Investigation into the Significance of Monitoring Drug Use from a Fingerprint

by

Mahado Ismail

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Faculty of Engineering and Physical Sciences
University of Surrey, Guildford, Surrey, GU2 7XH
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Declaration of Originality

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Abstract

The use of chemical analysis of fingerprints as an alternative approach for drug testing has become subject of recent publications. However, the significance of the detection of drugs in fingerprints compared to a background population of non-drug users has not yet been explored. In this research, the main area of research was to determine the forensic potential of detecting cocaine and heroin use through the analysis of fingerprints. Fingerprint samples deposited on paper were extracted using an extraction solution (10% dichloromethane in methanol) and analysed using liquid chromatography – mass spectrometry (LC-MS). This research showed that cocaine and benzoylecgonine were detected in 100 and 94% of natural fingerprint samples (n= 65) collected from drug users, and similarly, heroin and 6-acetylmorphine were detected in 98 and 100% of samples (n = 60). Cocaine and benzoylecgonine were also detected in 13 and 5% of natural fingerprints (n = 98) from a background population of non-drug users. In contrast, heroin and 6-acetylmorphine were detected in 0 and 1% of fingerprints from the background population. For cocaine, a threshold level was required to differentiate fingerprints from drug users and environmental exposure in non-drug users (at a ratio analyte (A) to internal standard (IS) 0.015). The analytes of interest could still be detected in fingerprint samples from drug users after a hand cleaning procedure, however this resulted in a lower detection rate compared to natural fingerprints. In contrast, the analytes were not present in fingerprints collected from non-drug users after handwashing (1% false positive rate for cocaine). Furthermore, cocaine, benzoylecgonine, heroin and 6-acetylmorphine can also be detected in fingerprint samples from dermal contact with the parent drug even after the use of hand cleaning procedures. The detection of illicit drugs in fingerprints is therefore not solely indicative of administration of a drug but does indicate that these analytes are not prevalent in a background population of non-drug users. Additionally, the detection of isoniazid and acetylisoniazid in fingerprint samples from tuberculosis medication showed the potential application of fingerprint testing to monitor adherence to drug treatments. The detection window of isoniazid and acetylisoniazid (<2 days) suggests that a fingerprint may confirm when a patient stops complying to their treatment. This demonstrates that a fingerprint test could confirm non-adherence to treatment, which can be used to help improve treatment plans for patients and improve success rates. Furthermore, the presence of cocaine and benzoylecgonine in urine and saliva collected from drug users was determined using
portable mass spectrometry to show the potential for on-site sample analysis for drug testing. Previous work has shown that illicit drugs can be detected in fingerprints, however the suitability of fingerprints for drug testing (namely cocaine and heroin use) has not yet been explored. This research provides information on the influence of the fingerprint sampling strategy, presence of contact residue and transfer of drugs between individuals. These aspects are important to consider in relation to the stages of fingerprint testing and highlight its strengths and weaknesses for further applications (e.g. workplace drug testing, rehabilitation centres and hospitals). To improve fingerprint testing, further work is required on the standardisation of a fingerprint collection procedure, including validation procedures.
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<tr>
<td>A</td>
<td>analyte</td>
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<tr>
<td>A/IS</td>
<td>analyte-to-internal standard ratio</td>
</tr>
<tr>
<td>ACN</td>
<td>acetonitrile</td>
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<tr>
<td>AGC</td>
<td>automatic gain control</td>
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<tr>
<td>CHCl₃</td>
<td>chloroform</td>
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<tr>
<td>CMS</td>
<td>compact mass spectrometer</td>
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<tr>
<td>CRM</td>
<td>certified reference material</td>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
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<td>DL</td>
<td>detection limit</td>
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<td>DOTS</td>
<td>direct observed treatment</td>
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<td>DRUID</td>
<td>Driving Under the Influence of Drugs, Alcohol and Medicines</td>
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<td>FFT</td>
<td>Fast Fourier Transform</td>
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<tr>
<td>FSI</td>
<td>Forensic Science Ireland</td>
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<tr>
<td>GC-MS</td>
<td>gas chromatography – mass spectrometry</td>
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<tr>
<td>H₂O</td>
<td>water</td>
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<td>HCl</td>
<td>hydrochloride</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<td>IS</td>
<td>internal standard</td>
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<td>IS/IE</td>
<td>ionisation suppression/ionisation enhancement</td>
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<td>LC</td>
<td>liquid chromatography</td>
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<td>LC-MS</td>
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<td>LOD</td>
<td>limit of detection</td>
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<td>LOQ</td>
<td>limit of quantification</td>
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<td>MeOH</td>
<td>methanol</td>
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<tr>
<td>MS</td>
<td>mass spectrometer</td>
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<td>NHS</td>
<td>National Health Service</td>
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<tr>
<td>p</td>
<td>probability level</td>
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<td>Q-ToF</td>
<td>quadrupole time-of-flight</td>
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<td>rcf</td>
<td>relative centrifugal force</td>
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<td>RSD</td>
<td>relative standard deviation</td>
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<td>SAMSHA</td>
<td>Substance Abuse and Mental Health Service Administration</td>
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<td>s</td>
<td>standard deviation</td>
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<tr>
<td>SIM</td>
<td>selected ion monitoring</td>
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<tr>
<td>SOP</td>
<td>standard operating conditions</td>
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<td>UHPLC</td>
<td>ultra high performance liquid chromatography</td>
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<td>World Health Organisation</td>
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~

