. It is still possible to buy GBL from many websites as well as 1, 4 Butanediol which is another GHB precursor that can also be converted into the drug.

When GHB is unavailable, GBL or 1, 4 Butanediol is sometimes consumed recreationally as the body will convert these substances to GHB so the same effect is achieved. GBL is a chemical which comes with the warning “Not for human consumption.”

Once GBL is purchased illicit GHB can be synthesised in a clandestine laboratory using Potassium or Sodium Hydroxide. Recipes to make GHB can be found on the internet be doing a simple Google search.

GHB is a central nervous system (CNS) depressant; in high doses its actions are similar to the hypnotics Benzodiazepines. In combination with alcohol, another CNS depressant the sedative effect is exacerbated. GHB also causes relaxation of the voluntary muscles. (Nicholson and Balster, 2001)

GHB is rapidly absorbed from the gastrointestinal tract and the intoxicating effects can be observed in around 5 to 15 minutes after a dose. (Drummer, 2001) Peak plasma concentrations are reached around 30-45 minutes after administration. (Drummer, 2001) The half life of a dose of GHB is around 27minutes (Li *et al*, 1998) One half life is the amount of time it takes for the drug to degrade by a half. Five half lives equal 97% elimination of the drug from the body. (Jickells and Nergrusz, 2008) This means that it is theoretically possible for all trace of the drug to have left the body 2 hours 15 minutes after administration.

GHB is oxidised by hepatic enzymes to succinic semialdehyde and then succinic acid, this is able to enter the Krebs cycle and therefore the ultimate end product of metabolism is carbon dioxide and water. Elimination occurs mainly through the lungs in the form of carbon dioxide; however it is a urine or blood sample which is used to detect the presence of GHB. (Li *et al*, 1998)

1-5% of GHB is excreted unchanged in the urine. If the urine is particularly acidic less will be excreted. (Hornfeldt *et al*, 2002)

Endogenous GHB, which is GHB which is naturally present in the body, is found in the serum of the human body unbound to protein. The basal ganglia is where the greatest concentration of endogenous GHB is found in the human body. Binding sites are found in several parts of the brain including the cortex, midbrain and the hippocampus. Areas such the cerebellum and medulla do not contain binding sites. (Li *et al*, 1998)

1.5 Raman Spectroscopy

Raman Spectroscopy is a type of vibrational spectroscopy used to determine information on chemical structure of a substance, it is used as an identification tool and can be used to determine quantitatively or semi-quantitatively the amount of a particular substance in a sample. (Smith and Dent, 2005) Raman Spectroscopy is complimentary to IR spectroscopy in the sense that Raman highlights the covalent bonds and structural carbon elements and IR highlights the functional groups. (West and Went, 2010)

A Raman spectrum is obtained by focussing a laser of monochromatic light on the area of the sample that is being analysed. Monochromatic light refers to light of a single wavelength.(Bell, 2006) Unlike Infrared and ultraviolet spectroscopy which uses absorbed and transmitted radiation, Raman Spectroscopy uses scattered light in order to determine information about the chemical bonds of the substance in question, (Jickells and Nergrusz, 2008)

1.5.1 Basic theory of Raman Spectroscopy

The light emitted from the laser impinges on the molecule and causes the electron cloud around the nucleus to distort and create a brief state known as the virtual state. This state can quickly reverse back and is not stable. Excitation happens in the visible or near infrared range. (Smith and Dent, 2005

The photons emitted from the molecule may scatter at the same frequency and wavelength as the laser frequency and wavelength. This is what happens with the majority of photons. (Jickells and Nergrusz, 2008) This is called elastic or Rayleigh scattering. No analytical information is obtained from Rayleigh scattering. (Bell, 2006)

Not all of the photons will scatter like this however; few scatter with a frequency which differs from the laser.

When the molecule relaxes back from the virtual state by emitting a photon and it relaxes back to a higher vibrational energy, this is inelastic scattering. The

emitted photon has a longer wavelength than the incident light so therefore it has a lower frequency. This is Stokes Raman scattering. (Jickells and Nergrusz, 2008) If the molecule relaxes back to a lower vibrational state emitting a photon which has a greater energy therefore a higher frequency than the incident light, this is referred to as anti-Stokes Raman scattering. (Jickells and Nergrusz, 2008)

It is typically the Stokes region of the spectrum that is used as they are more intense. Stokes scattering occur from the higher energy which is less frequent in molecules. The spectrum is usually shown as Raman intensity versus Raman shift, Raman Intensity is the amount of photon per second and Raman Shift is the shift in frequency of the emitted photon. (Jickells and Nergrusz, 2008)

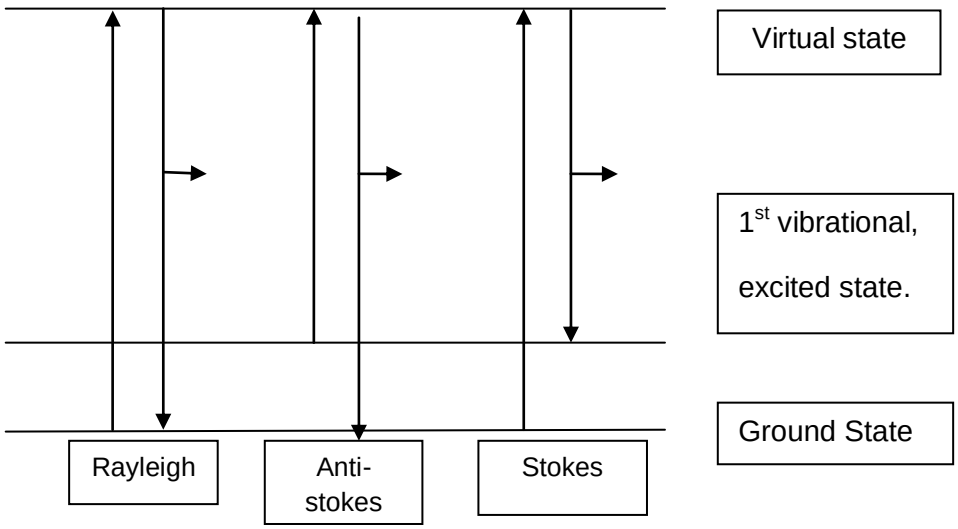

Figure 1.8. The three different types of scattering. (*Adapted from Bell,2006*)

There is no different in frequency in Rayleigh scattering. A molecule which is already in an excited state is further excited and relaxes back to the lower frequency in Anti- stokes scattering, this emits a photon of a higher energy. In Stokes scattering the molecule relaxes back to a higher frequency emitting a photon of a lower frequency.

1.5.2 Advantages of Raman Spectroscopy

Raman Spectroscopy has many favourable properties, as a technique it is easy to carry out and has a large range of uses with no or very little sample preparation required, other techniques such as GC/MS require the drug to be extracted from the sample matrix in order to be identified. (Jickells and Nergrusz, 2008) A spectrum can be obtained within seconds making it an extremely quick technique. (Smith and Dent, 2005) Analysis can be done on practically any type of sample, organic or inorganic, liquid, solid or vapour. The sample can be tiny particles or in bulk. (Smith and Dent, 2005) A Raman spectrum can be obtained from a sample as small as less than 1µm in diameter. (Jickells and Nergrusz, 2008) Another advantage of Raman spectroscopy is that it is non destructive and samples inside glass containers can be analysed reducing contamination. (Smith and Dent, 2005) As water gives a weak Raman signal, analysis of solutions or moist items can be carried out. (West and Went, 2010) Most Illicit drugs are strong Raman scatterers and give good spectra with good characteristic peaks. (Weyermann *et al*, 2011) Portable Raman spectrometers offer *in situ* analysis, which is ideal for investigating clandestine laboratories. With Raman the analysts do not need to be in direct contact with any potentially dangerous substances, it is a huge advantage if samples can be indentified without removing them from their packages. (Weyermann *et al*, 2011)

1.5.3 Disadvantages of Raman Spectroscopy

Fluorescence can be a problem when using Raman. Fluorescence can mask some weak Raman bands. (West and Went, 2010) The fluorescence can be caused by impurities present on the sample or the sample itself. Anti-Stokes scattering is preferred if this is the case as it avoids this interference. (Smith and Dent, 2005)

Another problem Raman faces is that the Raman effect is weak as only one in every 10^6–10^8 photons which scatter are Raman scattered so the instrument is required to be very sensitive and highly optimized. (Smith and Dent, 2005)

1.5.4. Surface Enhanced Raman Spectroscopy

Surfaced Enhanced Raman Spectroscopy is technique which enhances Raman Scattering. Molecules are absorbed on rough metal surfaces to create this enhancement. The technique can be used to detect a single molecule as it the enhancement factor can be as much as 10^{10} to 10^{11}. (Blackie *et al*, 2009)

As SERS is sensitive to the metallic surface employed, the shape and size of the nanoparticles used strongly affects the enhancement. The ideal size of metal nanoparticles or colloid used varies depending on what molecules are targeted. This means trial and error may have to be adopted in a SERS experiment. The enhancement effects exact mechanism is still debated in literature (Arocha, 2006)

1.5.5 Previous Research

There is a great deal of past research on the subject of detecting illicit drugs using Raman spectroscopy. Controlled substances are usually strong Raman scatterers and therefore produce characteristic peaks and informative spectra. (Weyermann *et al*, 2011) In 2003 Day *et al* carried out a study investigating the detection of drugs in latent and cyanoacrylate-fumed fingerprints; they concluded Raman spectroscopy was successful in detecting drugs of abuse in sweat rich latent fingerprints using photo bleaching to reduce fluorescence background. Sebum rich latent fingerprints presented some interfering bands however these bands did not interfere in the identification of the illicit substances. (Day *et al*, 2003) In the cyanoacrylate-fumed fingerprints the illicit substances were detected successfully as under normal sampling conditions, the sample was photo bleached in order to reduce fluorescence; interfering bands were present in the spectra due to the polymer however they did not prevent identification of drugs of abuse. (Day *et al*, 2003)

Hargreaves *et al* demonstrated that Raman Spectroscopy was able to identify a number of suspect powders in their containers were identified as illicit drugs *in situ* in an airport environment. This study was carried out using two different Raman Spectrometers. They concluded good quality spectra could be obtained

from Custom and Excise samples in 30 seconds and under providing there was not a highly fluorescent cutting agent. (Hargreaves *et al*, 2008)

Raman spectrometers involved in the detection of drugs are often Fourier transform (FT) and dispersive. FT spectrometers using a near intra red (NIR) laser and is often coupled with a microscope for trace samples. An advantage of FT-Raman is that it uses a 1064 nm laser which is less prone to fluorescence as most compound do not have excited states low enough in energy to fluoresce with this laser. This does mean scattering is weaker however. Weaker scattering results in longer detection time as the acquisition time is longer. Dispersive can use a UV, visible or NIR laser and a charge coupled detector (ccd) detector. (Smith and Dent, 2005)

One study was able to detect methamphetamine through plastic packaging such as polypropylene bags using FT-Raman with little interference from the bag. It was discovered the spectrometer could be used to distinguish between methamphetamine, amphetamine sulphate and ephedrine. It took around 1 minute for the compounds to be identified. Methamphetamine dissolved to be concealed in water or sodium chloride could be detected down to 1% (w/w) (Tsuchihashi *et al*, 1997)

Dispersive Raman spectroscopy can be used to detect MDMA, this has demonstrated good quality spectra and an acquisition of around 2 minutes using an 785 nm laser. This can be used to distinguish between isomers and bulking agents so is useful in composition analysis. (Bell *et al*, 2000)

Raman spectroscopy has been utilised in the screening of large seizures of ecstasy tablets. A study in 2000 demonstrated that Raman can be used to observe the inhomogeneity in tablets which had the same appearance and the same logo. This means that testing just one area of the tablet will not give a complete picture of what the pill contains, this much be considered when trying to obtain a full profile. It was shown that 400 of the pills could be grouped using the excipients highlighting that a batch may looked the same but can be extremely variable. (Bell *et al*, 2000) A larger study of pills was conducted in Northern Ireland. This study tested 1500 pills from a variety of sources. Acquisition time for the method used in this study was rapid at around 40

seconds. By profiling the impurities it was possible to tell if batches of pills from different seizures were likely to have come from the same source. Tablets with the same logos and appearance are assumed to be from the batch differed substantially in MDMA content and homogeneity. This reiterates the dangers of users believing they are taking the same pill as they have previously tried, this can lead to accidental overdose. (Bell *et al*, 2003)

As well as amphetamine type substances, Raman spectroscopy has also demonstrated it can discriminate between crack cocaine and cocaine hydrochloride without issues arising from common adulterants such as lidocaine and benzocaine. (Carter *et al*, 2000) A study also demonstrated that using thin layer chromatography (TLC) can separate out mixtures and spectra can be obtained from the TLC plate (Angel *et al*, 1999)

Weyermann *et al* used a portable Raman spectrometer to investigate controlled substances *in situ* at border controls. They found it to be a good screening device for powders and liquids due to it non-destructive nature and its ability to penetrate containers. It was noted that in order to acquire good spectra for illicit drugs dissolved in water or other liquids, the concentration of the drug, in most cases, has to be very high, therefore it is not sufficient for trace analysis. Weyermann *et al* observed that focalisation was very important in obtaining good quality spectra, this study used a probe and it was found that the focalisation varied from drug to drug, therefore a standard distance from the container was not adopted. The probe was pressed against the container then moved gradually away from it until optimal distance was found. (Weyermann *et al* 2011)

Burnett *et al* conducted a study into the concealment of cocaine in bottles of Rum for smuggling purposes. It was noted that confiscated samples typical contain between 50%-80% cocaine w/v. This study used a portable 785nm Raman as well as a 1064nm bench top instrument. The glass of the bottles had two chances to react with the radiation as the beam must travel through the glass to the sample then back through again to the detector doubling the opportunity to affect the spectra. The colour of the glass also affects the spectra, green glass will fluoresce at 785nm.

The study showed that colourless and brown glass give good results down to a concentration of 6% w/v, Plastic bottles had the same result. Green glass produced a spectra which completely masked the cocaine peaks on the 785nm instrument however the FT-Raman gave clear cocaine peaks down to 6% w/v.

The study concluded that cocaine in concentrations of 8%w/v and above in rum can be identified and therefore concentrations of 50%-80% should present no problem. (Burnett *et al* 2011)

The detection of drugs in drinks is also very useful in the prevention or evidence relating to drug-facilitated sexual assaults. GHB is heavily associated with drink spiking as it is difficult to detect and its sedative effects are exacerbated by alcohol making it an ideal date rape drug. A study demonstrated that GHB and its precursor GBL can be identified in alcoholic drinks in a number of containers such as glass and plastic using Raman spectroscopy. GBL is as important to detect as GHB as when in solution the two inter-convert. The study was able to detect the drugs lower than the common dose.(Brewster *et al*, 2009) However this study was limited as it did not include drink mixers such as soft drinks.

Surface enhanced Raman spectroscopy (SERS) has also demonstrated its use in the detection of drugs. Studies have shown it can be an effective way to identify amphetamine powder and ecstasy pills. (Sägmüller *et al*, 2001). The active ingredient of the drug was extracted using cyclohexane, this extraction worked well as components such as the colouring in the tablets were not present in the cyclohexane phase. This means there was less interference or fluorescence from excipients. SERS has also been utilised successfully for the detection of 2,5-dimethoxy-4-bromoamphetamine (DOB) which is potent at low doses. The use of a silver colloid enables DOB to be detected in a tablet down to a concentration of 15 ug. However if this technique cannot to used if MDMA is also in the pill as MDMA is present at a much higher concentration and the enhancement will have effected both compounds. The MDMA signal will drown out the weak DOB signal. (Bell *et al*, 2007) Another study showed that comparing the SERS spectra of drug heroin, methamphetamine and methadone users with non-users, a characteristic peak at 1030 cm-1 was present in the

users but not in the non users therefore users could be swiftly identified non-invasively (Anyu *et al*, 2009)

1.5.6 Previous research by the Author

In a previous study carried out by the author, KGHB, that is GHB manufactured in a clandestine laboratory using potassium hydroxide as a starting material, was detected in ethanol and some alcoholic drinks to a concentration as low as 3% v/v using a bench top DXR Raman Spectrometer. NaGHB (manufactured using sodium hydroxide as a starting material) was not detected in alcohol using a DXR Raman Spectrometer. GBL, GHB precursor, in alcohol was detected down to a concentration as low as 0.25% v/v. This study did not include mixers in any of the alcoholic drinks tested which is a great limitation, most people do not drink spirits straight and the addition of mixers increases the volume of the drink therefore diluting the drug further. Figure 1.9 shows a neat ethanol spectrum compared to an ethanol spiked with KGHB spectrum. The 930cm-1 peak was what determined the presence of KGHB in ethanol.

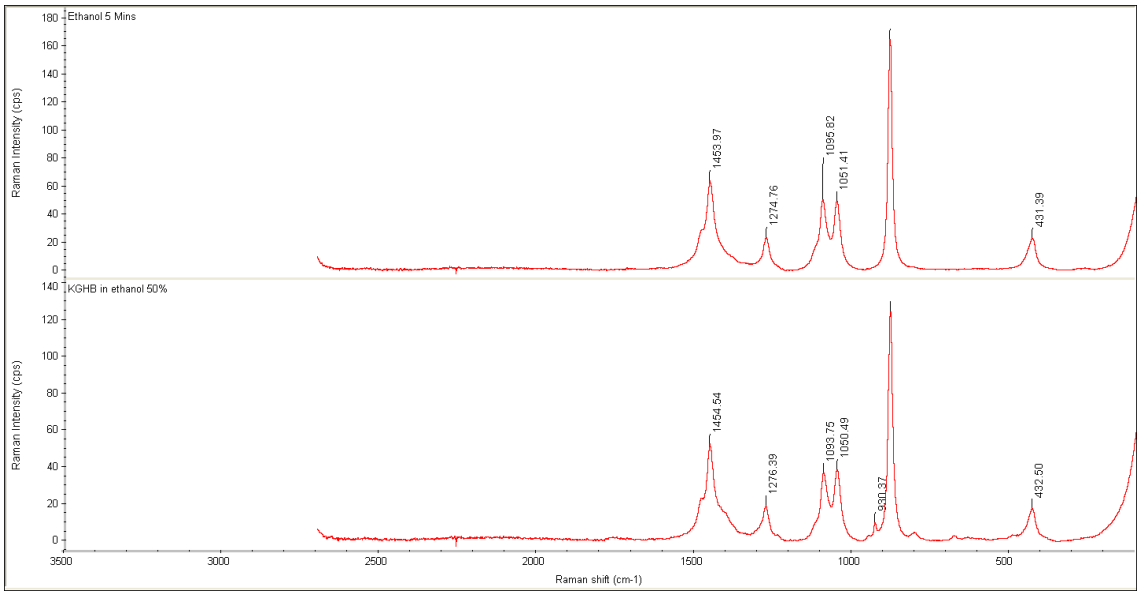

Figure 1.9: KGHB in Ethanol compared to neat ethanol spectra

Figure 1.10 demonstrates the limit of detection of KGHB in ethanol, it shows that the 930cm-1 peak was present at 3% v/v but could not be detected at a lower concentration.

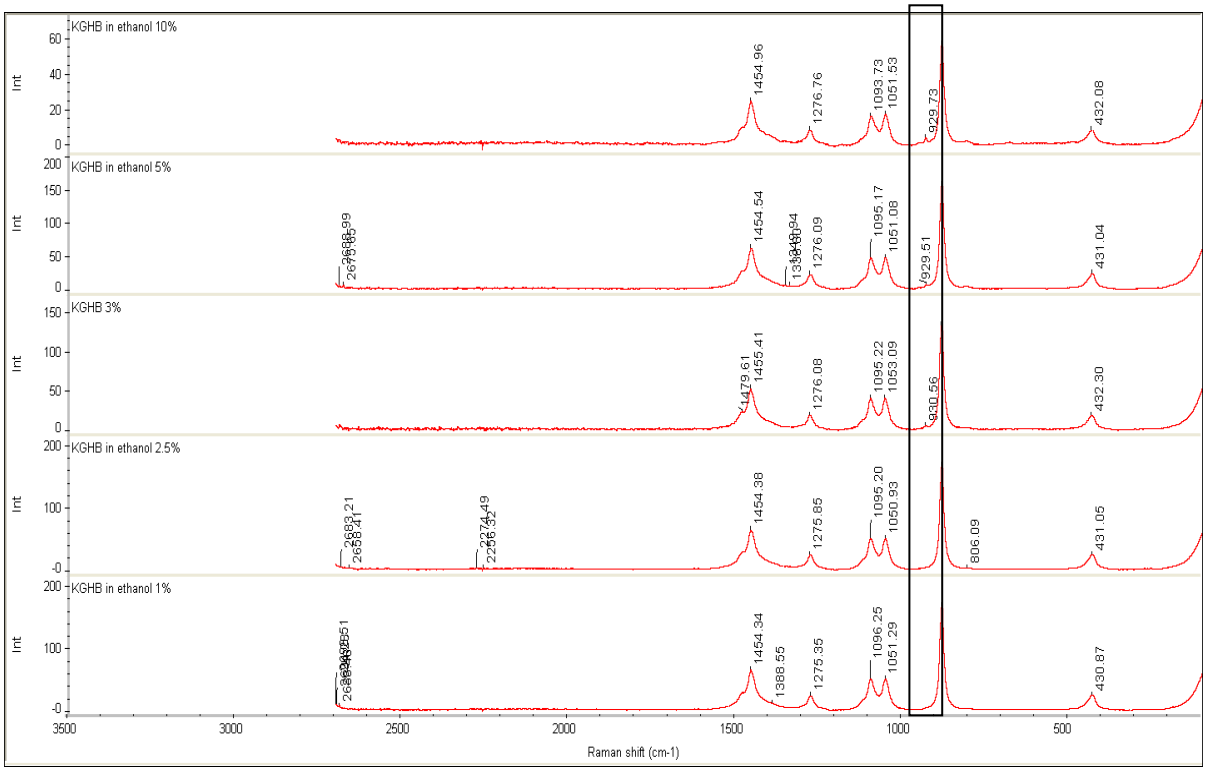

Figure 1.10: KGHB limit of detection in ethanol.

This study recognised that KGHB and GBL had very similar peaks. Table 1.1 demonstrates this by showing a comparsion of the peaks both substances have in common.

Table: 1.1: KGHB and GBL peak comparison

Peaks in common (cm^{-1})	
KGHB	**GBL**
1039	**1038**
931	**931**
875	**870**
803	**801**
680	**675**
496	**491**

This led to the conclusion that KGHB is likely to be not fully unconverted from GBL unlike NaGHB which has been completely converted. This is backed up by the presence of the 803cm^{-1} peak as this is probably a ring structure, GHB does not have a ring in it structure but GBL does.

1.6 TruScan Background

This study used a portable Raman Spectrometer called TruScan. This device was designed by a company called Ahura Scientific which Thermo Fisher Scientific bought over in 2010. (McBride, 2010 www.xconomy.com) The user manual produced by Ahura Scientific claims the instrument is light and portable as the unit weighs less than 4lbs, rugged and can be used in the field, sampling is easy as non contact analysis is possible which improves safety and reduces contamination of the sample, method development is fast and the device can easily be used by non technical staff. (TruScan User Manual, 2010)

Ahura Scientific and now Thermo Fisher Scientific claim that with TruScan and TruScan products “No matter how complex your raw material identification challenge, we put the solution in the palm of your hand.” This sounds promising but as illegal drugs are a mixture of a variety of substances, adulterants and

cutting agents, the device would need to be able to identify the illicit drug in these mixtures if it was to be used in drug identification based on its PASS/FAIL system which allows non technical users to use the instrument.

1.6.1 How TruScan works

TruScan shines a 785nm laser on the sample to receive a Raman measurement. The software which analyses the spectra and gives the PASS/FAIL result is a patent-pending package called DecisionEngine. DecisionEngine is designed to eliminate false identification, compare sample spectra to saved methods and if there is no significant Raman discrepancy then a PASS is reported, when there is a discrepancy a FAIL is reported. A p-value of 0.05 or greater passes the method. A p-value of 0.001 and 0.05 are at moderate risk of passing as the sample has similar characteristics to the method. p-value is used to reject or confirm a hypothesis. It is a measure of probability. For clarity, the TruScan names a reference spectrum, “a method.” This should not be confused which the experimental method.

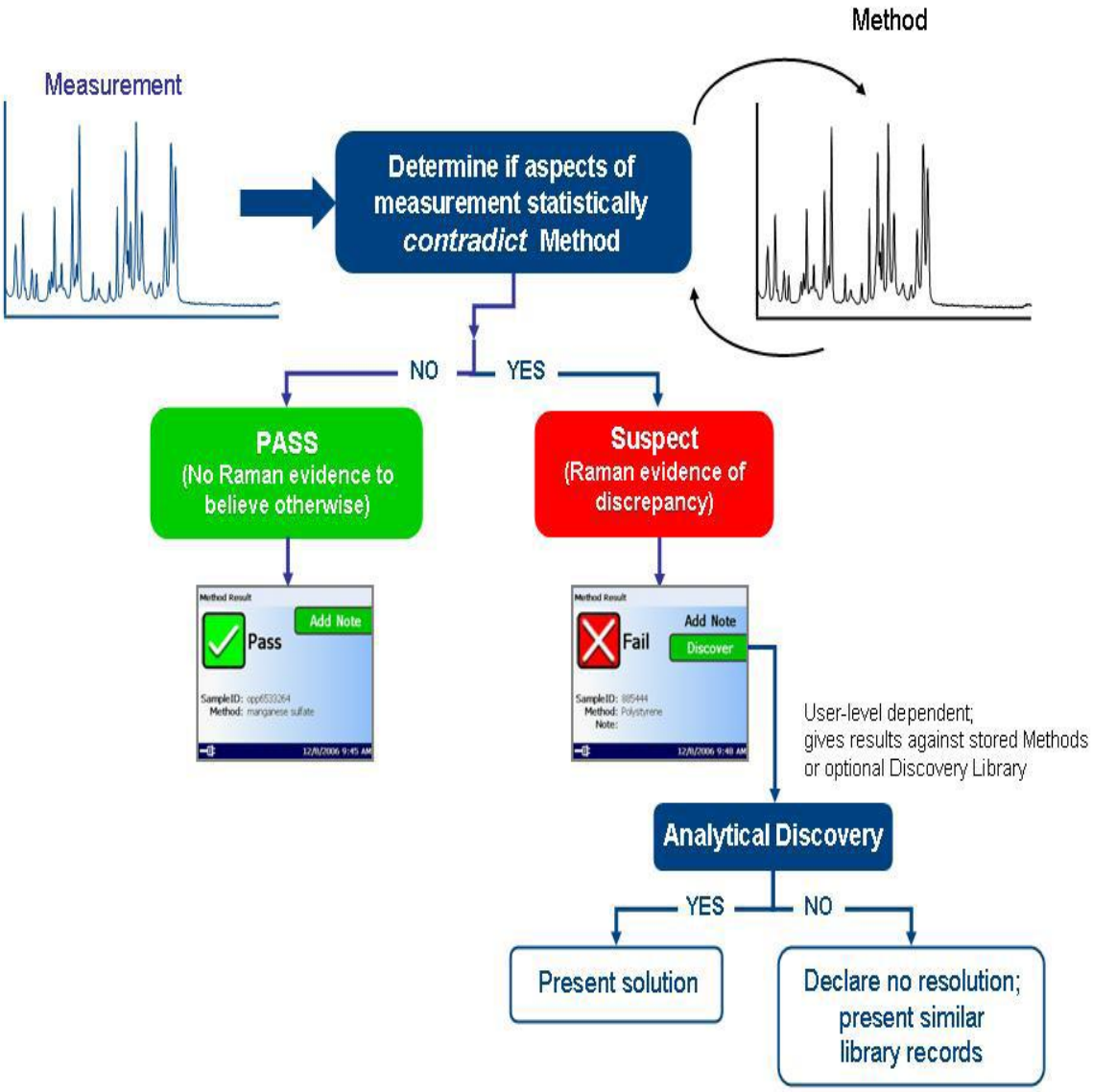

Figure 1.11: TruScan PASS/FAIL System (TruScan user manual, 2010)

1.7 Oral fluid

Oral fluid is defined as the fluid within the oral cavity; this is made up mostly of saliva, the secretions of the saliva glands but is also made up of small amounts of cellular debris, blood, food debris and gingival crevicular. Oral fluid is primarily secreted by three glands called the parotid, submaxillary and sublingual as well as other small glands. Various factors affect the flow of saliva such as emotional state, hunger and drug use. Saliva flow ranges from 0 to 6

mL per minute. Oral fluid can be tested to detect recent drug use. This is very relevant for testing motorists as only recent drug use is of interest. Oral fluid will not replace the need for urine drug testing where in cases a more historic view is required or hair drug testing in cases where a long term picture of drug use is required. Specimens collected by expectoration and by placing absorbants in the oral cavity are defined as oral fluid specimens. (Jickells and Nergrusz, 2008)

1.7.1 ROSITA

The first Roadside Testing Assessment (ROSITA) discovered that oral fluid was the most promising alternative specimen compared to sweat and urine, for a roadside drug screen. Urine testing gave satisfactory results as overall accuracy was over 95% and sensitivity and specificity was over 90% compared with a reference method but no device scored highly for all drug categories. Urine testing would require facilities such as a sanitary van to be able to take the sample at the roadside.

Most countries who took part in ROSITA preferred Oral fluid testing, with only one country favouring urine and one country favouring sweat. (Verstraete and Raes, 2006)

ROSITA 2 concentrated on oral fluid only. It states for a test to be fit for use it must be 95% accurate and over 90% sensitive and specific. All 9 devices tested in ROSITA 2 fell short of this and a very high number of failures were reported with some devices. This may have due to the viscosity of the saliva tested or the malfunctioning of the device. (Verstraete and Raes, 2006)

1.7.2 Advantages of oral fluid

A huge advantage of oral fluid drug testing is that it is quick and non invasive. (Drummer, 2006) Only small samples are required and can be analysed by LC-MS-MS, this adds the high sensitivity and specificity of a mass spectrometer to the liquid chromatograph. This technique is a confirmatory technique but can also be used as a preliminary test for drugs. (Drummer, 2006) This includes kits that can be used for onsite drug testing. Drug testing at the side of the road allows police offers to confidently arrest those driving under the influence and

reduces expensive laboratory analysis. This also eliminates whose who are not under the influence with minimal inconvenience. (Verstraete, 2005)

Another advantage of oral fluid is there is a relationship between oral fluid concentration and blood/plasma concentration. This can be calculated using the pH of the oral fluid and blood, the pKa of the drug and the protein binding of drug. The equilibrium favours blood for the acidic drugs and favours oral fluid of basic drugs. (Drummer, 2001) Theoretical ranges for some drugs have been calculated. For example the saliva: plasma for cocaine varies from 2.73-0.44, as saliva pH varies from 5.0-7.8. (Jickells and Nergrusz, 2008) Oral fluid is collected under direct supervision so this eliminates the opportunity to adulterate the sample.

1.7.3 Disadvantages of oral fluid

Stimulating the production of oral fluid by chewing gum or other agents will alters the pH and therefore the concentration of the drug. Stimulating oral fluid has been shown to reduce the concentration of the drug from two to four fold for methamphetamine and five-fold for cocaine. (Hillsgrove *et al*, 1993)

Some drugs such as MDMA reduce the secretion of oral fluid. This can mean that collecting just 1mL of oral fluid can take up to several minutes. Having a dry mouth due to improper hydration or the anxiety of the test can also hinder the process of drug testing in this way. The ROSITA project also noted that sometimes oral fluid was too viscous and therefore could not be used with some devices. Sometimes in this case a different sample such as blood may have to be taken. (Drummer, 2006)

An article published in 2011 details a study carried out by Real-Time Analyzers Inc, an American company that make, design and market Raman Spectrometers, establishing a method of detecting drugs in saliva for the use of identifying offending overdose drugs in a hospital setting. They successfully employed SERS to identify numerous drugs in saliva at the ng/mL concentration within 10 minutes. A Solid Phase Extraction capillary was combined with SERS

active capillary connected to a syringe driven sample system. A portable Raman Spectrometer was used. (Farquharson *et al* 2011)

1.8 North Review

The North review of the drink and drug driving law was published in 2010. It contains recommendations for the advancement of drug driving procedure. The second recommendation is that the government should commission more research into the prevalence of drug driving. Recommendation 11 states that type approval of an oral fluid device based on immunoassay or other test should be established quickly for use in police stations as a preliminary screen and used in accordance with the Road Traffic Act 1988. This would eliminate the need for a doctor or nurse to take the sample. Further blood or urine confirmatory tests would be carried out after a positive result in oral fluid. The review states that this process in police stations should be achieved within two years. (North, 2010)

Recommendation 17 then takes this further and states that the government should continue this technology and work on type approving a screening device to be used at the roadside. A positive result at the roadside would result in the individual being arrested then they would be obliged to provide a blood or urine specimen for confirmatory testing.

The review considers that the device may only be suitable in a controlled setting such as the police station and the issue of environmental contamination must be addressed and overcome. Other relevant recommendations from the North review include recommendation 13, 14 and 15. Recommendation 14 considers what levels of drugs and their active metabolites should be considered impairing and these prescribed levels should be included in the legislation. Recommendation 14 states that if the driver if found to have drugs in their body above the prescribed limit as they had taken a drug in accordance with medical advice, a stationary defence should be available. Recommendation 15 states that if no scientific census on the impairment level can be reached, a policy of zero tolerance should be introduced. (North, 2010)

1.9 Aim

The aim of this study is investigate the use of Raman Spectroscopy as a screening method for drugs of abuse. Raman Spectroscopy was chosen as in published literature it is praised for its quickness and its minimal sample preparation i.e. No extraction step required. It must be established if Raman can qualitatively detect drugs of abuse in mixtures and in oral fluid for individual drug testing which could be applied to suspected drug drivers. The drugs used in this study were chosen for their stimulant effects which are likely to cause risky behaviour such as driving under the influence. GHB was chosen as it is a notoriously difficult drug to detect in the body and may be administered to for DFSA. Previous work by the author can also offer a brief comparison between portable and bench top Raman instruments.

Chapter 2: Materials and Method

2.1 Materials

The drugs used in this study were purchased from Sigma Aldrich. The drugs used were cocaine hydrochloride, amphetamine sulphate, ketamine hydrochloride, methylenedioxyethylamphetamine hydrochloride (MDE), 3,4-methylenedioxymethamphetamine Hydrochloride (MDMA) and GBL, benzocaine and caffeine, also purchased from Sigma Aldrich were also used.

All the GHB used in this study was synthesised using an altered recipe obtained from the internet as the experiment aimed to mimic drinks spiked by GHB produced in a clandestine environment. The recipe was obtained from www.erowid.org after typing 'GHB recipes' into a Google search engine. (See Appendix 1)Potassium hydroxide was used for the synthesis. The GHB was not bought commercially

The GBL was obtained from Sigma-Aldrich. The colloid was obtained from Thermo Scientific.

All drugs used in this study were purchased, made and stored under the strict home office conditions detailed in the previous chapter. The university has a license from the home offence which states these conditions.

2.2 Instrument

The device used in this study was a Thermo Scientific TruScan Raman Spectrometer. It operated using a 785nm laser with a maximum power output of 300mW. It has a spectral range of 250 cm^{-1} to 2875 cm^{-1} . Spectral data was transferred to a PC with OMNIC software using a data card.

Figure 2.1: TruScan and DXR

The purpose of this figure is to demonstrate the size difference between the two instruments.

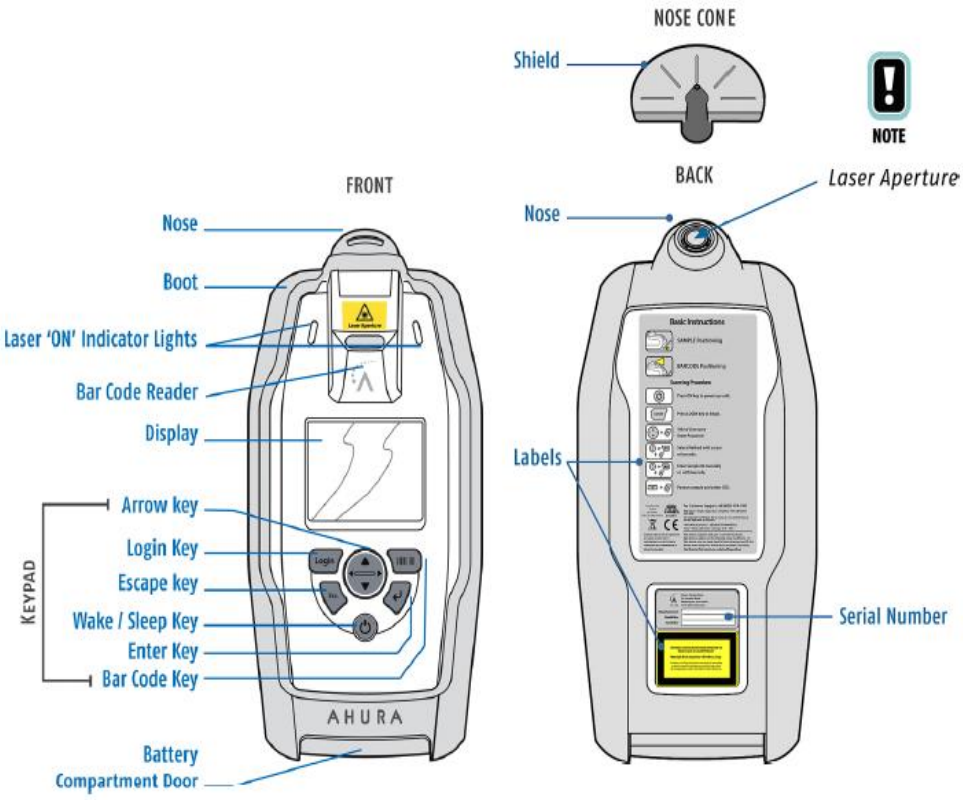

Figure 2.2: TruScan Portable Raman Spectrometer. (TruScan user manual, 2010)

2.3 Creating a Library

In order to use the TruScan Raman Spectrometer as an identification tool a library of materials was created. This was achieved by following the devised standard operating procedure, see appendix 2.

2.4 Production of liquid GHB

120 mL of GBL was added to a Pyrex glass container with 91 grams of Potassium hydroxide. 250 mL of warm distilled water was then added and the container was covered for the reaction to take place. The solution was slowly heated for an hour on a hot plate, taking care not to overheat or burn the solution. The solution was topped up with water to 1000 mL and 50-75 mL of vinegar was added until the pH was below 7.5.

2.5 KGHB in alcohol

To compare the TruScan device to the DXR Raman microscope used in a previous study, 0.5 mL of KGHB was added into a vial containing 1 mL of ethanol, this was mixed for 30 seconds using a vortex mixer, then analysed using the vial attachment on the TruScan.

2.6 Mixture Analysis

A 0.25 g white powder was made by mixing 0.125 g cocaine and 0.125 g benzocaine together. A Method for both cocaine and benzocaine was saved on the TruScan. A Run was carried out on this mixture sample using the above Standard Operating Procedure.

0.125 g of paracetamol and 0.125 g caffeine were added to this mixture producing a white powder with the weight of 0.5 g with 25% (w/v) of this being the illicit substance cocaine. This is a crude representation of what a street sample could consist of. Smith and Dodd reported that the mean purity of cocaine seized on the UK Street is 33% (Smith and Dodd, 2009) A method (i.e. reference spectra) for paracetamol and caffeine was saved on TruScan. This 0.5 g mixture was analysed using the TruScan SOP. Both 0.25 g and 0.5 g runs were carried out 4 times, with the laser focussed on a different area of the powder each time.