To my family, you have always been my guiding light.
Chapter 1 Introduction

1.0 Introduction

The possible use of fingerprints for drug testing purposes has been explored in recent research articles, due to the ease and non-invasive nature of sample collection. A fingerprint sample offers traceability as the identity of the donor is embedded in the fingerprint ridge pattern, making it difficult to falsify. There have been several approaches to the detection of drugs in fingerprints. However, only a few studies have investigated the detection of drugs from individuals who have administered a substance (Costa et al., 2017; Zhang et al., 2015; Jacob et al., 2008). This is of course different from the multitude of studies that have explored the detection of drugs in fingerprints from external contact with a substance (Groeneveld et al., 2015; Ng et al., 2009; West & Went, 2009; Ifa et al., 2008; Day et al., 2004). No research has investigated the suitability of fingerprints for drug testing and the challenges associated with the use of a fingerprint as a sampling matrix. This research investigates the suitability of fingerprints for the detection of drugs by evaluating the influence of the sampling strategy as well as contamination from dermal contact and secondary transfer of drugs. The significance of the detection of drugs in fingerprints is assessed by exploring the prevalence of the specific analytes of interest in a background population of non-drug users (as a negative control group). The use of fingerprint testing in a clinical setting is also investigated to potentially monitor compliance with tuberculosis medication. Additionally, the use of a portable mass spectrometer for drug testing is assessed using more conventional matrices such as urine and oral fluid (saliva).

1.1 Introduction to Fingerprints

The palmar surface of the hands and fingers and the plantar surface of the feet and toes consists of friction ridges. Upon contact with a surface, perspiration excreted via the pores in the fingertips is transferred onto the surface, thereby leaving an imprint of the friction ridge pattern which is called a fingerprint (Lee & Gaensslen, 2001). The term fingerprint is used for impressions which are collected under controlled conditions (Forensic Science Regulator, 2013). Fingerprints have a key role in forensic investigation (as a form of biometrics) for the purpose of positively identifying an individual due to the accessibility and ease of classification (Lee & Gaensslen, 2001). No two fingerprints have been shown to
be identical and as such it is assumed that fingerprints are a unique characteristic to every individual (Nayak et al., 2010).

1.1.1 Fingerprint composition

Fingerprints may consist of naturally secreted material, such as sweat, sebum or lipids and foreign materials picked up on the hands (e.g. cosmetics, perfumes and food residues). The exact composition of a fingerprint deposit is always unknown (Lee & Gaensslen, 2001). The composition of sweat can be influenced by various factors, including diet and genetics (Cadd et al., 2015). Sweat is excreted via three secretory glands in the skin, including eccrine, apocrine and sebaceous glands (Lee & Gaensslen, 2001). Eccrine glands are located all over the body, but a greater density is found on the soles of the feet and palms of the hands (Holder et al., 2011). The composition of a fingerprint is therefore dominated by secreted material from eccrine sweat glands. Eccrine sweat glands contribute to temperature regulation by secreting sweat and metabolic waste products (e.g. urea) (Holder et al., 2011). Eccrine sweat is composed of approximately 98% water as well as organic and inorganic constituents (Cadd et al., 2015; Lee & Gaensslen, 2001). These constituents include potassium, sodium, calcium, chloride, urea, creatinine, amino acids, proteins, lipids and lactate (Cadd et al., 2015; Holder et al., 2011; Lee & Gaensslen, 2001).

Sebaceous glands are located throughout the body, except for the palms of the hands and soles of the feet (Lee & Gaensslen, 2001). These glands are most abundant on the face, scalp, anus, nose, mouth and external portions of the ear (Holder et al., 2011). Sebaceous glands secrete sebum, which is primarily composed of lipids, including glyceride, fatty acids, wax esters, squalene and cholesterol (Cadd et al., 2015; Holder et al., 2011; Lee & Gaensslen, 2001). Although these glands are not present on the fingertips, sebum can be found in these places as a result of transfer from other parts of the body (e.g. touching the face). Apocrine sweat glands are primarily found in the axillary and pubic area (Holder et al., 2011). Very few studies have been able to analyse secretions from the apocrine glands due to contamination from the eccrine and sebaceous glands (Lee & Gaensslen, 2001). Constituents of the apocrine glands include proteins, cholesterol, carbohydrates and iron (Holder et al., 2011; Lee & Gaensslen, 2001).

In addition to compounds secreted from the secretory glands, fingerprints can also contain many semi-exogenous and exogenous materials, such as cosmetic products, illicit substances, explosives and food residue (Girod et al., 2012). Constituents of cosmetic
Products, such as skin lotion, hair products and perfumes have previously been identified in fingerprints (Weyermann et al., 2011; Croxton et al., 2010; Mong et al., 1999). It was found that fingerprints from female participants contained a significant amount of constituents likely to originate from cosmetic products, such as hydrocarbons (tetracosane to triacontane) from petroleum jelly (Mong et al., 1999). Additionally, octyl methoxycinnamate, which is a common constituent in cosmetic products has also been detected in fingerprints from a female participant (Weyermann et al., 2011). Although fatty acids and wax esters have been detected in fingerprints, these are also naturally present from endogenous compounds in fingerprints. Therefore the source of these constituents (exogenous versus endogenous) is difficult to differentiate (Girod et al., 2012).

In addition to the detection of cosmetics, other semi-exogenous compounds, such as nicotine (from smoking cigarettes), has also been identified in fingerprints (Benton et al., 2010). It was shown that nicotine was detected in fingerprints from smokers but could also be detected in non-smokers through contact with smokers or contaminated surfaces. However, a significant difference in the levels of nicotine was observed between smokers and non-smokers.