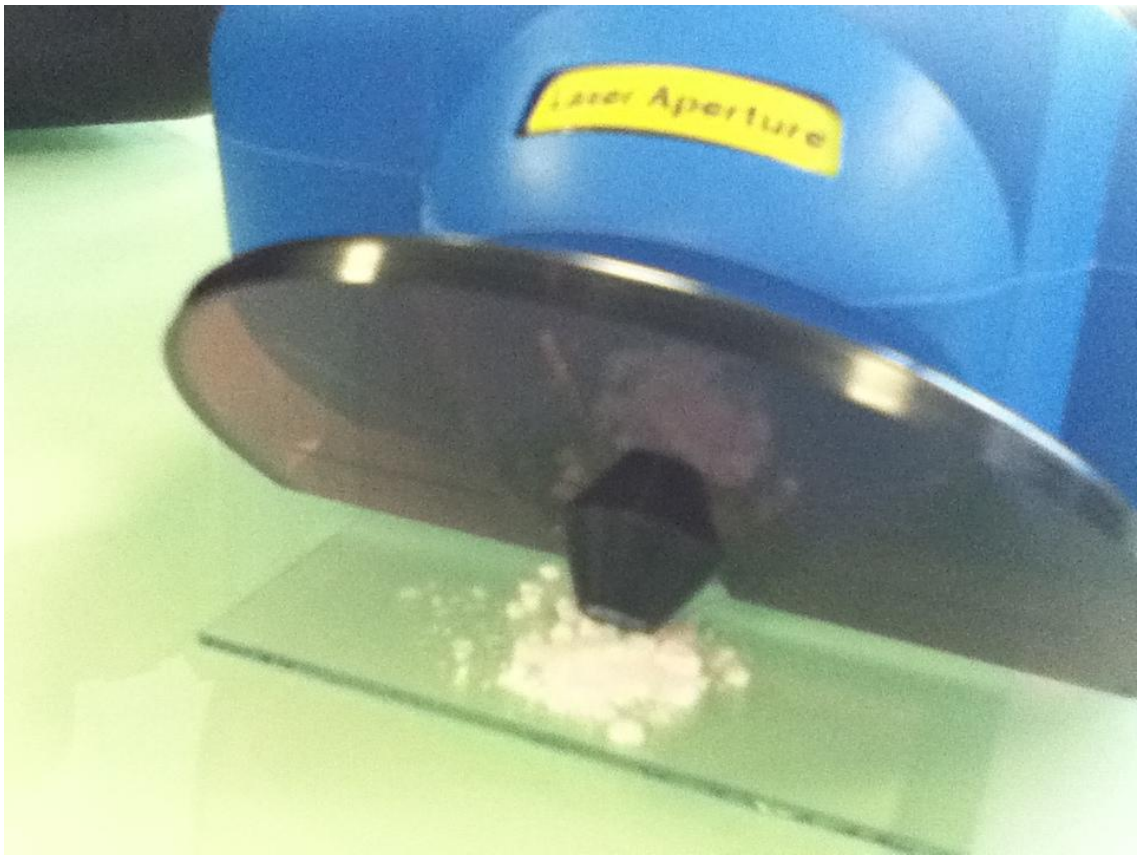

Figure 2.3:TruScan focussed on white powder. This figure demonstrates how the powder was presented to the TruScan device. It was put onto a glass slide, with all the powder in one concentrated area of the slide and the nose cone was very close to the powder when a run was carried out.

2.7 Oral Fluid Analysis

The Oral fluid was collected from the donor by expectoration which means the oral fluid was spat out into a beaker. The oral fluid was collected over a period two days and 10 mL was collected. The oral fluid was stored in a sealed container in the fridge. The author was the donor for ease and to be sure no drugs were present in the donors body. The donor was not permitted to provide oral fluid within 10 minutes of eating or drinking. Litmus strips were used to regularly check the pH of the oral fluid. The oral fluid was disposed off and re-collected if there were any pH changes.

1 mLl of KGHB was used to spike 1 mL of oral fluid; this was mixed for 10 seconds using a vortex mixer. This was ran using the TruScan SOP and the vial attachment.

2 mg of cocaine hydrochloride was used to spike 1mL of oral fluid; this was mixed for 10 seconds using a vortex mixer. This was analysed by following the TruScan SOP and using the vial attachment.

This was repeated for amphetamine sulphate and ketamine hydrochloride.

2.8 SERS sample preparation and method

A 0.1M sodium acetate buffer was prepared for the SERS method. This was produced by dissolving 6.8g of sodium acetate in 400 mL of distilled water; Hydrochloric acid was used to adjust the pH. The volume was made up 500 mL using distilled water.

The final SERS sample preparation decided upon was to spike 0.5 mL of drug in 0.5 mL of oral fluid, this was mixed with 1mL of buffer for 30 seconds. 40 µl of this mixture was pipetted into an eppendorf tube, 40 µl of 29 nm gold colloid was added and this was centrifuged for 10 minutes at 14000 RPM. 40 µl of the pellet at the bottom of the eppendorf tube was pipette into a dimple tray and the pointed nose cone was attached to the Truscan device before analysis.

Many variations of this method were attempted including different sizes of colloid (51,64, 90 nm) Different nose cones used and no nose cone attached. Sampling from the supernatant instead of the pellet after centrifugation was also attempted. For the Truscan run different methods attempted included drying the sample on a glass slide at room temperature, pipetting the sample into a capillary tube and focussing the laser down the length of the capillary tube or focussing it through glass of the capillary tube. The glass slide and capillary tube gave the same large glass hump with no peaks present in the spectra so were not suitable.

Chapter 3: Results

Raman Spectroscopy causes vibrations which produce spectra highlighting the covalent bonds and the structural carbon elements of the molecule. This can be used to give a “chemical fingerprint” of the molecule

Comparing a spectrum to reference spectra is how a substance is identified. The numbers assigned to each peak assists in the identification.

3.1 Creating a Library

The library of chosen drugs was successfully created; this was confirmed by comparing all spectra to spectra in the literature. Weyermann *et* al attributed peaks at 1003cm^{-1} and 1022 cm^{-1} to cocaine which corresponds to the double peak in the spectrum in figure 3.1.The reference spectra’s or Methods were all saved successfully. See appendix 2 for reference spectra for all drugs used in this study including details of the corresponding peaks in mentioned in the literature.

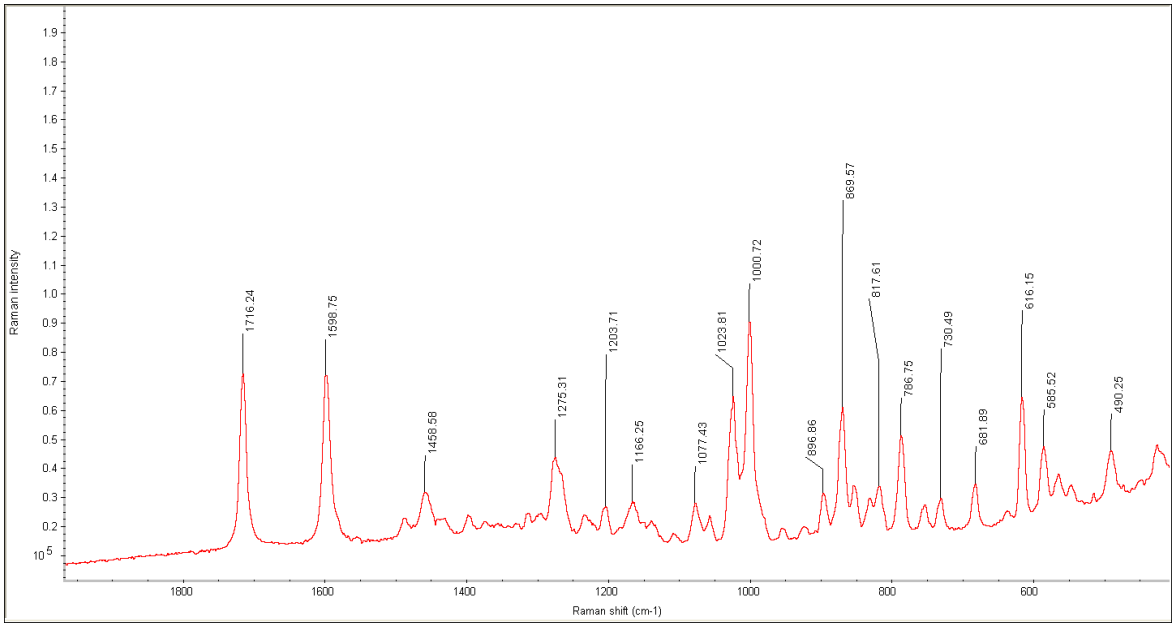

Figure 3.1: Cocaine Hydrochloride reference spectra.

3.2 KGHB in Alcohol

50% v/v KGHB in ethanol was not detected using the TruScan device. KGHB can be detected in ethanol using a DXR bench-top Raman Spectrometer as a peak at 930cm^{-1} is evident. However this peak is more characteristic to GBL. GBL and GHB are equilibrium when in a liquid. Brewter *el al* considers peak 931cm^{-1} to be GBL. (Brewster *et al*, 2009)

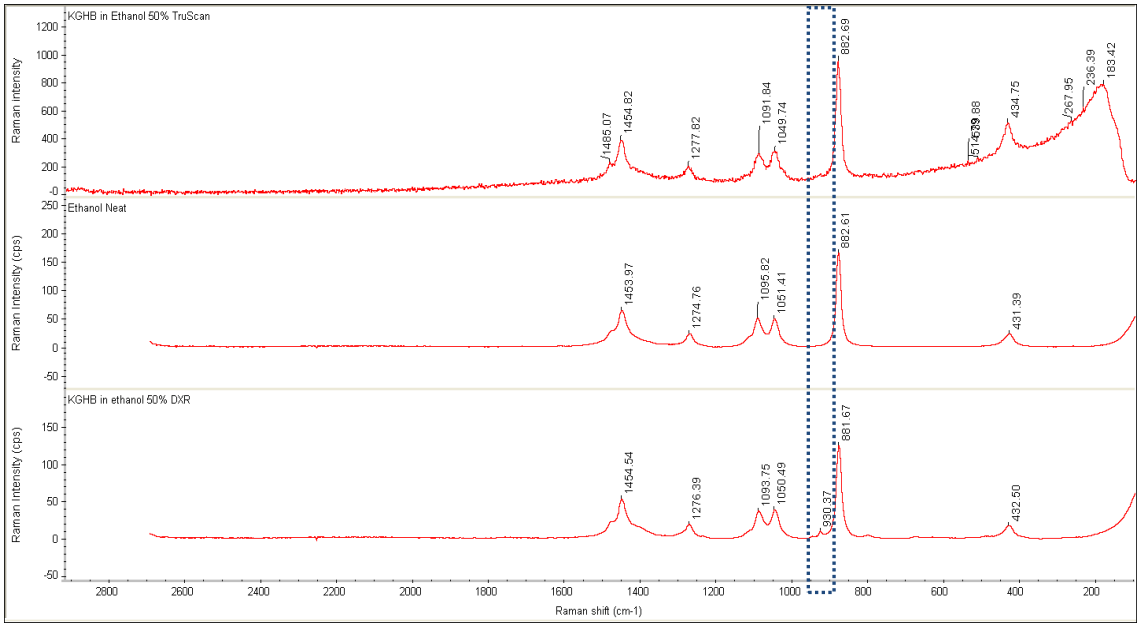

Figure 3.2: KGHB and Ethanol comparison

3.3 Mixture Analysis

The TruScan device identified all three runs of the 0.25g powder composed 0.125g of cocaine and 0.125g of benzocaine as benzocaine. This means it matched the mixture spectra to the benzocaine spectra contained in its library only. It did not report a match to the cocaine spectra in its library.

Table 3.1 lists the peaks in the neat cocaine and benzocaine spectra and the peaks that occurred in each run of the mixture. The lists allow the peaks to be compared easily and common peaks to cocaine and benzocaine can be identified quickly.

Table 3.1: Cocaine and benzocaine mix run results.

Cocaine	Benzocaine	Run 1	Run 2	Run 3
1716.24	-	1713.79	-	1716.77
-	1682.47	1682.46	1683.52	1682.34
-	1604.67	1605.45	1604.77	1604.66
1598.75	-	-	-	-
-	1575.63	1575.46	-	1574.47
1458.58	-	-	-	1433.31
-	1447.40	1446.65	-	-
-	1368.74	1367.65	-	1367.96
-	1311.21	1311.19	1312.41	1310.55
-	1281.76	1281.98	1281.94	1280.41
1275.31	-	-	-	-
1203.71	-	-	-	-
-	1172.14	1171.57	1173.16	1171.95
1166.25	-	-	-	-
-	1111.33	1110.50	-	-
1077.43	-	-	-	1075.75
1023.81	-	-	1023.76	1024.33
1000.72	-	999.17	1000.71	1001.71
896.86	-	-	-	-
869.57	-	-	-	-
-	864.05	863.13	863.29	863.64

817.96	817.61	817.12	820.27	818.50
786.75	-	788.16	-	785.63
730.49	-	-	-	-
681.89	-	-	-	-
-	640.25	-	639.32	-
616.15	-	615.40	616.72	616.13
585.52	-	-	-	-
-	506.16	506.05	508.05	505.78
490.95	-	-	-	-
-	402.10	-	400.23	-
372.33	-	-	-	-
359.26	-	-	-	-
342.67	-	-	-	-
-	317.53	317.89	318.17	317.74
281.85	-	-	-	-
	158.74	-	-	-

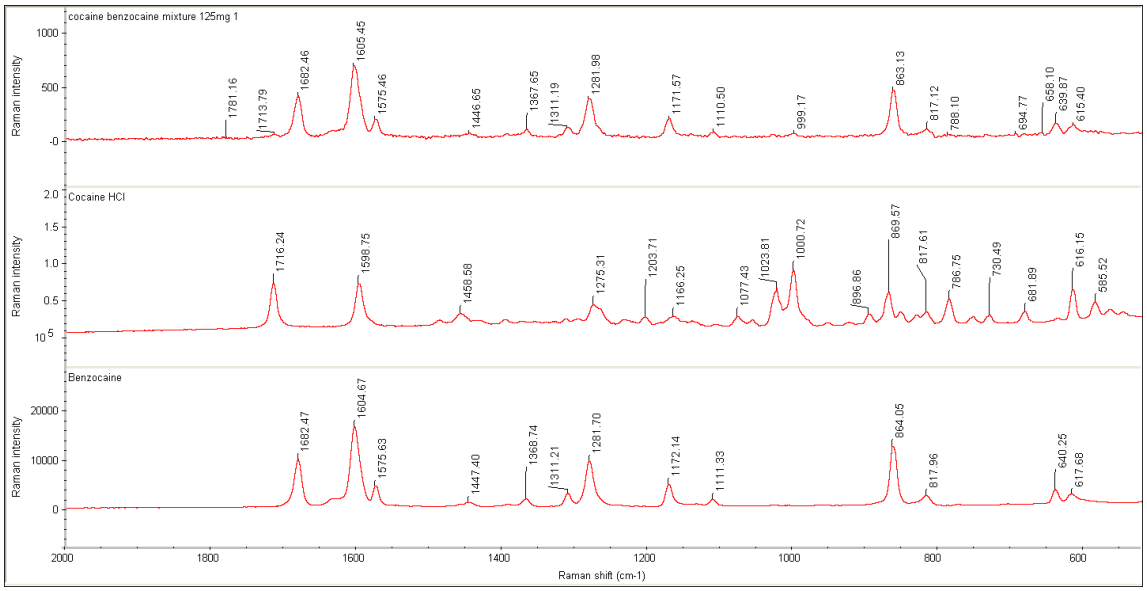

Figure: 3.3: Cocaine, benzocaine mix 1 compared with cocaine and benzocaine reference spectra.

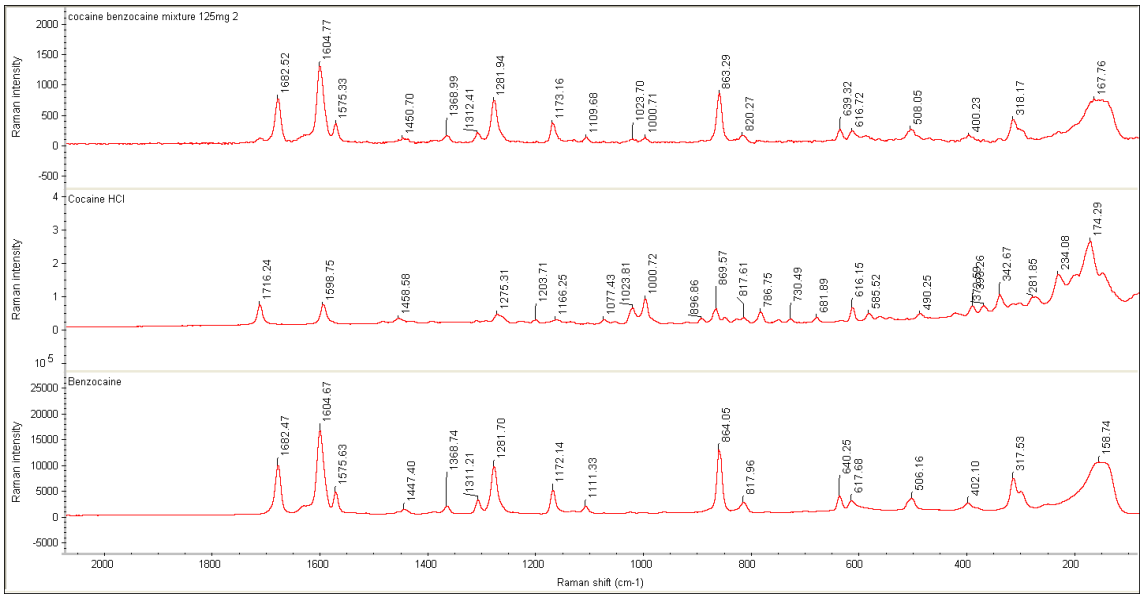

Figure: 3.4: Cocaine, benzocaine mix 2 compared with cocaine and benzocaine reference spectra.

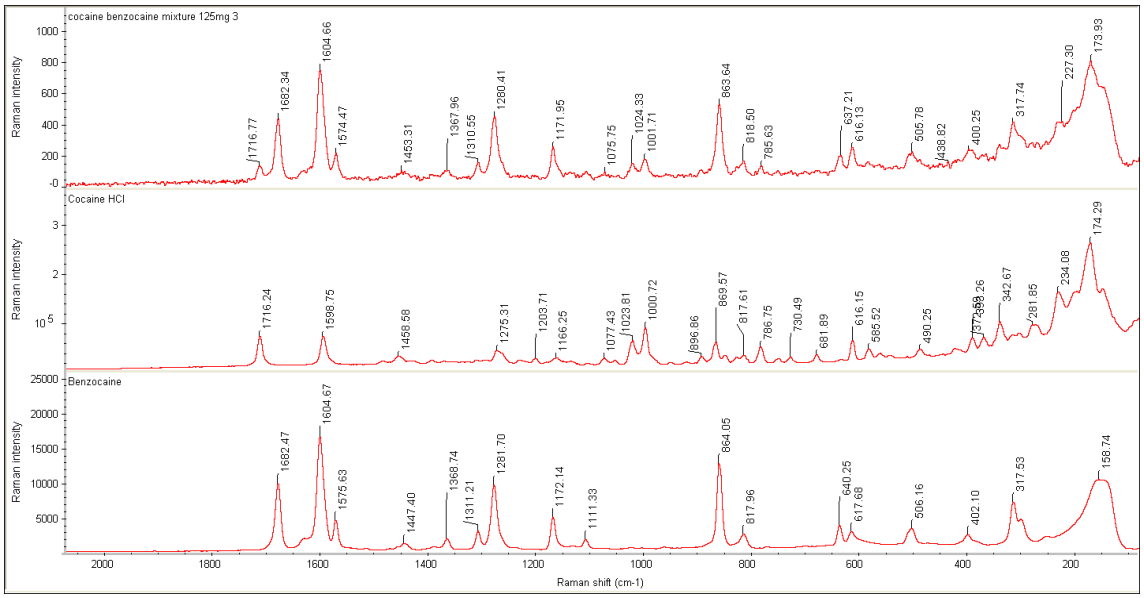

Figure: 3.5: Cocaine, benzocaine mix 3 compared with cocaine and benzocaine reference spectra.

The Truscan device identified all three runs of the mixture composing of 0.125g cocaine hydrochloride, 0.125g benzocaine and 0.125g caffeine as benzocaine. This means it matched the mixture spectra to the benzocaine spectra contained in its library only. It did not report a match to the cocaine or caffeine spectra in its library.

Table 3.2 lists the peaks in the neat cocaine, benzocaine and caffeine spectra and the peaks that occurred in each run of the mixture. The lists allow the peaks to be compared easily and common peaks to cocaine and benzocaine can be identified quickly.

Table 3.2: Cocaine, benzocaine, Caffeine mix run results

Cocaine	Benzocaine	Caffeine	Run 1	Run 2	Run 3
1716.24	-	-	1715.01	1715.66	1715.02
-	-	1698.99	-	-	-
-	1682.47	-	1682.50	1682.33	1682.24
-	-	1655.88	-	-	-
-	1604.67	-	1604.08	1602.77	1604.78
1598.75	-	-	-	-	-
-	1575.63	-	1574.81	1574.74	1574.99
-	-	1555.69	-	-	-
1458.58	-	1459.02	1462.91	1460.10	-
-	1447.40	-	1440.78	-	1447.30
-	-	1407.67	-	-	-
-	1368.74	1360.37	-	1370.88	1369.36
-	-	1328.63	-	-	-
-	1311.21	-	1310.91	1310.71	1311.17
-	1281.76	1285.37	1281.66	1280.81	1281.43
-	-	1241.25	-	-	-
1275.31	-	-	-	-	-
1203.71	-	-	-	-	-
-	1172.14	-	1170.64	1170.77	1171.71
1166.25	-	-	-	-	-
-	1111.33	-	1109.11	-	1112.09

1077.43	-	1071.81	-	1077.62	-
1023.81	-	1022.82	1022.81	1023.29	1023.66
1000.72	-		1001.57	1000.90	1002.12
-	-	928.58	929.54	-	-
896.86	-	-	893.64	896.01	-
869.57	-	-	-	-	-
-	864.05	-	863.45	863.00	864.02
817.96	817.61	-	818.73	819.90	818.23
-	-	802.13	-	-	-
786.75	-	-	781.95	787.61	-
-	-	741.75	742.28	736.98	-
730.49	-	-	-	-	-
681.89	-	-	-	683.33	-
-	640.25	645.15	640.04	639.32	639.78
616.15	-	-	614.97	616.86	616.42
585.52	-		-	585.06	-
-	-	557.99	557.57	558.06	557.90
-	506.16	-	505.39	507.44	507.40
490.95	-	-	-	487.52	-
-	-	484.45	482.70	-	-
-	-	445.41	445.28	450.77	-
-	402.10	-	-	-	402.94
-	-	391.26	397.46	394.77	-

372.33	-	369.28	-	373.35	-
359.26	-	-	-	-	-
342.67	-	-	-	342.87	-
-	317.53	315.16	317.71	318.49	317.71
281.85	-	-	-	282.04	-
-	-	224.95	-	-	-
174.29	-	-	-	173.41	-
-	-	168.26	168.91	-	166.36
	158.74		-	153.14	-

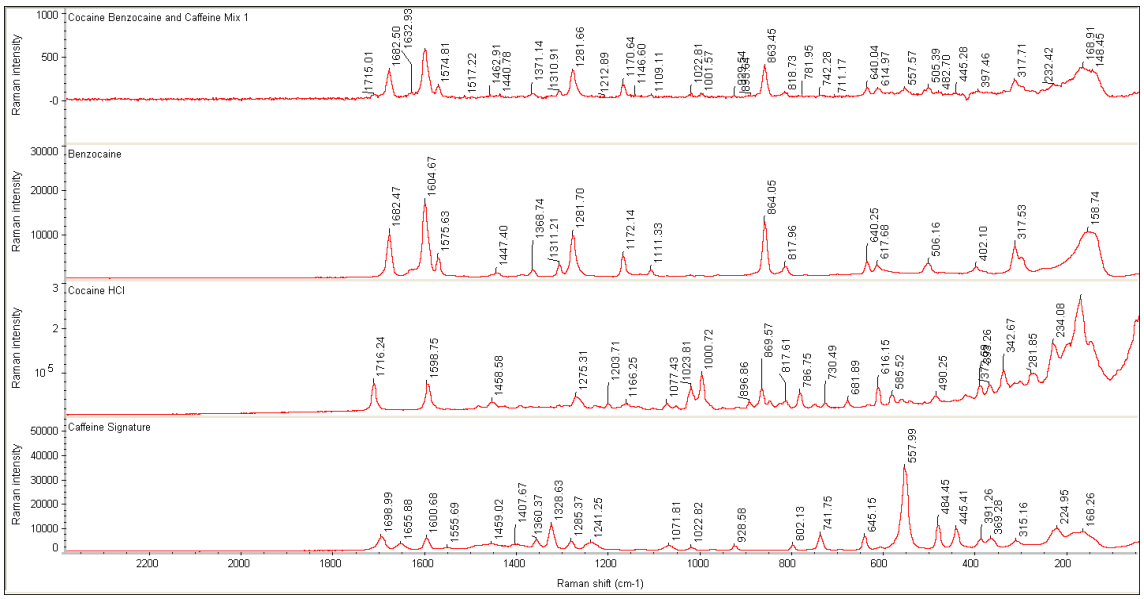

Figure: 3.6: Cocaine, benzocaine, caffeine mix 1 compared with reference spectra

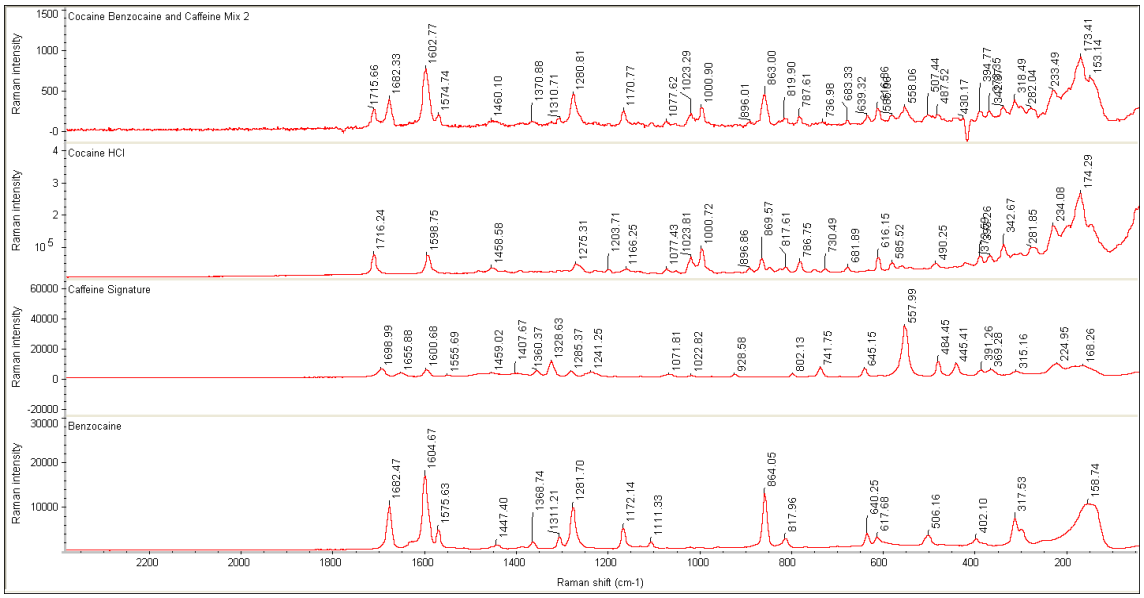

Figure: 3.7: Cocaine, benzocaine, caffeine mix 2 compared with cocaine and benzocaine reference spectra.

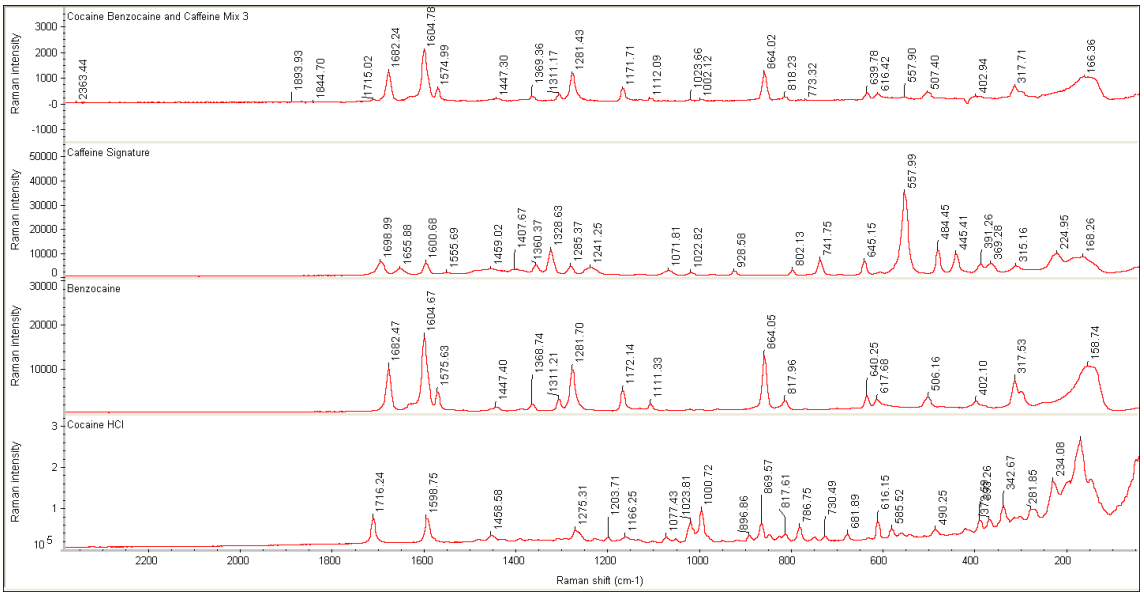

Figure: 3.8: Cocaine, benzocaine, caffeine mix 3 compared with reference spectra.

The Truscan device identified all three runs of the mixture composing of 0.125g cocaine hydrochloride, 0.125g benzocaine, 0.125g caffeine and 0.125g paracetamol as benzocaine.This means it matched the mixture spectra to the benzocaine spectra contained in its library only. It did not report a match to the cocaine, caffeine or paracetamol spectra in its library.

Table 3.3 lists the peaks in the neat cocaine, benzocaine, caffeine and paracetamol spectra and the peaks that occurred in each run of the mixture. The lists allow the peaks to be compared easily and common peaks to cocaine and benzocaine can be identified quickly

Table 3.3: Cocaine, benzocaine, Caffeine, Paracetamol mix run results

Cocaine	Benzocaine	Caffeine	Paracetamol	Run 1	Run 2	Run 3
1716.24	-	-	-	1715.89	-	1715.90
-	-	1698.99	-	-	-	-
-	1682.47	-	-	1682.31	1682.83	1682.30
-	-	1655.88	1649.83	1648.15	1650.98	1652.60
-	-	-	1613.19	-	-	-
-	1604.67	1600.68	-	1603.93	1604.20	1601.72
1598.75	-	-	-	-	-	-
-	1575.63	-	-	1574.89	1575.03	1575.33
-	-	-	1562.98	-	1560.09	-
-	-	1555.69	-	-	-	-
-	-	-	1515.59	1516.12	-	-
1458.58	-	1459.02	-	1455.75	-	1454.78
-	1447.40	-	1446.91	-	1448.87	-
-	-	1407.67	-	-	-	-
-	1368.74	1360.37	1372.08	1368.82	-	1371.40
-	-	1328.63	1325.13	1327.40	-	1326.75
-	1311.21	-	-	1312.46	1311.73	-
-	1281.76	1285.37	1278.34	1281.37	1281.84	1279.12
1275.31	-	-	-	-	-	-
-	-	-	1256.53	-	-	-
-	-	1241.25	1237.37	1236.77	1238.99	1236.75

1203.71	-	-	-	1205.98	-	1202.86
-	1172.14	-	-	1170.62	1171.56	1171.04
1166.25	-	-	1168.92	-	-	-
-	1111.33	-	1105.38	-	1111.71	1108.93
1077.43	-	1071.81	-	1070.62	-	1077.17
1023.81	-	1022.82	1017.29	1025.30	1020.59	1023.36
1000.72	-		-	1001.36	999.65	1000.87
-	-	-	969.77	971.36	965.92	-
-	-	928.58	-	924.95	-	930.19
896.86	-	-	-	898.64	-	895.94
869.57	-	-	-	-	-	-
-	864.05	-	-	862.42	862.80	863.79
-	-	-	858.73	-	-	-
-	-	-	834.99	832.23	-	830.98
817.96	817.61	-	-	818.08	818.52	819.74
-	-	802.13	-	-	-	-
-	-	-	798.18	796.70	798.16	-
786.75	-	-	-	785.90	-	786.81
-	-	741.75	-	-	742.48	-
730.49	-	-	-	739.53	-	730.81
-	-	-	711.82	709.61	-	-
681.89	-	-	-	680.99	680.30	680.79
-	-	-	652.48	-	-	-

-	640.25	645.15	-	640.17	640.38	640.27
616.15	-	-	-	616.46	616.80	616.53
585.52	-		-	584.04	582.73	584.99
-	-	557.99	-	557.62	555.96	557.34
-	506.16	-	-	504.58	504.48	504.09
490.95	-	-	-	-	-	
-	-	484.45	-	485.71	485.60	487.55
-	-	-	466.46	468.66	-	-
-	-	445.41	-	447.61	445.33	445.83
-	402.10	-	-	-	-	-
-	-	391.26	392.54	392.70	394.72	393.35
372.33	-	369.28	-	372.05	-	371.70
359.26	-	-	-	-	-	-
342.67	-	-	-	339.51	-	341.96
-	-	-	330.40	-	-	-
-	317.53	315.16	-	317.33	317.84	318.07
281.85	-	-	-	276.64	-	271.72
-	-	224.95	-	-	-	-
-	-	-	214.63	-	-	-
174.29	-	-	-	174.08	-	174.59
-	-	168.26	-	-	165.64	-
-	158.74	-	153.12	151.28	-	-

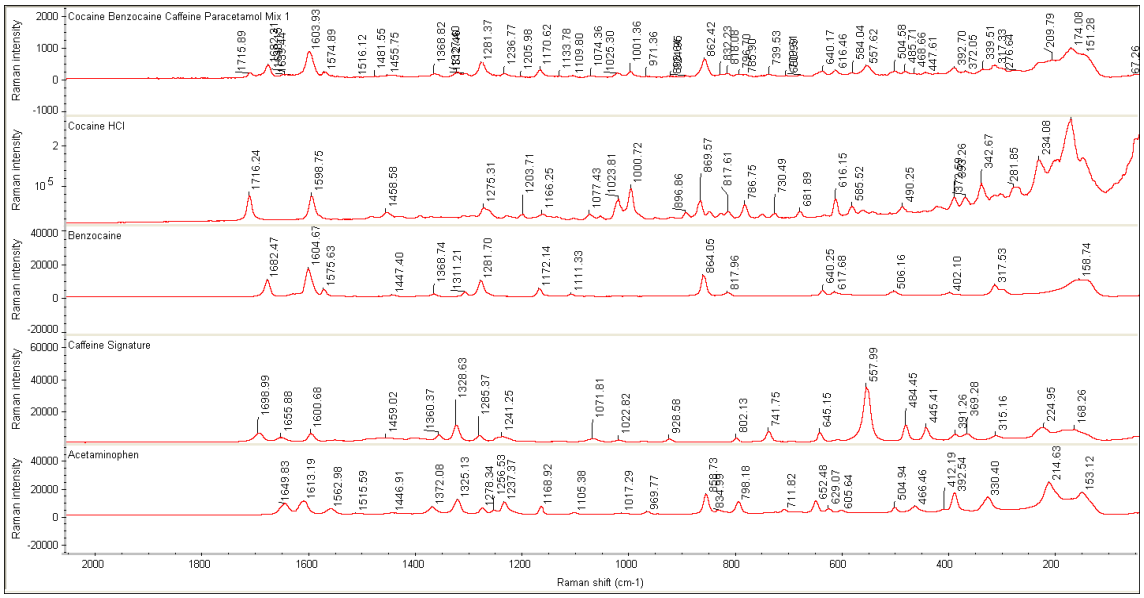

Figure: 3.9: Cocaine, benzocaine, caffeine and paracetamol mix 1 compared with reference spectra.

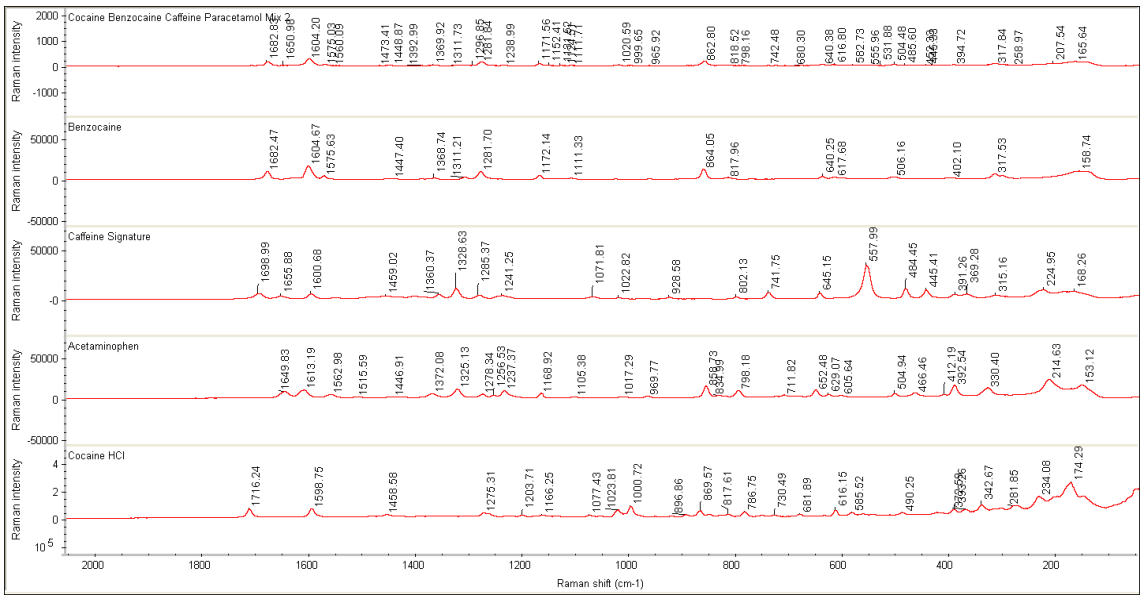

Figure: 3.10: Cocaine, benzocaine, caffeine and paracetamol mix 1 compared with reference spectra.

3.4 Oral fluid analysis

1 mL KGHB in 1 mL of oral fluid produced a KGHB spectra and the TruScan device reported a pass for GHB.