Fingerprints have also been investigated for compounds related to gunshot residues after carrying out a controlled firing experiment (Gilchrist et al., 2012). Fingerprint samples showed increased levels of nitrate and nitrite compared to control fingerprints. However, levels of other gunshot residue constituents (cyanate) were not significantly increased. It is therefore possible that the observed increased level of nitrate and nitrite originated from induced endogenous sweat secretion rather than gunshot residue during the firing experiment. However, exogenous explosives have been detected and quantified in fingerprints (Gilchrist et al., 2015; Love et al., 2013).

Finally, traces of drugs have also been detected in fingerprints, including methadone, lorazepam, caffeine, cold medicine and cocaine (Costa, 2017; Bailey et al., 2015; Kuwayama et al., 2014; Kuwayama et al., 2013; Goucher et al., 2009; Jacob et al., 2008). These studies have shown that fingerprints can contain traces of drugs due to the consumption of these substances and thereby integrated into the metabolic pathway. Further discussion on the detection of drugs in fingerprints is detailed in section 1.2.6.
1.2 Introduction to Drugs

1.2.1 Drug misuse and treatment

Drug misuse continues to negatively affect the health, wellbeing and quality of life of individuals. Between 2016 and 2017, around 1 in 12 (8.5%) adults aged 16 – 59 years old in England and Wales had taken a drug in the last year, which equates to an approximate 2.8 million people (Home Office, 2017). However, this percentage is more than doubled (19.2%) for young adults (aged 16 – 24 years old), and equates to 1.2 million people (Home Office, 2017). The economic and social cost of drug supply in England and Wales is an estimated £10.7 billion a year, and just over half of this is attributed to drug related crime, such as burglary, robbery and shoplifting (HM Government, 2017).

The second most commonly used drug in the last year (after cannabis) among adults aged 16 – 59 years old was powder cocaine (Home Office, 2017). Approximately 2.3% of adults (760,000 people) and 4.8% of young adults (297,000) have taken cocaine, based on a 2016/17 survey carried out by the Home Office. Although cocaine is one of the most commonly used drugs, the trend in cocaine use has remained relatively flat since 2009-10 (Home Office, 2017).

In 2016-17, 279,793 individuals received treatment for drug misuse through drug and alcohol services (HM Government, 2017). Most individuals were treated for opiate use. In addition, the lowest rate of successful completion (26%) was observed for opiate users compared to non-opiate and alcohol, and alcohol only use (Knight et al., 2017). The remaining individuals were discharged without completing treatment. This highlights the importance of the treatment programmes and quality of support in services for these individuals to improve the outcomes observed. Over the last few years, less individuals are entering treatment aged under 25 (Knight et al., 2017). However, there is an increase in the amount of young adults under 25 entering treatment for cocaine use (Knight et al., 2017).

Drug misuse is commonly observed among individuals with mental health problems (HM Government, 2017). It has been shown that up to 70% of individuals in substance misuse treatment also experience mental illness (e.g. schizophrenia) (HM Government, 2017). In addition, the availability of treatment for these individuals is usually limited. As a result, 33% of individuals in mental health treatment who committed suicide had a history of drug
use, but only 7% were in contact with drug and alcohol treatment services (HM Government, 2017).

The number of deaths (3,744) related to drugs in England and Wales is the highest number it has been since comparable records began in 1993 (Office for National Statistics, 2017). This includes deaths due to accidents and suicide involving drug poisoning, as well as drug abuse and drug dependency (Office for National Statistics, 2017). The majority of drug related deaths 2,593 (69%) were due to drug misuse, which equates to 0.5% of all deaths (Office for National Statistics, 2017). Most of the drug related deaths were for people aged 30 – 49 years old. Comparison between genders showed that 73% of drug related deaths were for males (Office for National Statistics, 2017). Over half of the deaths related to drugs involved an opiate, mainly heroin/morphine. Additionally, the number of deaths related to cocaine use has increased 16% (to 371 deaths) since 2015 (Office for National Statistics, 2017).

1.2.2 Classification of illegal drugs
In the UK, the Misuse of Drugs Act 1971 is the main regulation to control and classify drugs (Leg Gov, 1971). The term ‘controlled drugs’ was introduced and refers to substances that are dangerous or otherwise harmful when misused. The main purpose of the Act is to prevent the misuse of controlled drugs, and thus it is not lawful to import, export, supply, possess and produce controlled drugs (Leg Gov, 1971). The Act classifies controlled drugs into 3 categories, namely class A, B and C, based on the harmfulness of the substance, with class A considered to be the most harmful. The penalties for the possession, supply and production of controlled substances depends on the class of the drug, with the highest penalties associated with class A drugs (Table 1.1) (Leg Gov, 1971). The lawful possession and supply of controlled drugs for legitimate purposes are regulated under the Misuse of Drugs Regulations 2001 (Leg Gov, 2001).

The rise of new psychoactive substances causes great concern as the long-term effects are unknown. These substances are produced by modification of the chemical structure of other psychoactive substances (e.g. ecstasy or cocaine). Due to the difference in chemical structure, these substances are not regulated under the Misuse of Drugs Act 1971. However, a new regulation has been introduced called the Psychoactive Substances Act 2016 (Leg Gov, 2016). This Act was initiated so that the production, import, export, supply and
possession of any substance intended for human consumption that can produce psychoactive
effect is not lawful (Leg Gov, 2016).

Table 1.1: Penalties associated with the possession, supply and production of controlled
drugs in the United Kingdom (Leg Gov, 1971).