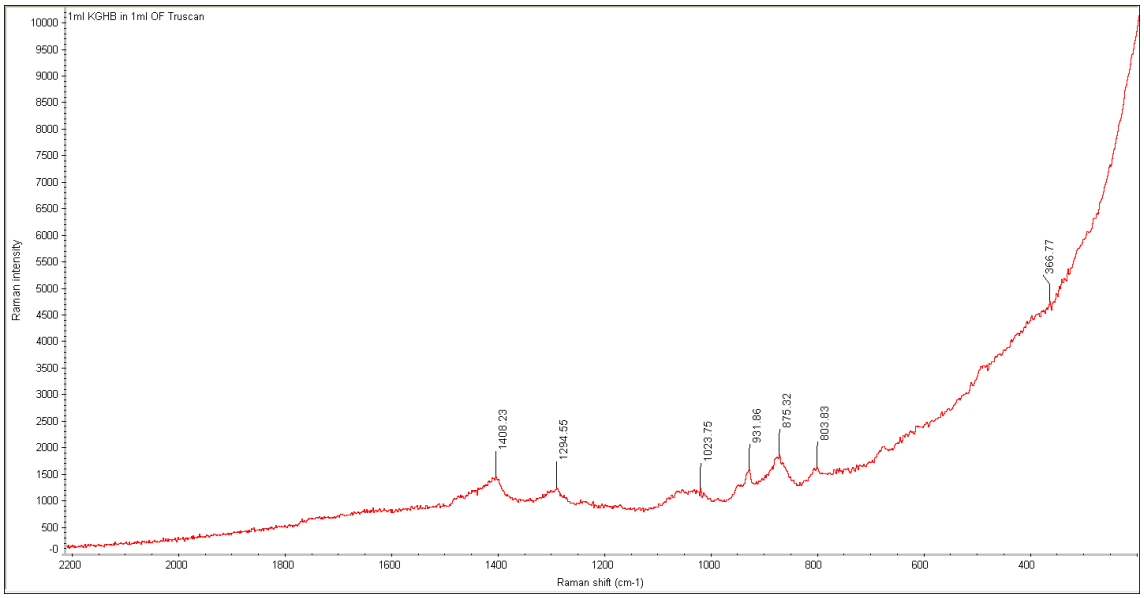

Figure 3.11: The Truscan device showed peaks for 1 mL GHB in 1 mL of oral fluid.

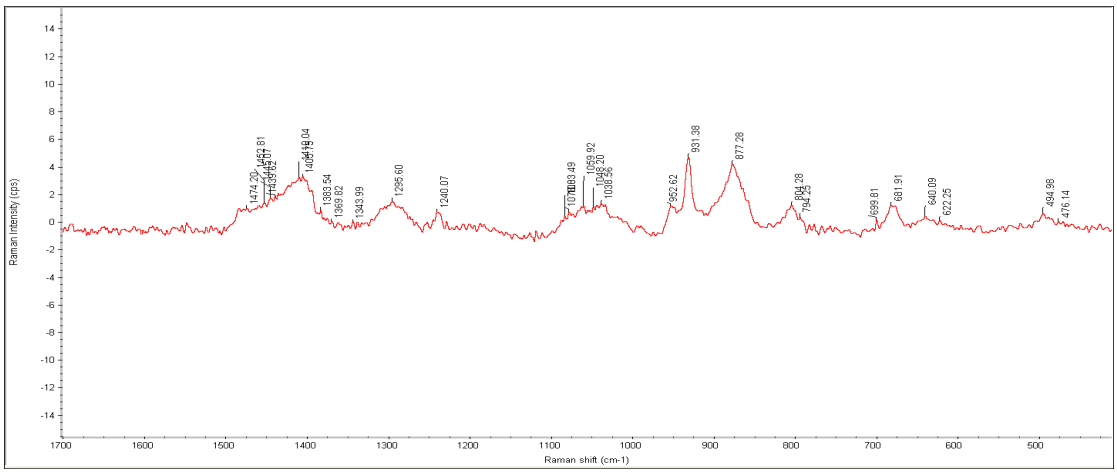

Figure 3.12: Pure KGHB Spectra taken from the authors' previous study.

Table 3.4 compares the significant peak numbers in the spiked oral fluid with the peak number present in neat KGHB. The spiked oral fluid sample displays all of the same peaks as the neat oral fluid so it can be safely assumed the KHGB can be detected.

Table 3.4 Common Peaks Neat KGHB and KGHB in Oral Fluid

1 mL KGHB in 1 mL OF	KGHB
Oral Fluid	**Neat**
1408.23	1405.79
1294.55	1296.60
1023.75	-
931.86	931.38
875.32	877.28
803.86	804.28

The Truscan device did not show any peaks for cocaine and amphetamine in oral fluid.

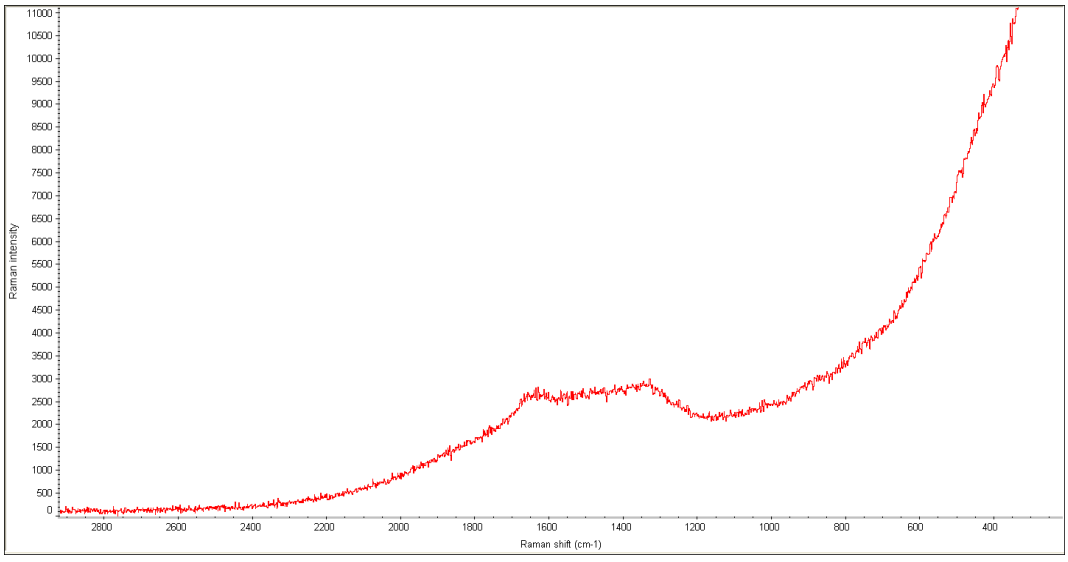

Figure 3.13: 2 mg of Cocaine Hydrochloride in 1 mL oral fluid.

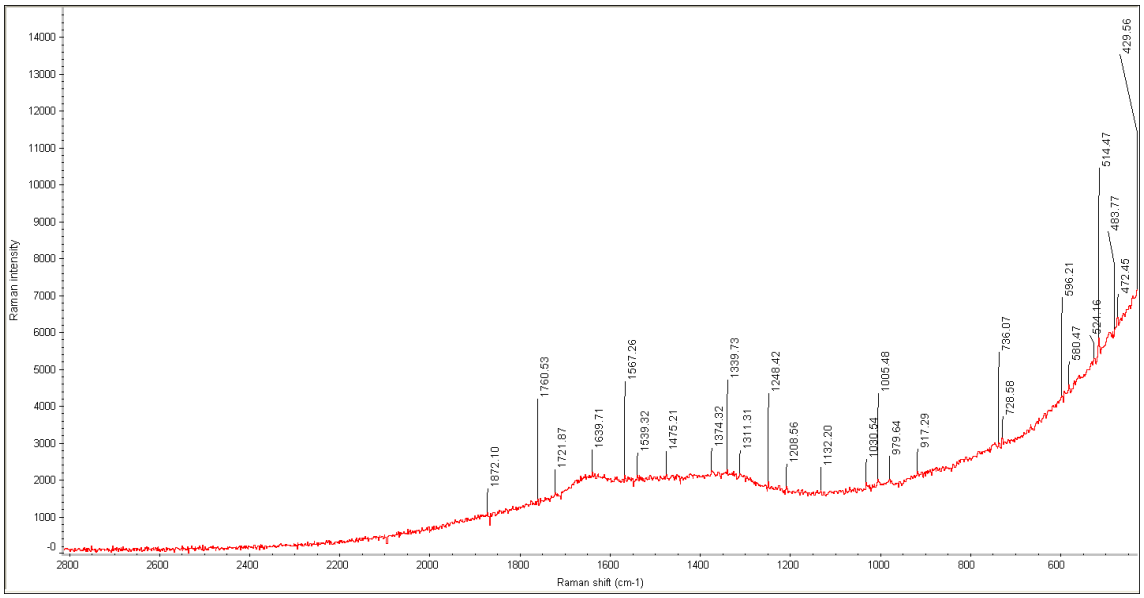

Figure 3.14: 2 mg amphetamine sulphate in 1 mL oral fluid

No more than 2 mg of cocaine and amphetamine was added to oral fluid as high amounts would not give a realistic situation. Metabolised drugs would not be as high a 2 mg in a user's oral fluid.

3.5 Limit of Detection of KGHB in Oral Fluid

The TruScan device reported a pass for GHB at 90% but failed at 80% and below.

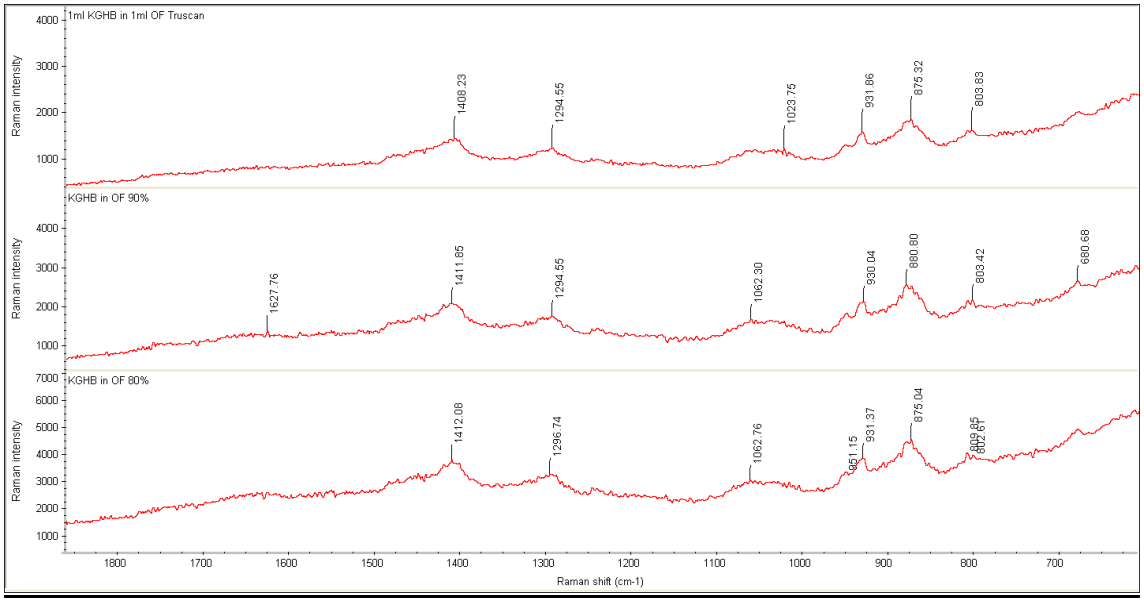

Figure 3.15 KGHB Limit of Detection 1

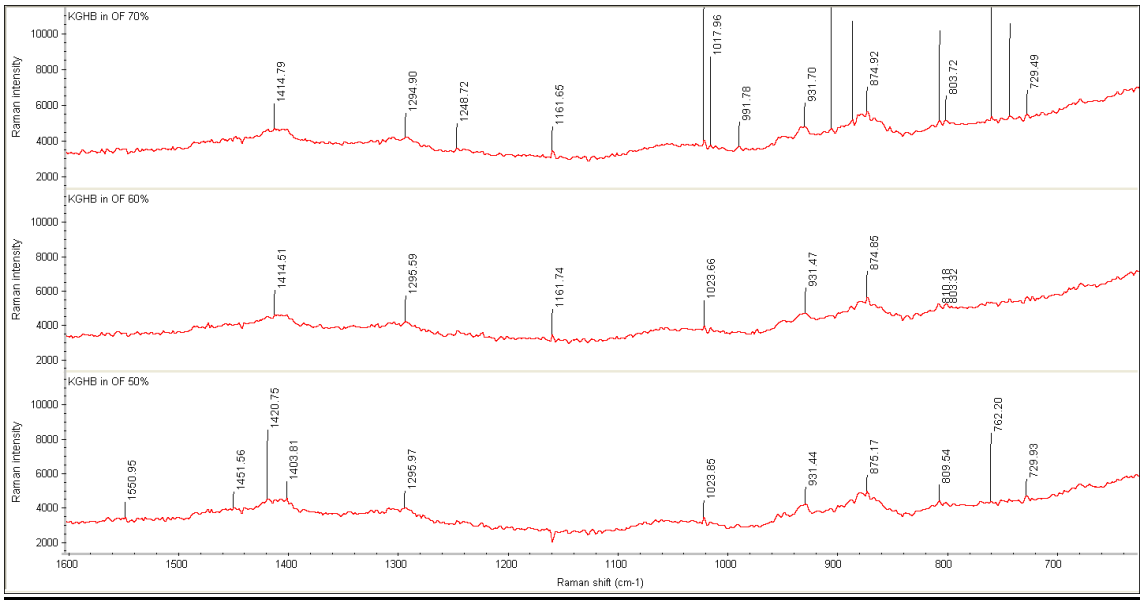

Figure 3.16 KGHB Limit of Detection 2

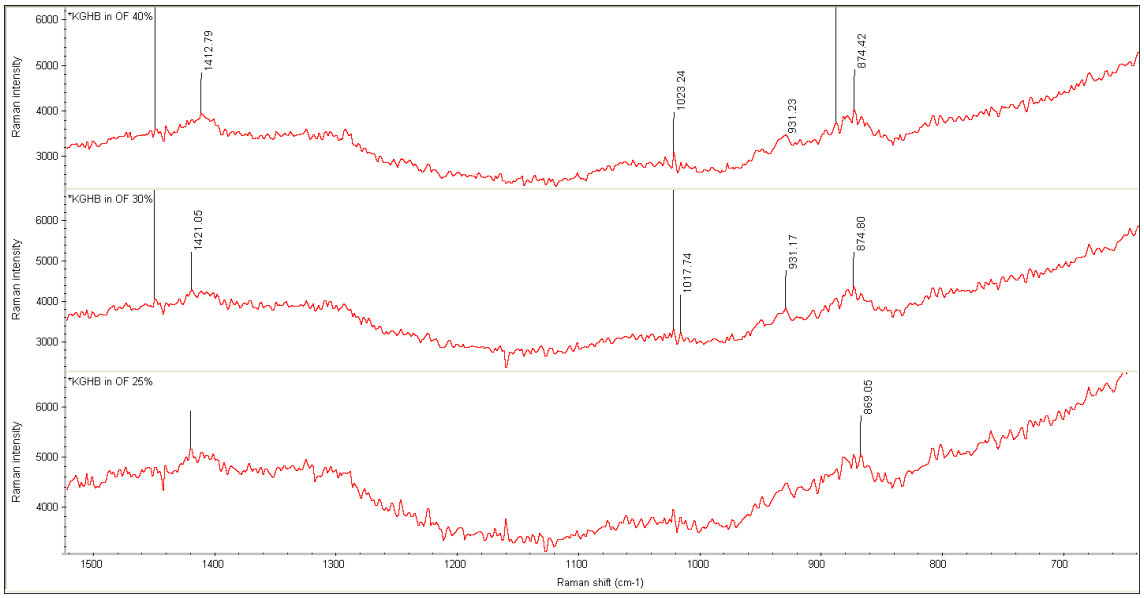

Figure 3.17 KGHB Limit of Detection 3

The limit of detection of GHB in oral fluid is around 30% at the very lowest, as the 931 and 803 cm^{-1} peaks can still, just, be identified.

3.6 SERS and Oral fluid analysis

Spiking oral fluid with 0.5 mL of GHB and carrying out the SERS method produced characteristic peaks.

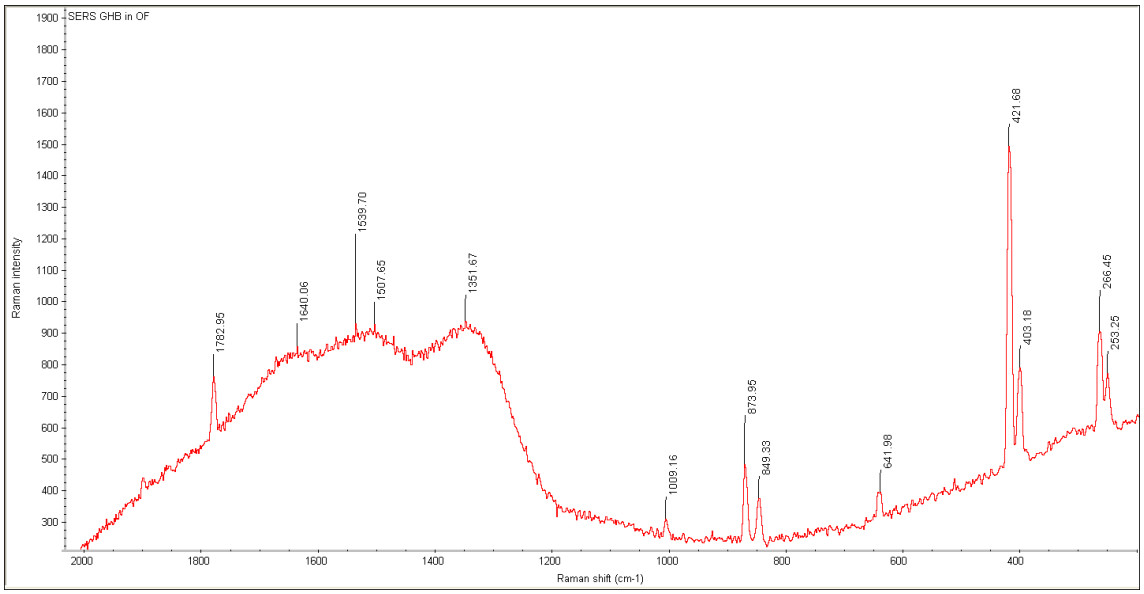

Figure 3.18: 0.5 mL GHB in 0.5 mL oral fluid.

SERS method proved to be less ideal as although it provided several peaks for KGHB, there is only one peak present which is common to KGHB. This is explained by comparing the blank dimple tray spectra to the SERS KGHB in oral fluid spectra.

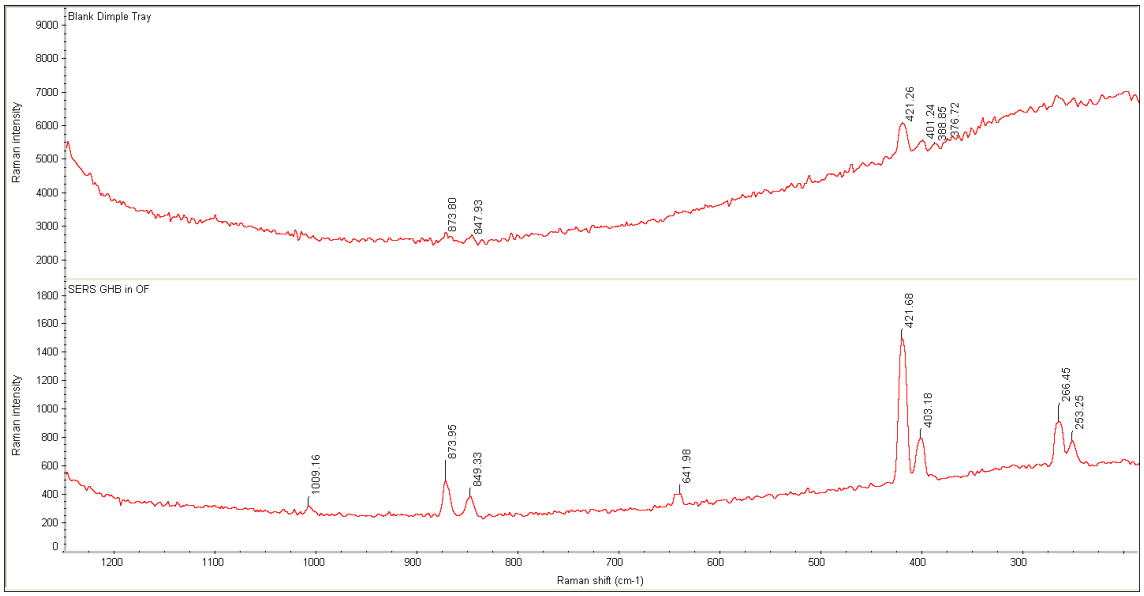

Figure 3.19: Blank dimple tray and SERS KGHB in OF comparison.

Table 3.5: Common peaks to KGHB, KGHB in oral fluid and SERS KGHB in oral fluid.

KGHB	KGHB in OF	SERS KGHB in OF
-	-	1782.95
-	-	1640.06
-	-	1539.70
1406.05	1408.23	-
-	-	1351.67
1296.94	1294.55	-
1239.73	-	-
1039.80	-	-
-	1023.75	-
-	-	1009.16
931.35	931.86	-
875.92	875.32	873.95
-	-	849.33
803.74	803.83	-
680.18	-	-
-	-	641.98
-	-	421.68
-	-	403.18
-	-	266.45
-	-	253.25

Other drugs spiked in oral fluid did not produce any peaks when the SERS method was applied.

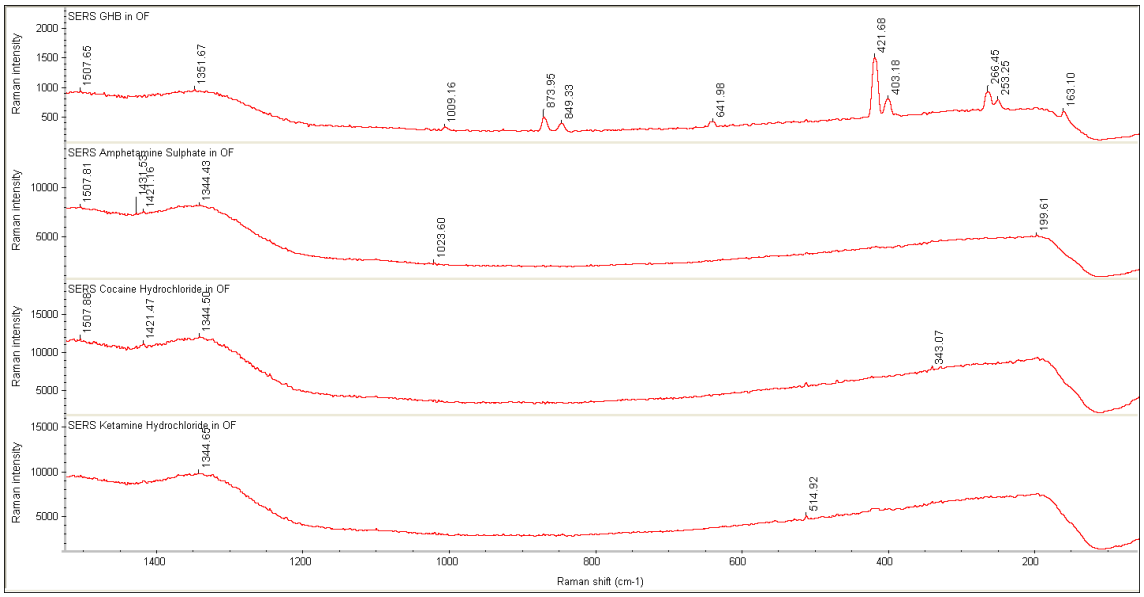

Figure 3.20: Comparison of SERS spectra 1

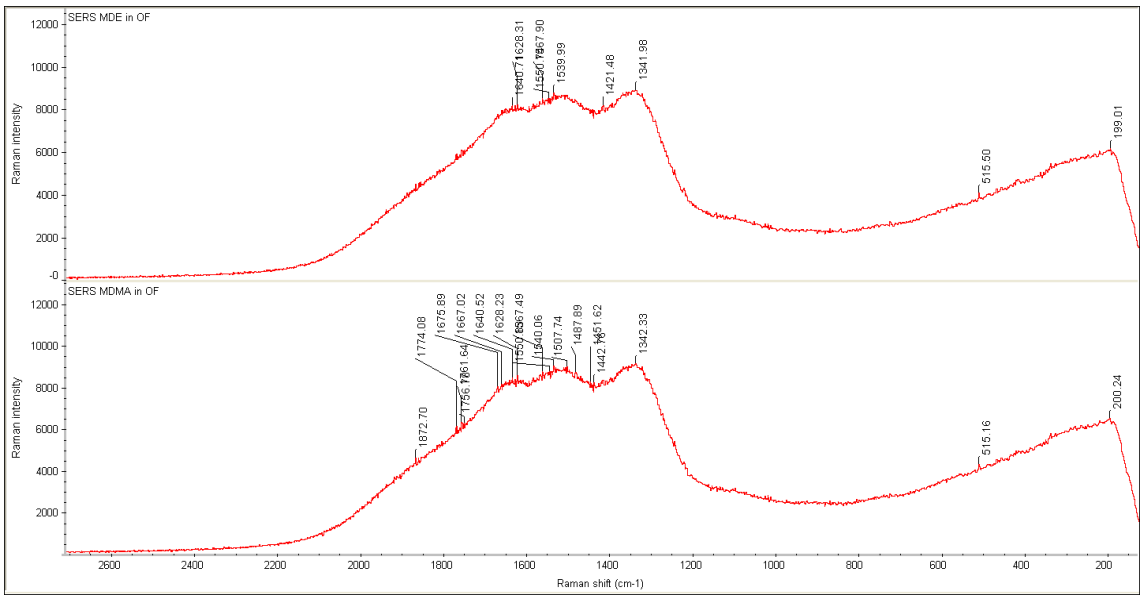

Figure 3.21: Comparison of SERS spectra 2

Chapter 4: Discussion

4.1 Method Optimisation

In order to obtain optimum results many variations of the method were attempted including different sizes of colloid (51,64, 90 nm) Different nose cones used and no nose cone attached. Sampling from the supernatant instead of the pellet after centrifugation was also attempted. For the Truscan run different methods attempted included drying the sample on a glass slide at room temperature, pipetting the sample into a capillary tube and focussing the laser down the length of the capillary tube or focussing it through glass of the capillary tube. The glass slide and capillary tube gave the same large glass hump with no peaks present in the spectra so were not suitable.

4.2 Library

Creating the library to gain reference spectra was a time consuming process. It could take up to several hours to obtain a single reference spectra or signature, as referred to in the TruScan's manual. For use in the field, the maximum amount possible of controlled substances would have to be added to the library. As the results of this study show, once cutting agents and adulterants are added, it is not always likely that the TruScan software will report the result as the controlled substance present in the sample. For this reason a library of common cutting agents and adulterants should be included also, further adding to the time consuming process. The controlled drug may not be reported by the software but the cutting agent result gives a clue it is likely an illicit drug is there. This however requires inspection of the spectra on Omnic software on a PC and this cannot be performed on the TruScan device.

The TruScan manual claims the installed software; DecisonEngine on the device takes environmental factors such as light into consideration when a spectra is being obtained. However, covering the sample up whilst the laser is on, reduces the time taken. This study used items as black weighing boats to

cover up the sample in order to speed up the process. This applied to obtaining a signature used to create the reference spectra as well as performing a simple run. This also applied to the vial attachment, although it appears enclosed and not subject to external light interference, covering it up reduced the time considerably.

4.3 KGHB in alcohol

50% v/v KGHB in ethanol is not detectable using the TruScan device. The DXR Raman spectrometer shows a characteristic 930.37cm^{-1} peak denoting the presence of KGHB. There is no way of distinguishing the TruScan Spectra from the neat ethanol spectra. This raises questions about TruScans sensitivity. Its failure to identify a substance defining peak causing a false negative is concerning as the DXR easily picks up the peak.

Table 4.1: Handheld and Bench-top Raman comparison

Specification Comparison		
Specification	**DXR**	**TruScan**
Raman Spectrum Range	50 and 3300 cm^{-1}	250cm^{-1} to 2875cm^{-1}
Laser Excitation Wavelength	780 nm	785 nm
Laser output	Maximum 14mW	Maximum 300mW

The high laser output seems excessive at 300mW. On a number of occasions the powder samples were burned, black holes were present on the sample where the laser was focussed and there was a burning smell. No way of adjusting the laser power was identified from reading the manual as well as investigating the menus present on the device.

4.4 Mixture analysis

Three runs were carried out on each mixture, each on a different location of the powder as the cocaine content will vary between areas as crudely cutting the substance in this way would not to produce a uniform mixture. This is evident in table 4.2.

Table 4.2: Number of peaks common to cocaine reference spectra.

	Number of Run			
Mixture Composition	**1**	**2**	**3**	
Cocaine and benzocaine	5	4	8	**Number of peaks in common with cocaine**
Cocaine, benzocaine and caffeine	6	16	5	
Cocaine, benzocaine, caffeine and paracetamol	16	6	16	

Every run featured a peak at 817cm^{-1} which has been counted in table 4.2, this peak is common to both cocaine and benzocaine. Every run detailed in the table also peaked at the 1000cm^{-1} region. This is a characteristic cocaine peak however it is not exclusive to cocaine as it also occurs in amphetamine and other drugs therefore its presence alone is not enough to conclude a cocaine result. Other characteristic peaks must also be present.

Table 4.3: Number of peaks common to benzocaine reference spectra.

	Number of Run			
Mixture Composition	**1**	**2**	**3**	**Number of peaks in common with benzocaine**
Cocaine and benzocaine	14	11	11	
Cocaine, benzocaine and caffeine	15	13	15	
Cocaine, benzocaine, caffeine and paracetamol	13	13	13	

The above table also included the 817cm^{-1} peak that is common to cocaine and benzocaine.

Peaks in the region of 1604, 1281, 864 cm^{-1} are the most intense peaks on the mixture spectras. These peaks are common to benzocaine. The TruScan software obviously considers these peaks important in identification. The intensities of these peaks are weighted more than the occurrence of common peaks as the tables show, i.e. 16 common cocaine peaks to 13 benzocaine peaks.

The careful wording of “raw material” in the manual makes perfect sense as the device is not capable of identifying simple mixtures.

4.5 Oral fluid analysis

KGHB was the only drug to be detected at a high concentration in oral fluid. (equivalent of 300 mg/L, 4 mg/L is the optimum detection limit for GHB in oral fluid) 1 mL GHB in 1 mL oral fluid give clearly defined peaks which are common to pure KGHB. As noted in a previous study, the 803 cm^{-1} peak is present in the pure KGHB spectra as well as in KGHB in oral fluid spectra. This peak is likely to corresponding to a ring structure. As GHB does not have a ring within its structure it is likely to be unconverted GBL or GHB and GBL in equilibrium. GBL is considered a stronger Raman scatterer than GHB. This possible explains why a spectra containing characteristic peaks have not be obtained. Adding a liquid to a liquid also may be a factor. All other drugs used in this study were powder dissolved in oral fluid.

4.6 Limit of detection in oral fluid

KGHB can be detected down to 30% v/v in oral fluid. This is still a high concentration as it corresponds to a limit of around 300 mg/L in saliva. Oral fluid tests need to be sensitive enough to detect the presence of a drug down to the ng level. It is established in the literature that detection limit cut-off level of GHB in oral fluid is around 4 mg/L, (Verstraete, 2004) this is something to aim for in screening devices however this is well below the 300 mg/L detection limit established in this country. A study carried out by Kintz *et al* found that if an individual was administered 60 mg/kg of GHB on an empty stomach, it would be expected that around 257 mg/L GHB would be present in saliva after 20 minutes. (Kintz *et al*, 2001) This means that in theory if the saliva sample was taken quickly after administration i.e. 20 minutes or under, it is possible for the Raman method detailed in this study to detect it. However this depends on variables, such as the dose of GHB administered. The dose required would almost certainly sedate the individual. This is hugely limited by the short time frame.

4.7 SERS and oral fluid analysis

The TruScan device has clearly picked up peaks from the dimple tray, therefore the laser has by-passed the solution that was intended to be analysed. This is a potential issue with the TruScan device; it is very difficult to understand exactly what the laser is focussing on. This is could also explain why containing samples in capillary tubes would not work as it was very difficult to focus the laser on the desired area. As the DXR Raman has a microscope, where the laser is focussed is very apparent and the user has more control over the precise area of the sample.

All other drugs did not give any peaks at all.

A likely explanation to the failure of SERS is that the method detailed in this study failed to combine the drug molecules with the gold colloid molecules to achieve an enhanced effect. It is likely that the colloid and equipment used were not the most suitable.

4.8 Further Research

This study has shown that the TruScan device does not have any real potential in the detection of illicit drugs and therefore no further work using this device would be recommended. Raman Spectroscopy could be a potential drug detection device as it has many favourable properties. Further research on other Raman devices may be of value. Other devices may have the sensitivity required and prove promising as a first screening technique before destructive testing is carried out.

A method of detecting drugs of abuse in saliva using SERS has already been discussed in a published article however the article was vague and difficult to understand how the method could be reproduced. However, Real-Time Analyzers, Raman manufacturers, who published the article, claim to have a successful technique which could be promising. If this research could be replicated independently and successfully, this would be a great step forward.

The SERS area of this research could be replicated using commercially available SERS kits which are available from companies such as Thermo Scientific. These kits would possibly be more reactive with the drug molecules and produce indentifying Raman peaks. This could be further improved upon by using a different Raman Spectrometer which allows the operator to focus the laser beam precisely.

Chapter 5: Conclusion

The study concluded that TruScan is able to identify a pure drug i.e. not a powder containing a percentage of an illegal drug, when reference spectra have been saved in the TruScan library. Creating a library is very time consuming.

KGHB, which is easily indentified using the 930cm^{-1} peak in an ethanol spectra on the DXR, cannot be identified on the TruScan. The TruScan is therefore less sensitive and less favourable than other Raman spectrometers.

KGHB was the only drug tested that was identified in oral fluid using the TruScan. However the concentration was high, around 75 times higher than an optimum cut-off level for a screening device. (i.e 300 mg/L compared to 4 mg/L) Applying this to a real life situation, the oral fluid would have to be tested around 20 minutes after a high dose of KGHB had been administrated. GHB is quickly eliminated from the body and the TruScan is not sensitive enough to detect GHB in oral fluid down to the ng level.

With regard to mixture analysis, it appears TruScan will report the substance in a mixture with the most intense peaks as a positive. Peak intensity is favoured over occurrences of common peaks.

The SERS method employed in this study only gave peaks for KGHB. It did not give peaks for any of the other drugs tested. The peak numbers were not peak numbers usually associated with GHB however. The exact same method was carried out for all drugs.

The police would not be able to use the TruScan device for drug screening or roadside testing of oral fluid for several reasons. The device is not able to identify even high concentrations of drugs in oral fluid, with the exception of KGHB, even this is limited as it requires a very high dose and sample to be taken 20 minutes after administration. There is a danger of missing concentrations of KGHB in the range of 4-300 mg/L. KGHB in alcohol would go undetected with TruScan. The device is unable to report a positive for the illegal substance, cocaine in a white powder mixture. Analysis of spectra is required, this cannot be done by police and the spectra have to be transferred to a PC

with Omnic software as the TruScan screen is unable to show detailed spectra and peak numbers.

Chapter 6: References

Advisory Council on Misuse of Drugs http://drugs.homeoffice.gov.uk/drugs-laws/acmd/ accessed 17/01/10

Angel, S. M., Carter, J. C., Stratis, D. N., Marquardt, B. J., & Brewer, W. E. (1999). Some new uses for filtered fiber-optic Raman probes: in situ drug identification and in situ and remote Raman imaging. *Journal of Raman spectroscopy*, *30*(9), 795-805.

Anyu, C., Lin, H., Jinghua, L., ZiJian, C., Yi, J., Dian, Q., & Hong, W. (2009, January). Detecting Narcotic Usage Using Surface-Enhanced Raman Spectroscopy on Saliva Samples. In *World Congress on Medical Physics and Biomedical Engineering, September 7-12, 2009, Munich, Germany* (pp. 71-74). Springer Berlin Heidelberg.

Aroca, R. (2006). *Surface-enhanced vibrational spectroscopy*. England: John Wiley & Sons.

BBC News. Italian River 'full of cocaine' http://news.bbc.co.uk/1/hi/world/europe/4746787.stm accessed 25/01/2011.

Bell, S. E., Burns, D. T., Dennis, A. C., & Speers, J. S. (2000). Rapid analysis of ecstasy and related phenethylamines in seized tablets by Raman spectroscopy. *Analyst*, *125*(3), 541-544.

Bell, S. E., Burns, D. T., Dennis, A. C., Matchett, L. J., & Speers, J. S. (2000). Composition profiling of seized ecstasy tablets by Raman spectroscopy. *Analyst*, *125*(10), 1811-1815.

Bell, S.E.J, Beattie, J.R,McGarvey,J.J,Peters,K.L,Sirimunthu,N.M.S,Speers,S.J. (2004) Development of sampling methods for Raman analysis of solid dosage forms of therapeutic and illicit drugs. *Journal of Raman Spectroscopy*. **35** 409-417

Bell, S. E. J., Beattie, J. R., McGarvey, J. J., Peters, K. L., Sirimuthu, N. M. S. and Speers, S. J. (2004), Development of sampling methods for Raman analysis of solid dosage forms of therapeutic and illicit drugs. *Journal of Raman Spectroscopy*.., 35: 409–417.

Bell, S. (2006) *Forensic Chemistry*. New Jersey; Pearson. Prentice Hall

Bell, S. E., Fido, L. A., Sirimuthu, N., Speers, S. J., Peters, K. L., & Cosbey, S. H. (2007). Screening Tablets for DOB Using Surface-Enhanced Raman Spectroscopy. *Journal of forensic sciences*, *52*(5), 1063-1067.

Bennett, M. J., & Steiner, R. R. (2009). Detection of Gamma-Hydroxybutyric Acid in Various Drink Matrices via AccuTOF-DART. *Journal of forensic sciences*, *54*(2), 370-375.

Blackie, E. J., Ru, E. C. L., & Etchegoin, P. G. (2009). Single-molecule surface-enhanced Raman spectroscopy of nonresonant molecules. *Journal of the American Chemical Society*, *131*(40), 14466-14472.

Brewster, V., Edwards, H. G., Hargreaves, M. D., & Munshi, T. (2009). Identification of the date-rape drug GHB and its precursor GBL by Raman spectroscopy. *Drug testing and analysis*, *1*(1), 25-31.

Burnett, A. D., Edwards, H. G. M., Hargreaves, M. D., Munshi, T., & Page, K. (2011). A forensic case study: the detection of contraband drugs in carrier solutions by Raman spectroscopy. *Drug testing and analysis*, *3*(9), 539-543.

Carter, J.C, Brewer, W.E & Angel, S.M. (2000) Raman Spectroscopy for the in Situ Identification of Cocaine and Selected Adulterants. *Applied Spectroscopy* 54(12), 1876-1881.

Ciolino, L. A., Mesmer, M. Z., Satzger, R. D., Machal, A. C., McCauley, H. A., & Mohrhaus, A. S. (2001). The chemical interconversion of GHB and GBL: forensic issues and implications. *Journal of forensic sciences*, *46*(6), 1315-1323.

Curran, H. V., & Monaghan, L. (2001). In and out of the K-hole: a comparison of the acute and residual effects of ketamine in frequent and infrequent ketamine users. *Addiction*, *96*(5), 749-760.

Daly, M. (2010) Drug gangs boost cocaine purity to compete with internet highs. *Drugscope.* 25 155-156.

Day, J. S., Edwards, H. G., Dobrowski, S. A., & Voice, A. M. (2004). The detection of drugs of abuse in fingerprints using Raman spectroscopy I: latent fingerprints. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *60*(3), 563-568.

Day, J. S., Edwards, H. G., Dobrowski, S. A., & Voice, A. M. (2004). The detection of drugs of abuse in fingerprints using Raman spectroscopy II: cyanoacrylate-fumed fingerprints. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *60*(8), 1725-1730.