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug</th>
<th>Possession</th>
<th>Supply and production</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Crack cocaine, cocaine, ecstasy (MDMA), heroin, LSD, magic mushrooms, methadone, methamphetamine (crystal meth)</td>
<td>Up to 7 years in prison, an unlimited fine or both</td>
<td>Up to life in prison, an unlimited fine or both</td>
</tr>
<tr>
<td>B</td>
<td>Amphetamines, barbiturates, cannabis, codeine, ketamine, methylphenidate (Ritalin), synthetic cannabinoids, synthetic cathinones (e.g. mephedrone, methoxetamine)</td>
<td>Up to 5 years in prison, an unlimited fine or both</td>
<td>Up to 14 years in prison, an unlimited fine or both</td>
</tr>
<tr>
<td>C</td>
<td>Anabolic steroids, benzodiazepines (diazepam), gamma hydroxybutyrate (GHB), gamma-butyrolactone (GBL), piperazines (BZP), khat</td>
<td>Up to 2 years in prison, an unlimited fine or both (except anabolic steroids – it’s not an offence to possess them for personal use)</td>
<td>Up to 14 years in prison, an unlimited fine or both</td>
</tr>
<tr>
<td></td>
<td>Some methylphenidate substances (ethylphenidate, 3,4-dichloromethylphenidate (3,4-DCMP), methylphenidate (HDMP-28), isopropylphenidate (IPP or IPPD), 4-methylmethylphenidate, ethylphenidate, propylphenidate) and their simple derivatives</td>
<td>None, but police can take away suspected temporary class drug</td>
<td>Up to 14 years in prison, an unlimited fine or both</td>
</tr>
</tbody>
</table>

*New drugs placed under a temporary banning order, while the Advisory Council on the Misuse of Drugs (ACMD) decides on the classification of these substances.

Additionally, since November 2011, a temporary class drug order has been initiated in the United Kingdom for new psychoactive substances (Leg Gov, 1971). The use of the ‘temporary class drug order’ enables the government to prohibit these harmful substances for 12 months whilst the Advisory Council on the Misuse of Drugs (ACMD) determines how to permanently classify these (Leg Gov, 1971).
1.2.3 Cocaine and chemistry

Cocaine is extracted from the leaves of the coca plant (*Erythroxylum coca*), which primarily grows in South American countries, such as Peru, Bolivia and Colombia (Negrusz & Cooper, 2013). It is a central nervous system stimulant that is used for its short-term neurological effects, including euphoria, pleasure, feeling of power, increased energy and awareness (Negrusz & Cooper, 2013). However, cocaine abuse is followed by many consequences once the effect of the drug gradually disappears, such as anxiety, fatigue and depression (Wild, 2013).

According to the Misuse of Drugs Act 171, cocaine is classified as a class A controlled drug (Leg Gov, 1971). The molecular structure of cocaine is illustrated in Figure 1.1. It is a weak base as the nitrogen atom only has three bonds and has a molecular weight of 303.36 g/mol (Karch, 2016).

![Molecular structure of cocaine](image)

**Figure 1.1:** Molecular structure of cocaine.

1.2.3.1 Production of cocaine

Cocaine is a widely abused drug that is available in two forms, a hydrochloride (HCl) salt (powder cocaine) and a free base form (crack cocaine) (Negrusz & Cooper, 2013). The production of illicit natural cocaine primarily involves three steps, including (i) extraction of crude coca paste from the coca leaf, (ii) purification of crude coca paste to cocaine base and (iii) conversion of cocaine base to the hydrochloride salt (Negrusz & Cooper, 2013). Cocaine hydrochloride is prepared by dissolving the alkaloid (naturally occurring chemical compound containing carbon, hydrogen, nitrogen and oxygen) in hydrochloric acid. When the free base form reacts with the hydrochloric acid, the nitrogen atom accepts a hydrogen atom (H⁺) and forms the hydrochloride salt of cocaine (Figure 1.2). Cocaine hydrochloride is a water-soluble, white crystalline powder that decomposes when heated (McQueen *et al.*, 2010). Cocaine hydrochloride is converted to crack cocaine by adding baking soda (sodium bicarbonate) to aqueous cocaine hydrochloride and heating until the free base (deprotonated...
amine form of the compound) starts to precipitate into small pellets (Negrusz & Cooper, 2013; McQueen et al., 2010; Conaboy, 1997). The removal of the hydrochloride allows the drug to be administered via smoking due to the lower melting point (Hope et al., 2005). In contrast, cocaine hydrochloride is typically administered via nasal insufflation. Both cocaine hydrochloride and crack cocaine are harmful, however the route of administration of crack cocaine delivers a more intense effect compared to nasal insufflation (Wild, 2013).

Cocaine is commonly cut using adulterants such as caffeine, levamisole and lidocaine to enhance weight and volume (Karch, 2016; Negrusz & Cooper, 2013). Additionally, cocaine contains numerous impurities from the co-extraction of coca alkaloids, solvent and processing chemicals used in the production process. These can include breakdown products (metabolites) of cocaine, such as benzoylecgonine, eegonine methyl ester, norcocaine, norecgonine (Bogusz, 2008; Casale & Klein, 1993).

![Molecular structures of cocaine hydrochloride (powder cocaine) and crack cocaine](image)

Figure 1.2: Molecular structures of cocaine hydrochloride (powder cocaine) and crack cocaine (Negrusz & Cooper, 2013).