Diercks, T., Coles, M., & Kessler, H. (2001). Applications of NMR in drug discovery. *Current opinion in chemical biology*, *5*(3), 285-291.

Drummer, OH. (2001). *The forensic pharmacology of drugs of abuse.* London; Hodder Arnold.

Drummer, O. H. (2006). Drug testing in oral fluid. *Clinical Biochemist Reviews*, *27*(3), 147.

Dumont, G. J. H., Sweep, F. C. G. J., Van der Steen, R., Hermsen, R., Donders, A. R. T., Touw, D. J.,& Verkes, R. J. (2009). Increased oxytocin concentrations and prosocial feelings in humans after ecstasy (3, 4-methylenedioxymethamphetamine) administration. *Social neuroscience*, *4*(4), 359-366.

EMCDDA (2009). Annual report 2009: state of the drug problem in Europe. Luxembourg Office for Official Publications of the European Communities. Luxembourg.

EMCDDA (2009). Drugs in focus-responding to drug driving in Europe. Luxembourg Office for Official Publications of the European Communities. Luxembourg.

Farquharson S, Shende C, Sengupta A, Huang H, Inscore F. Rapid Detection and Identification of Overdose Drugs in Saliva by Surface-Enhanced Raman Scattering Using Fused Gold Colloids. (2011) *Pharmaceutics*. 3(3):425-439.

Foye, WO. Lemke, TL. Williams, DA (2007). *Foye's Principles of medicinal chemistry*. 6th edition. Baltimore. MD. Lippincott Williams and Wilkins.

Gallardo, E., Barroso, M., & Queiroz, J. A. (2009). LC-MS: a powerful tool in workplace drug testing. *Drug testing and analysis*, *1*(3), 109-115.

GHB Recipes, www.erowid.org accessed 15/06/11

Gilhooly, T. C., & Daly, A. K. (2002). CYP2D6 deficiency, a factor in ecstasy related deaths?. *British journal of clinical pharmacology*, *54*(1), 69.

Hargreaves, M, D. Page, K. Munshi, T. Tomsett, R. Lynch, G. Edwards, H,G,M. (2008) Analysis of seized drugs using portable Raman Spectroscopy in an airport environment – a proof of principle study. *Journal of Raman Spectroscopy.* 39 (7) 873-880

Hoare (2009). Drug misuse declared: Findings from 2008/2009 British Crime Survey, England and Wales. Home Office Statistical Bulletin. London:Home Office

Holzgrabe, U., & Malet-Martino, M. (2011). Analytical challenges in drug counterfeiting and falsification—The NMR approach. *Journal of pharmaceutical and biomedical analysis*, *55*(4), 679-687.

Home Office, http://www.homeoffice.gov.uk/drugs/drugs-law/Class-a-b-c/ accessed 15/01/10

Hornfeldt, C. S., Lothridge, K., & Upshaw-Downs, J. C. (2002). Forensic science update: gamma-hydroxybutyrate (GHB). *Forensic Science Communications*, *4*(1).

Jackson, P., & Hilditch, C. (2010). A review of evidence related to drug driving in the UK: a report submitted to the North review team. *London, UK: Clockwork Research LTD, Transport Df*.

Jickells, S. Negrusz, A. (2008). *Clarkes Analytical Forensic Toxicology.* London. Pharmaceutical press.

Kalant, H. (2001). The pharmacology and toxicology of “ecstasy”(MDMA) and related drugs. *Canadian Medical Association Journal*, *165*(7), 917-928.

Kang, J., Gu, H., Zhong, L., Hu, Y., & Liu, F. (2011). The pH dependent Raman spectroscopic study of caffeine. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *78*(2), 757-762.

Karch, S. (2008). *Drugs abuse handbook.* 2nd edition. CRC Press.

Kato, K., Hillsgrove, M., Weinhold, L., Gorelick, D. A., Darwin, W. D., & Cone, E. J. (1993). Cocaine and metabolite excretion in saliva under stimulated and nonstimulated conditions. *Journal of analytical toxicology*, *17*(6), 338-341.

King, L. A. (2009). *Forensic chemistry of substance misuse: a guide to drug control*. Royal Society of Chemistry.

Kintz, P., Goullé, J. P., Cirimele, V., & Ludes, B. (2001). Window of Detection of γ-Hydroxybutyrate in Blood and Saliva. *Clinical chemistry*, *47*(11), 2033-2034.

Klemenjak, W., Braun, E., Alvarez, J., Bernhoft, I. M., & Fjerdingen, L. (2005). Final programme report: IMMORTAL.

Li, J., Stokes, S. A., & Woeckener, A. (1998). A tale of novel intoxication: a review of the effects of γ-hydroxybutyric acid with recommendations for management. *Annals of emergency medicine*, *31*(6), 729-736.

MacLeod, P. Page, L. Kinver, A. Iliasov, A. (2010*) Scottish Crime and Justice Survey 2008-9: Drug Use.* Scottish Government Social Research.

McBride, R. (2010) Thermo Fishers $145M buyout of Ahura Scientific, good news for Arch, Castile and other venture backers. www.xconomy.com, Accessed 17/11/11.

Misuse of Drugs Act 1971 (Amendment) Order 2009; http://www.opsi.gov.uk/si/si2009/draft/ukdsi_9780111486610_en_1, accessed 26/01/2011

Misuse of Drugs Regulations 2001, http://www.legislation.gov.uk/uksi/2001/3998/introduction/made accessed 3/10/11)

Myant, K. Hope, S. McIntosh, J. O’Brien, T. McKeganey, N. Stradling, S. (2006) *Illicit drugs and driving*. Scottish Executive Social Research.

Nicholson, K. L., & Balster, R. L. (2001). GHB: a new and novel drug of abuse. *Drug and alcohol dependence*, *63*(1), 1-22.

North, P. (2010). Report of the Review of drink and drug driving law. Great Britain. *Department for transport*.

Nutt, D. J., King, L. A., & Phillips, L. D. (2010). Drug harms in the UK: a multicriteria decision analysis. *The Lancet*, *376*(9752), 1558-1565.

O'Connor, L. (2010) The detection of GHB and GBL in alcohol using Raman Spectroscopy. Edinburgh Napier University, Unpublished

Rang, H. P., Dale, M. M., Ritter, J. M., & Moore, P. K. (2003). *Pharmacology.* New York. Churchill Livingstone.

Ravna, A. W., Sylte, I., Kristiansen, K., & Dahl, S. G. (2006). Putative drug binding conformations of monoamine transporters. *Bioorganic & medicinal chemistry*, *14*(3), 666-675.

Road Traffic Act 1988. http://www.legislation.gov.uk/ukpga/1988/52 Accessed 02/02/2011.

Sägmüller, B., Schwarze, B., Brehm, G., & Schneider, S. (2001). Application of SERS spectroscopy to the identification of (3, 4-methylenedioxy) amphetamine in forensic samples utilizing matrix stabilized silver halides. *Analyst*, *126*(11), 2066-2071.

Saunders, N. (1993). *E for Ecstasy.* London. Nicholas Saunders

Smith, E. Dent, G. (2005) *Modern Raman Spectroscopy: A practical approach.* UK. John Wily and Sons.

Smith,F., Siegel, J. (2010). *Handbook of forensic drug analysis.* Elsevier Science and Technology.

Speedy, T., Baldwin, D., Jowett, G., Gallina, M., & Jehanli, A. (2007). Development and validation of the Cozart® DDS oral fluid collection device. *Forensic science international*, *170*(2), 117-120.

Stafford, P. (1993) *Psychedelics Encyclopaedia*. Ronin Publishing.

The Medicines Act 1968, http://www.legislation.gov.uk/ukpga/1968/67 accessed 20/1/12

The Railways and Transport Safety Act 2003. http://www.legislation.gov.uk/ukpga/2003/20/schedule/7 accessed 05/02/2011.

TruScan Manual (2010) *Ahura Scientific.*

Tsuchihashi, H., Katagi, M., Nishikawa, M., Tatsuno, M., Nishioka, H., Nara, A., and Petty, C. (1997). Determination of methamphetamine and its related compounds using Fourier transform Raman spectroscopy. *Applied spectroscopy*, *51*(12), 1796-1799.

Verstraete, A. G. (2004). Detection times of drugs of abuse in blood, urine, and oral fluid. *Therapeutic drug monitoring*, *26*(2), 200-205.

Verstraete, A. G. (2005). Oral fluid testing for driving under the influence of drugs: history, recent progress and remaining challenges. *Forensic Science International*, *150*(2), 143-150.

Verstraete. AG., Raes, E. (2006) ROSITA-2 Project Final Report. Belgium.Ghent University

Weiner, A. L., Vieira, L., McKay Jr, C. A., & Bayer, M. J. (2000). Ketamine abusers presenting to the emergency department: a case series. *The Journal of emergency medicine*, *18*(4), 447-451.

West, M. J., & Went, M. J. (2011). Detection of drugs of abuse by Raman spectroscopy. *Drug testing and analysis*, *3*(9), 532-538.

Weyermann, C., Mimoune, Y., Anglada, F., Massonnet, G., Esseiva, P., & Buzzini, P. (2011). Applications of a transportable Raman spectrometer for the *in situ* detection of controlled substances at border controls. *Forensic science international*, *209*(1), 21-28.

Wills, S. (2005) *Drugs of Abuse.* 2nd edition. London. Pharmaceutical Press.

Winger.G., Woods.JH., Hofmann.F., (2004) *A handbook on drug and alcohol abuse. The Biomedical Aspects.* New York. Oxford University Press.

Appendix 1: GHB Recipes obtained from the internet.

How to make GHB:

You will need :

1. 135 grams (120 mL) of gamma butyrolactone

2. 63 grams of Sodium Hydroxide or 91 grams of Potassium Hydroxide

3. Papers to test pH

1. Place the content of the gamma butyrolactone bottle in a stainless steel or pyrex glass saucepan.

Do not use aluminum cookware to make GHB.

2. Place the content of the NaOH or KOH bottle in the same saucepan.

3. Put SLOWLY around a half cup of warm distilled water in it. Put a cover (fast! the reaction may be immediate) on it but not tight.

4. Wait a little it will start reacting on itself. If it doesn't (after 2-3 minutes), heat it a LITTLE (once it reacts remove it from the stove).

4.5 If there is some NaOH not dissolved, stir it up till it is.

5. (This step is optional, some like it like that and others prefer to heat the solution a little.) After it's finished. Start heating it slowly. You will see it starting boiling. Don't overheat! It can burn. Do it for one hour. Don't forget to add water if you make it boil for a long time.

5.5. Between step 4 and 6 you might see a white compound on the side of the saucepan (it doesn't happen everytime). Don't throw it away, it's GHB. When you will add water, it will dissolve.

6. When you are finished, put it in a measuring cup (Pyrex) and fill it with water (when I'm in a hurry to taste it I use ice) to 1000 mL (a little more than 4 cups). That way you'll have around 1 grams per teaspoon.

7. Measure the PH. If it's higher than 7.5 add vinegar to lower it to below 7.5 It can take 50 - 75 mL of vinegar.

9. To store it I use a mason glass jar with a plastic cover. I draw the poison logo on it (very important! you don't want a kid to take a full glass of GHB). I place it in the fridge, the taste is better when it's cold.

Appendix 2: TruScan Standard Operating procedures.

The following standard operating procedure for the TruScan Raman Spectrometer was devised in order to create the library.

Press button to turn on the device.

Press login to start as directed on screen.

Select Jack_admin using the enter key

Enter “K” as the password

Select the appropriate attachment for the sample i.e vial holder, tablet holder or nose cone. Sometimes no attachment is needed e.g. when sample is in thick glass bottle. Refer to Page 23 of Manual for “Best Practise for making measurement”

Reference spectra are referred to as “Methods” on TruScan, these have to be added to create the library.

2.1 Creating a Method

Select Tools from the main menu then click on Signature. This opens a further menu, select Acquire from this menu. This can take some time. It was found that the best and quickest way to obtain a Signature of a powder was to put a small amount of powder on a glass slide, focus the laser on an even area on the powder with the nose cone attachment on then black out most of the surrounding light by resting black weighing boats against the plastic shield of the nose cone.

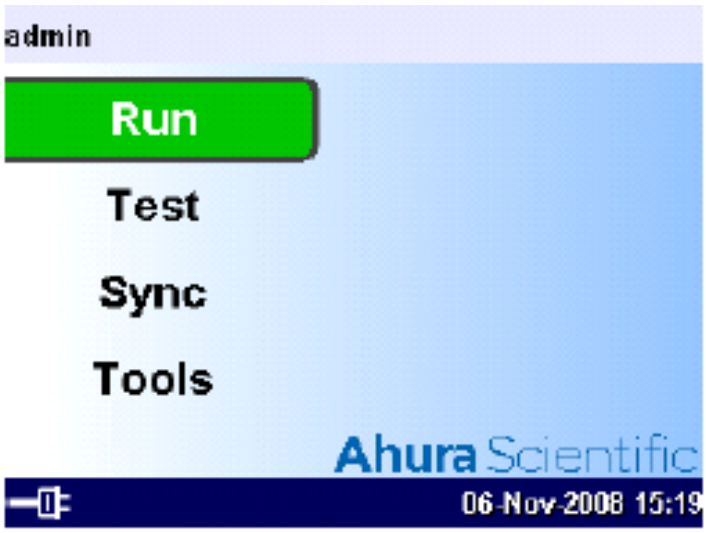

Figure 2.3: TruScan Main Menu

Once the Signature run is finished collecting go to Tools in the main menu then Signature then Inactive, click Activate as and give the sample an appropriate name.

Connect the TruScan device to the PC using the CF Ethernet adapter and cable. The adapter fits in to the card slot in the battery compartment at the bottom of the device. The cable fits into this and a USB port on the PC.

Once the device is connected, Open the Web Admin Utility which is labelled New Internet Shortcut on the PC desktop.

Log in to this program the same way as logging into TruScan device (see above)

Select Method Management from the list, then select Add New Method.

Enter a name of the Method into the Method name field and click Enabled in Status field .

Highlight the corresponding Signature in the “Unattached Signatures” window and click the arrow between the two windows to attach Signature to the Method.

Click Save Changes.

It is now possible to run a sample against this Method.

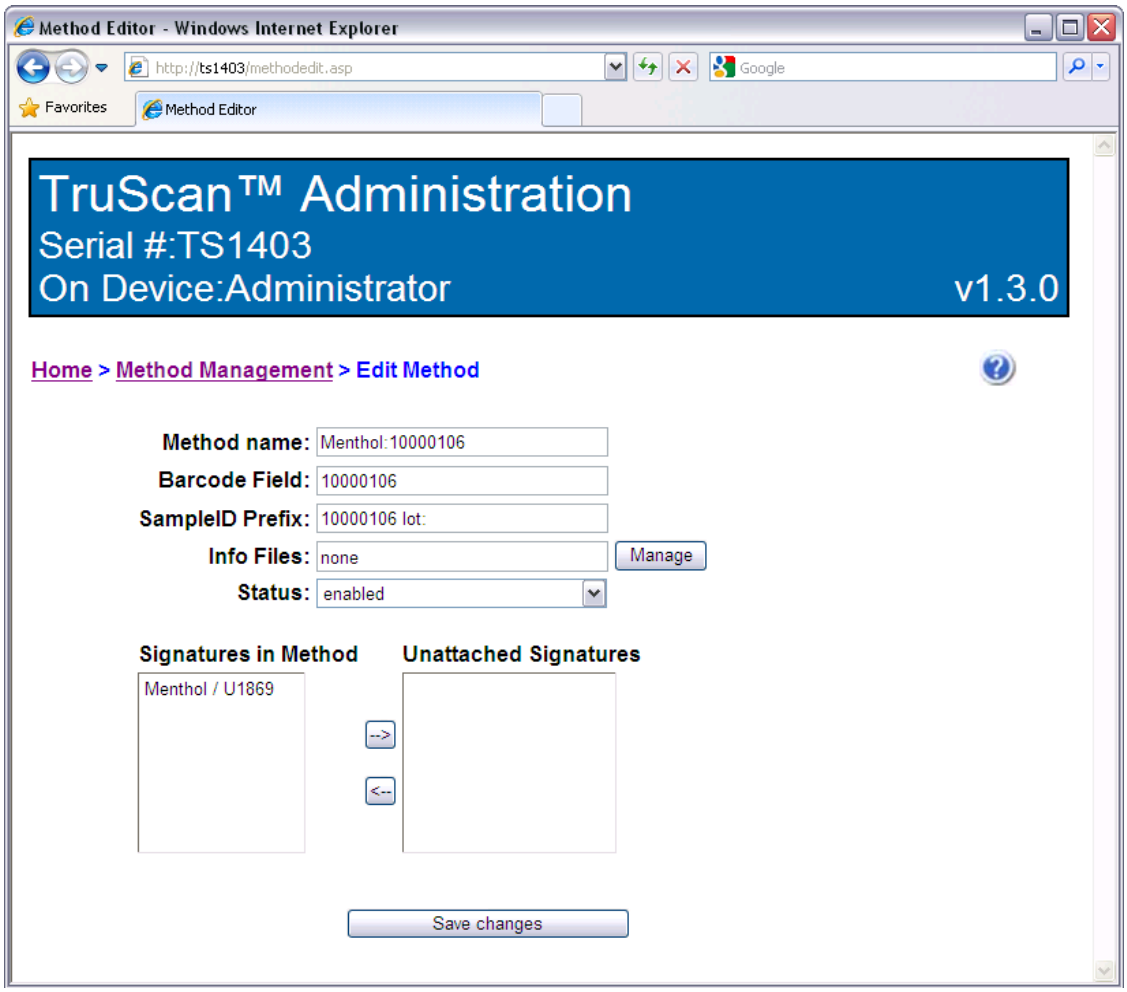

Figure 2.4: Method Management on PC

2.2 Running a Sample

Press button to turn on the device.

Press login to start as directed on screen.

Select Jack_admin using the enter key

Enter “K” as the password

Select the appropriate attachment for the sample i.e vial holder, tablet holder or nose cone. Sometimes no attachment is needed e.g. when sample is in thick

glass bottle. Refer to Page 23 of Manual for “Best Practise for making measurement”

Select Run from the main menu

Select the Method most appropriate to the sample

Enter Sample ID if required

Select Go and wait for analysis

Result will be a pass or fail.

If the result is a fail, select Discover to see if any positive matches are found.

Click on any matches to view spectra and a library spectra comparison.

Transferring Run Spectra to Omnic on PC

The CF card must be inserted into the card slot within the battery compartment at the bottom of the device.

Select Tools from the main menu

Select Review Runs

Select the Run from the list and select Export to Card from the pop- up menu.

Once this is successful, eject the card by pressing in the square button next to the card slot.

Insert the card into the USB card reader and insert into USB port on PC

A folder will open on the PC when the card reader is connected, select TruScan Runs.

Select the Run of interest. The Runs do not have logical names other than the name of the person signed in. They often have to be identified by time and date.

Right click the desired Run and select openOmnicFiles from the pop-up menu.

The Spectra will appear in the Omnic program window and can be saved.

Appendix 3: Reference Spectra

All spectra in this appendix are of the neat compound and have been acquired by the TruScan device.