1.2.3.2 Metabolism of cocaine

Metabolism is the process of conversion of one chemical species to another (Jambhekar & Breen, 2009). Drug metabolism primarily occurs in the liver, because it contains a high concentration of metabolic enzymes (Poole Arcangelo & Peterson, 2006). However, kidneys also contribute to the metabolism of drugs, mostly for the parent drug (Jambhekar & Breen, 2009; Poole Arcangelo & Peterson, 2006). Most drugs are metabolised from an active form to a less active or inactive form, although exceptions occur (e.g. cannabis and codeine) (Negrusz & Cooper, 2013; Poole Arcangelo & Peterson, 2006). The products of drug metabolism are more polar, which enhances the water solubility and excretion of the drugs (Gibson & Skett, 2001).
Following administration, cocaine is primarily converted to major metabolites benzoylecgonine and ecgonine methyl ester and then finally to ecgonine as shown in Figure 1.3 (Karch, 2016; Khojasteh et al., 2011; Kolbrich et al., 2006; Platt, 2000; Gorelick, 1997). Cocaine is metabolised by both enzymatic and non-enzymatic (influenced by temperature and physiological alkaline pH) pathways (Karch, 2016; Baselt, 1983). Benzoylecgonine is the primary metabolite of cocaine in urine and plasma following nasal insufflation, intravenous, oral and smoked administration routes (Bogusz, 2008). It is formed through hydrolysis at the methyl ester bond of cocaine through both enzymatic and non-enzymatic pathways (Platt, 2000). Another major metabolite is ecgonine methyl ester, which is formed through enzymatic hydrolysis at the benzoyl ester bond of cocaine (Bogusz, 2008). Further hydrolysis of benzoylecgonine and ecgonine methyl ester produces ecgonine. Additionally, the co-administration of cocaine and alcohol can lead to the formation of cocaethylene.

Figure 1.3: Metabolic pathway of cocaine to major metabolites benzoylecgonine and ecgonine methyl ester (Bogusz, 2008).

In addition to the presence of cocaine, benzoylecgonine is predominantly used to indicate cocaine use in drug testing as the concentrations present in urine and plasma are considerably higher (by a factor of 10 or 11) than those of ecgonine methyl ester or any other metabolite (Karch, 2016; Kolbrich et al., 2006).
1.2.3.3 Detection window of cocaine in biological matrices

Benzoylecgonine, one of the main metabolites of cocaine, has been detected in urine for 1–2 days after a single intravenous dose (at 20 mg) (Cone et al., 1989). Peak concentrations of benzoylecgonine ranged from 4000–9000 ng/ml and occurred within the first 12 hours after administration. Another study reported that cocaine administrated via the intranasal route (single dose at 1.5 mg/kg) could be detected in urine for approximately 8 h (based on n = 6 participants) (Hamilton et al., 1977). In contrast, benzoylecgonine was detected for up to 2–3 days. Peak concentrations for cocaine and benzoylecgonine were 24 and 75 µl/ml, respectively. Similar results were obtained by another study, which reported that after a single dose administration of cocaine, benzoylecgonine has been detected in urine (for n = 6 participants) up to 2 days (max 2.5 days) after intravenous (dose 25 mg), intranasal (dose 42 mg) or smoked administration routes (dose 42 mg) (Cone et al., 2003). Peak concentrations observed were highly variable but were highest for benzoylecgonine, followed by ecgonine methyl ester and cocaine, respectively. In chronic users, benzoylecgonine was detected for 10 to 22 days in urine after last administration (Weiss & Gawin, 1988).

Cocaine is the main analyte detected in oral fluid (saliva) and appears almost immediately after intravenous, intranasal or smoked administration (Cone et al., 1997). The major metabolites, benzoylecgonine and ecgonine methyl ester appear within 15 min of cocaine use (Negrusz & Cooper, 2013). In oral fluid, cocaine has been detected up to 3.9 h after intravenous administration (dose 25 mg) with peak concentrations ranging from 285–1303 ng/ml, 5.7 h after intranasal administration (dose 32 mg) with peak concentrations ranging from 75–59600 ng/ml and 3.7 h after smoking (dose 42 mg) with peak concentrations ranging from 94–12582 ng/ml using a single dose (Cone et al., 1997). Peak concentrations for benzoylecgonine ranged from 14–43 ng/ml after intravenous administration, 28–1931 ng/ml after intranasal administration and 17–49 ng/ml after smoking (Cone et al., 1997). Another study reported that the mean detection time (n = 7) of cocaine in oral fluid after intravenous injection (dose 44.8 mg) and smoking (dose 40 mg) was up to 7.5 h and 8.5 h (maximum up to 12 h), respectively (Jenkins et al., 1995). Saliva concentrations rapidly declined after intravenous administration and ranged from 428–1927 ng/ml (Jenkins et al., 1995). In contrast, after smoking the peak concentrations for benzoylecgonine ranged from 15852–504880 ng/ml. In chronic users, the detection time of cocaine in oral fluid can be up to 10 days during abstinence (Cone & Weddington, 1989). Benzoylecgonine
concentrations ranged from 510 – 204500 ng/ml (n = 6) during this period. Another study measured the levels of cocaine, benzoylecgonine and ecgonine methyl ester in oral fluid for 12 h (monitoring period) in participants who frequently used cocaine (Moolchan et al., 2000). The concentration of benzoylecgonine and ecgonine methyl ester in later samples were higher than those observed for cocaine, which is consistent with the longer half-lives (7.9 h for cocaine, 9.2 h for benzoylecgonine and 10.0 h for ecgonine methyl ester) (Moolchan et al., 2000). Mean concentrations for cocaine and benzoylecgonine in oral fluid were 22.5 ± 49.5 ng/ml and 371.8 ± 484.7 ng/ml, respectively.

In blood plasma, cocaine can be detected up to 11.5 h (max 24.1 h) after a subcutaneous low dose (75 mg/70 kg) and 9.5 h (max 47.6 h) after a high dose (150 mg/70 kg) (Scheidweiler et al., 2010). Maximum cocaine concentrations after the low dose ranged from 108.6 – 434.1 µg/ml (n = 19) and 253.5 – 1153.9 µg/ml (n = 14) after the high dose. Additionally, maximum benzoylecgonine concentrations ranged from 190.1 – 411.2 µg/ml after a low dose and 336.3 – 832.0 µg/ml after a high dose (Scheidweiler et al., 2010). The main metabolites benzoylecgonine and ecgonine methyl ester were detected up to 48 h (max 48 h) and 24 h (max 48 h), respectively following low and high doses. In serum, the maximum elimination time of benzoylecgonine from chronic drug users (n = 6) was 5.1 h (maximum 7.2 h) (Reiter et al., 2001). The concentration of benzoylecgonine present in serum ranged from 27.0 – 207.0 ng/ml. The elimination time of cocaine in serum was not determined due to the short half-life of cocaine in blood (ca. 6 h).

1.2.4 Heroin and chemistry

Heroin (also referred to as diacetylmorphine or diamorphine) is derived from the plant *Papaver somniferum* L. (Negrusz & Cooper, 2013). It is one of the synthetic derivatives of the opium poppy, which are classified as opioids. The most commonly abused opioid derivative is heroin. It is administered through intravenous or subcutaneous injection, inhalation of vaporised heroin or nasal insufflation (snorting) (Wild, 2013; Negrusz & Cooper, 2013). Heroin acts as a depressant of the central nervous system and includes effects such as euphoria, analgesia, constipation, reduction in anxiety, respiratory depression and pinpoint pupils (Negrusz & Cooper, 2013). Regular heroin use leads to tolerance and physical dependence, thus higher doses are required to produce the same effect (Wild, 2013). Heroin is classified as class A controlled drug (Leg Gov, 1971). The molecular structure of heroin is illustrated in Figure 1.4.
Chapter 1: Introduction

1.2.4.1 Production of heroin

Heroin is synthesised from morphine which is contained in the seed of the opium poppy (Karch, 1998). The production of heroin from the opium poppy includes four steps, namely (i) the cultivation of opium poppy, (ii) collection of raw opium from the opium seed, (iii) isolation of morphine from raw opium and (iv) the acetylation of morphine to heroin (Karch, 1998). The seed heads of the plant are cut to release the liquid that contains morphine. The liquid dries on the seed head which is then collected as raw opium (Negrusz & Cooper, 2013). Morphine is extracted from the raw opium and acetylated using acetic anhydride to produce heroin (Negrusz & Cooper, 2013; Karch, 1998). Heroin can contain opium alkaloids including papaverine, noscapine and 6-acetymorphine (metabolite of heroin) as impurities from the production process (Karch, 1998). Cutting agents for heroin can include sugars, paracetamol and caffeine (Negrusz & Cooper, 2013).

1.2.4.2 Metabolism of heroin

Heroin is rapidly metabolised through enzymatic hydrolysis of the esters bond to form 6-acetylmorphine and subsequently morphine (Negrusz & Cooper, 2013; Karch, 1998; Kamendulis et al., 1996). The metabolic pathway for the major metabolites of heroin are shown in Figure 1.5. The free phenolic hydroxyl groups of morphine undergo further metabolism to produce water-soluble metabolites by conjugation with glucuronic acid to form morphine-3-glucuronide and morphine-6-glucuronide (Karch, 1998). Metabolism primarily occurs in the liver and the majority (90%) is excreted in the urine and the remaining 10% in faeces (Karch, 1998).

Figure 1.4: Molecular structure of heroin.

Heroin
Molecular weight 369.41 g/mol
Molecular formula C_{21}H_{23}NO_{5}
1.2.4.3 Detection window of heroin in biological matrices

6-Acetylmorphine has been detected in urine up to 2.3 h (max 5.1 h) after a low dose (3 – 7 mg) and up to 4.5 h (max 11.2 h) after a high dose (10.5 – 13.9 mg) of heroin administered intravenously or smoked (Smith et al., 2001). Morphine was detectable up to 4.3 h (max 10.1 h) after a low dose (3 – 7 mg) and 34.4 h (max 53.5 h) after a high dose (10.5 – 13.9 mg). Peak concentrations for morphine and 6-acetylmorphine in urine ranged from 1392 – 9250 ng/ml and 6.1 – 568 ng/ml, respectively. According to the study, the results obtained were not significantly dependent on the route of administration. Additionally, for chronic users the detection time for 6-acetylmorphine in urine was on average 5 h (max 34.5 h) with maximum concentrations ranging from 35 – 3699 ng/ml (Reiter et al., 2001). In contrast, the average detection time for morphine was 118.8 h (max 270.3 h) after the last administration to the body with maximum concentrations ranging from 280 – 2105929 ng/ml (Reiter et al., 2001).
6-Acetylmorphine has been detected in oral fluid for 1 – 2 h after smoked administration (dose 10.5 mg) and 1 – 4 h after intravenous administration (dose 12 – 20 mg) (Jenkins et al., 1995). Peak concentrations in oral fluid after smoking ranged from 3534 (2.6 mg) to 20580 ng/ml (5.2 mg). After intravenous administration, concentrations ranged from 6 (10 mg heroin HCl) to 30 ng/ml (12 mg heroin HCl). In chronic users, morphine and 6-acetylmorphine have been detected in oral fluid up to 3 and 8 days, respectively after last administration (Vindenes et al., 2014).