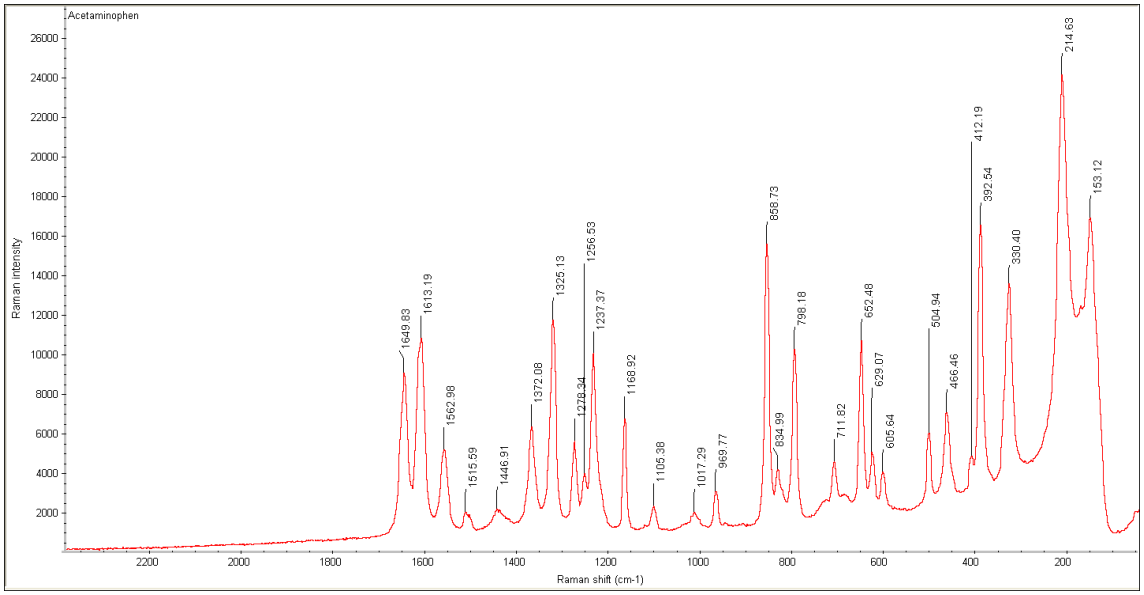

Figure A1: Acetaminophen (paracetamol) (Solid)

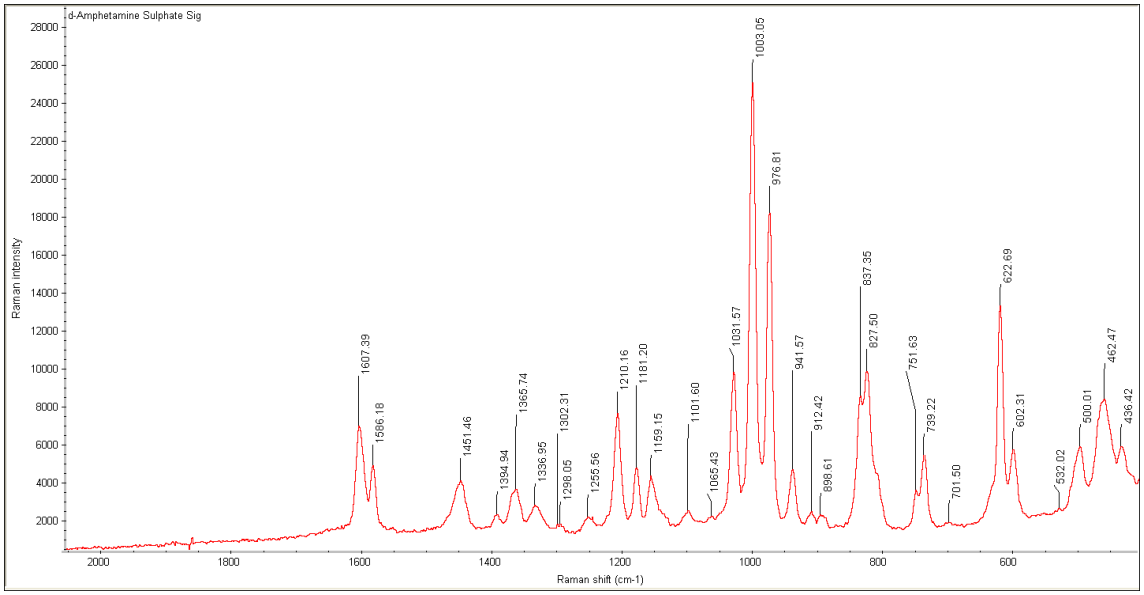

Figure A2: Amphetamine (Solid)

In literature characteristic amphetamine peaks are 1030 cm^{-1}, 1003 cm^{-1} and 970 cm^{-1} (Weyermann *et al*, 2011)

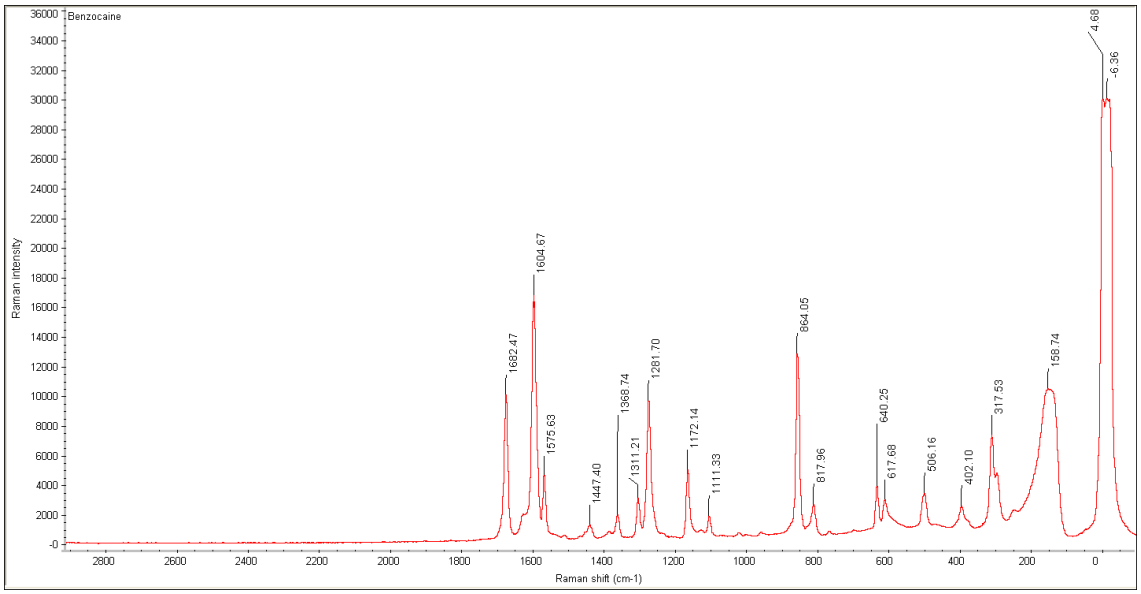

Figure A3: Benzocaine (Solid)

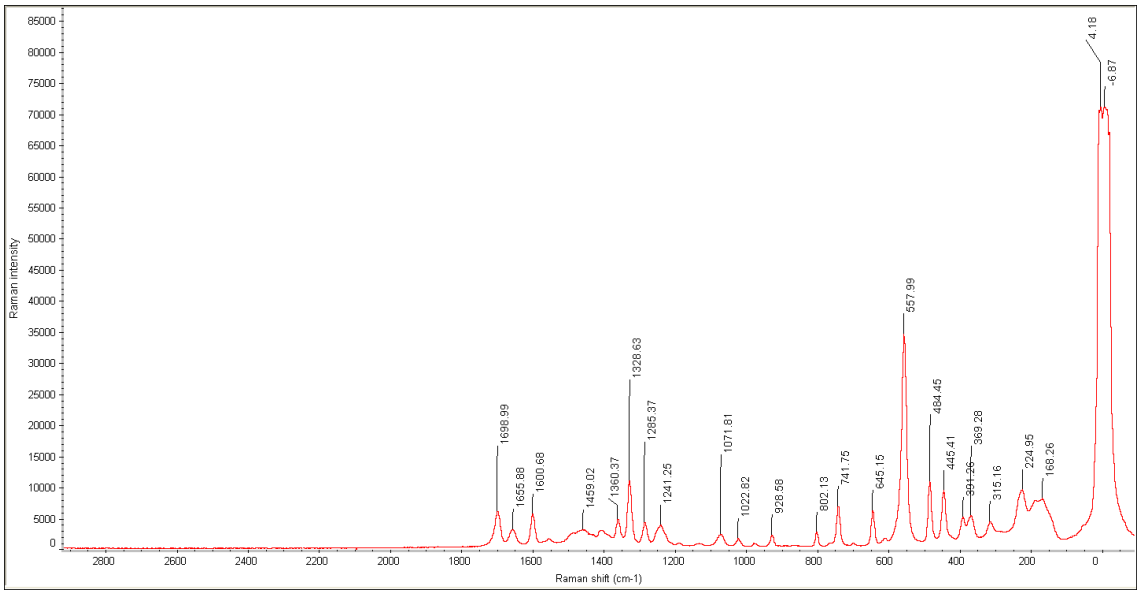

Figure A4: Caffeine (Solid)

In literature characteristic caffeine peaks are 1327 cm^{-1} and 555 cm^{-1}(Kang *et al* 2011)

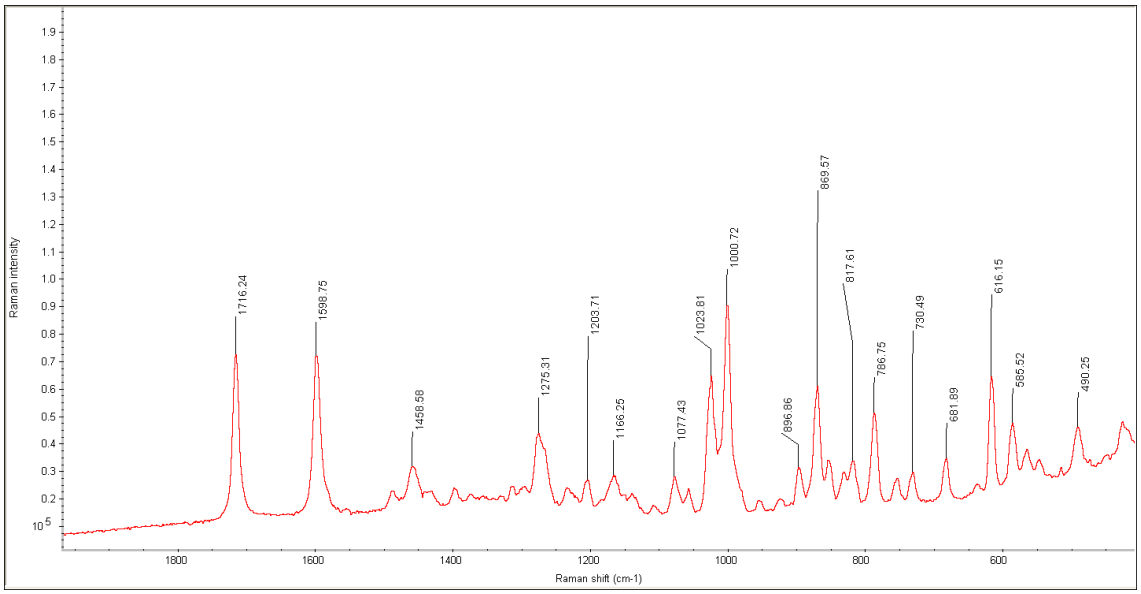

Figure A5: Cocaine Hydrochloride (Solid)

In literature characteristic cocaine peaks are 1022 cm^{-1}, 1003 cm^{-1} and 869 cm^{-1} (Weyermann *et al*, 2011)

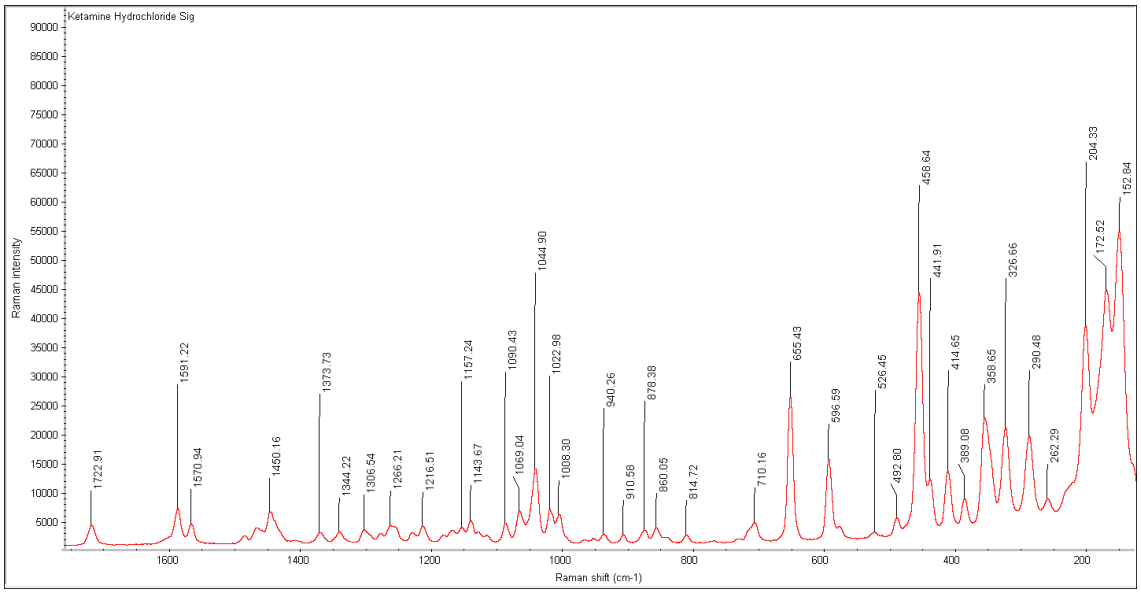

Figure A6: Ketamine Hydrochloride (Solid)

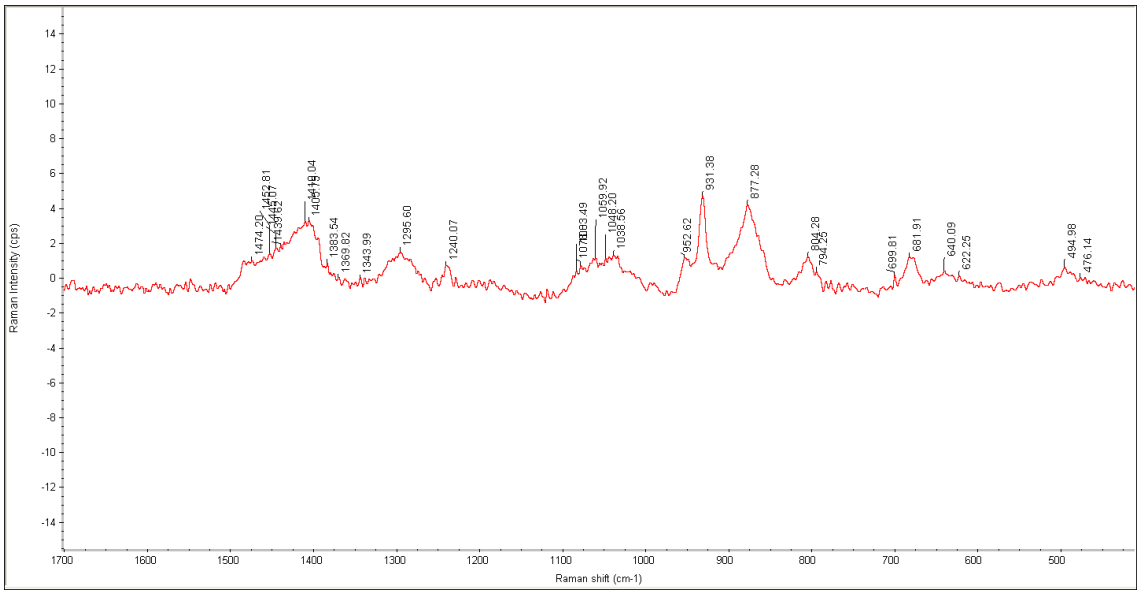

Figure A7: KGHB (Liquid)

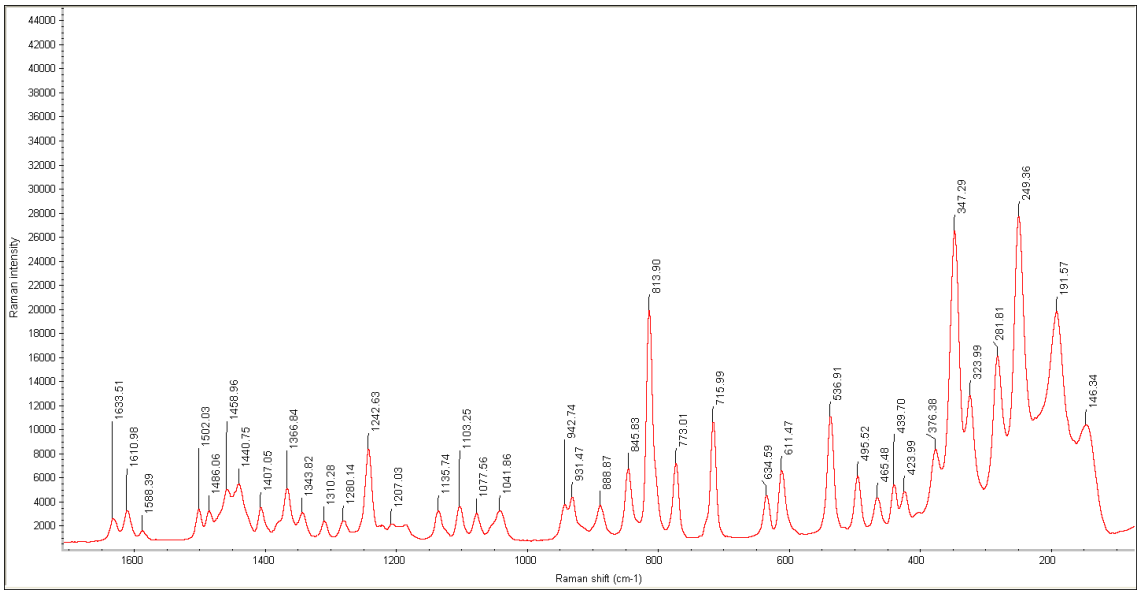

Figure A8: MDE Hydrochloride (Solid)

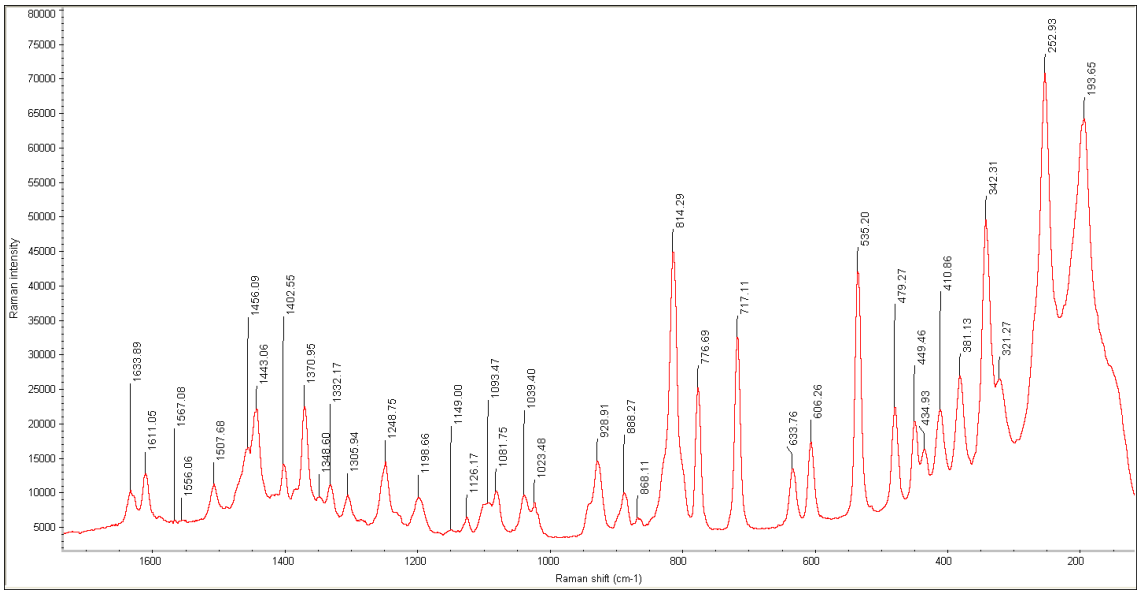

Figure A9: MDMA (Solid)