The detection time of heroin in whole blood was reported to be longer after intravenous injection compared to smoked administration (Jenkins et al., 1994). After smoking 10.5 mg of heroin, morphine was detected in whole blood up to 22 min – 2 h (Jenkins et al., 1994). In contrast, the active metabolite 6-acetylmorphine was detected up to 30 – 60 min. After intravenous administration of 12 or 20 mg of heroin, the detection time in whole blood was extended to 30 min – 2 h for 6-acetylmorphine and up to 8 h for morphine (Jenkins et al., 1994). In addition, 6-acetylmorphine and morphine have been detected in blood plasma up to 45 min and 12 h, respectively after 9 mg of intranasal administration (Goldberger et al., 1993). In chronic users (n = 51), morphine has been detected up to 5 days after the last administration to the body, with peak concentrations ranging from 29 – 1264 ng/ml (Reiter et al., 2001).

1.2.5 Biological matrices

There are numerous biological matrices that can be used for the detection of drugs of abuse, including blood (plasma, serum and whole blood), urine, hair and oral fluid. The matrix selected for drug testing purposes is influenced by a variety of factors, including ease of collection, analytical and testing considerations and the interpretation of results (Caplan & Goldberger, 2001). A summary of liquid chromatographic – mass spectrometry (LC-MS) approaches for the analysis of cocaine and heroin use across various matrices including performance characteristics is detailed in Table 1.2. Various sample preparation methods are used for the extraction of drugs from biological matrices, including liquid extraction, solid phase extraction (SPE) and protein precipitation. Overall, the sensitivity of the methods across the matrices is in the ng/ml range. The relative detection window of drugs in various biological matrices is shown in Figure 1.6 (Caplan & Goldberger, 2001). The elimination time depends on the dose of drug taken, method of administration, single or chronic drug use, number of drugs taken simultaneously, health and genetics of the user. The ability to
detect drugs also depends on the method of analysis, which affects the sensitivity (limit of detection and cut-off levels).

![Figure 1.6: Relative detection window of drugs in various biological matrices (Caplan & Goldberger, 2001).](image)

At NHS Drug and Alcohol Services, urine is the primary matrix used to determine drug use for individuals in treatment for drug dependency. Urine is preferred due to the ease of collection and longer detection window in comparison to blood. Additionally, a large volume can be obtained for analysis. Therefore, urine has been widely used in doping control, workplace drug testing, criminal justice and drug abuse treatment programmes (Allen, 2011; Bosker & Huestis, 2009; Chawarski et al., 2007). However, urine testing is also notorious for the potential for adulteration or substitution (Negrusz & Cooper, 2013).

Blood (mainly plasma or serum) can also be used for drug testing purposes. A principal advantage of a blood sample is that it is very difficult to adulterate and relates to the level of impairment, thus indicating recent drug use (Negrusz & Cooper, 2013; Dasgupta, 2010). This is because it is collected by venepuncture and needs to be collected by trained personnel (Negrusz & Cooper, 2013). In contrast, oral fluid is an alternative matrix that is more commonly used for drug testing (Bosker & Huestis, 2009). The main attribute of oral fluid samples is the ease and non-invasive sample collection. It is of particular interest in workplace drug testing and roadside drug testing (Negrusz & Cooper, 2013). Oral fluid is able to indicate recent drug use due to the rapid absorption from the blood into the salivary glands (Allen, 2011). However, the detection window for oral fluid is short and limited sample volume (1 – 5 ml) is collected (Negrusz & Cooper, 2013; Allen, 2011). Interestingly, it is difficult to differentiate heroin use from morphine or codeine administration using urine testing. However, 6-acetylmorphine is solely indicative of heroin use and is readily detected in oral fluid samples (Bosker & Huestis, 2009).
Table 1.2: LC-MS procedures and performance characteristics for the analysis of cocaine and heroin use in biological matrices.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Sample type</th>
<th>Extraction method</th>
<th>Column type</th>
<th>Mobile phase</th>
<th>Instrumentation</th>
<th>Type of measurement</th>
<th>Limit of detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC, BZE, MOR, 6-AM including other analytes</td>
<td>Urine</td>
<td>SPE</td>
<td>BEH C18 column (50 x 2.1 mm, 1.7 ( \mu \text{m} ))</td>
<td>Solvent A: 5 mM ammonium bicarbonate buffer (pH 10)</td>
<td>Acquity UPLC coupled to a Waters Quattro premie Xe mass spectrometer</td>
<td>Quantitative</td>
<td>0.001 - 0.02 ( \mu \text{g/ml} )</td>
<td>(Berg et al., 2009)</td>
</tr>
<tr>
<td>COC, BZE, EME, AME, CE, NCOC, MOR, HER, 6-AM</td>
<td>Hair</td>
<td>SPE</td>
<td>Atlantis® T3 C18 column (150 x 2.1 mm, 3 ( \mu \text{m} ))</td>
<td>Solvent A: 2mM ammonium formate (pH 3)</td>
<td>Perkin-Elmer Series 200 HPLC coupled to an AB Sciex API 2000 triple quadrupole mass spectrometer</td>
<td>Quantitative</td>
<td>0.005 and 0.030 ng/mg</td>
<td>(Imbert et al., 2014)</td>
</tr>
<tr>
<td>COC, BZE, MOR, 6-AM including other analytes</td>
<td>Hair and oral fluid</td>
<td>Protein precipitation</td>
<td>Alltima C18 column (250 x 4.6 mm, 5 ( \mu \text{m} ))</td>
<td>Solvent A: Acetonitrile + 5 mM formic acid</td>
<td>Perkin-Elmer Series 200 HPLC cuplted to an AB Sciex API 2000 triple quadrupole mass spectrometer</td>
<td>Quantitative</td>
<td>Hair LOD: 0.2 - 2.8 ng/ml</td>
<td>(Seri et al., 2009)</td>
</tr>
<tr>
<td>BZE, MOR</td>
<td>Human serum, urine and post-mortem (PM) blood</td>
<td>Protein precipitation</td>
<td>Luna C18 column (150 x 2 mm, 5 ( \mu \text{m} ))</td>
<td>Solvent A: 10 mM ammonium acetate in 95.5 (v/v) water/methanol + 0.1% acetic acid</td>
<td>Shimadzu HPLC coupled to an API 4000 Qtrap mass spectrometer</td>
<td>Qualitative</td>
<td>Serum: 0.01 - 0.67 ng/mL</td>
<td>(Dziadosz et al., 2018)</td>
</tr>
</tbody>
</table>

COC: cocaine; BZE: benzoylecgonine; EME: ecgonine methyl ester; AME: anhydromethylecgonine; CE: cocaethylene; NCOC: norcocaine; HER: heroin; MOR: morphine; 6-AM: 6-acetylmorphine
<table>
<thead>
<tr>
<th>Analytes</th>
<th>Sample type</th>
<th>Extraction method</th>
<th>Column type</th>
<th>Mobile phase</th>
<th>Instrumentation</th>
<th>Type of measurement</th>
<th>Limit of detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC, BZE, EME, ECG, NCOC, CE, EEE, NEEE</td>
<td>Whole blood</td>
<td>SPE</td>
<td>Atlantis® T3 C18 column (150 x 2.1 mm, 3 µm)</td>
<td>Solvent A: 0.1% formic acid in water</td>
<td>Shimadzu HPLC coupled to an AB Sciex triple TOF™ mass spectrometer</td>
<td>Quantitative</td>
<td>0.2 - 0.9 ng/ml LOQ: 1.9 - 320 ng/ml</td>
<td>(Chen et al., 2017)</td>
</tr>
<tr>
<td>COC, BZE, 6-AM, MOR including other analytes</td>
<td>Oral fluid</td>
<td>SPE</td>
<td>Allure PFP propyl column (50 x 2.1 mm, 5 µm)</td>
<td>Solvent A: 0.1% formic acid, 2 mM ammonium acetate, and 2% acetonitrile in water</td>
<td>Agilent 1100 HPLC coupled to a Sciex API 3200 mass spectrometer</td>
<td>Quantitative</td>
<td>LOQ: 0.4 - 2.0 ng/ml</td>
<td>(Cone, 2012)</td>
</tr>
<tr>
<td>HER, 6-AM, MOR including other analytes</td>
<td>Urine</td>
<td>SPE</td>
<td>Restek Pinnacle® DB C18 column (100 x 2.1, 3 µm)</td>
<td>Solvent A: 10 mM ammonium acetate + 0.1% formic acid in water</td>
<td>Shimadzu LC-20AD HPLC coupled to an MDS Sciex API 3200 mass spectrometer</td>
<td>Quantitative</td>
<td>LOQ: 2 - 80 ng/ml</td>
<td>(Knight et al., 2014)</td>
</tr>
<tr>
<td>COC, BZE, NCOC, HER, MOR, 6-AM, M3G, M6G including other analytes</td>
<td>Human plasma</td>
<td>SPE</td>
<td>Zorbax bonus RP column (150 x 4.6 mm, 5 µm)</td>
<td>Solvent A: 5 mM ammonium formate, pH 4.0 Solvent B: acetonitrile</td>
<td>Agilent 1100 HPLC coupled to Sciex API 3000 mass triple quadrupole mass spectrometer</td>
<td>Quantitative</td>
<td>Not specified</td>
<td>(Rook et al., 2005)</td>
</tr>
</tbody>
</table>

COC: cocaine; BZE: benzoylecgonine; EME: ecggonine methyl ester; ECG: ecggonine; CE: cocaethylene; NCOC: norcocaine; EEE: ecggonine ethyl ester; NCE: norcocaethylene; NEEE: norecgonine ethyl ester; HER: heroin; MOR: morphine; 6-AM: 6-acetylmorphine; M3G: morphine-3-glucuronide; M6G: morphine-6-glucuronide
<table>
<thead>
<tr>
<th>Analytes</th>
<th>Sample type</th>
<th>Extraction method</th>
<th>Column type</th>
<th>Mobile phase</th>
<th>Instrumentation</th>
<th>Type of measurement</th>
<th>Limit of detection</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| COC, BZE, EME, NCCO, ECG         | Whole blood and urine | SPE                 | Zorbax SB-C18 column (30 x 2.1 mm, 3 µm) | Solvent A: 5% acetonitrile + 0.05% formic acid  
Solvent B: acetonitrile + 0.05% formic acid | Agilent 1100 HPLC coupled to a Waters Quattro micro quadrupole mass spectrometer | Quantitative                     | Whole blood LOD: 0.001 - 0.003 mg/kg  
LOQ: 0.05 - 0.008 mg/kg | (Johansen & Bhatia, 2007) |
| HER, 6-AM, MOR, M3G, M6G         | Human plasma        | Protein precipitation | X-Bridge phenyl column (150 x 4.6 mm, 5 µm) | Solvent A: 5 mM ammonium formate, pH 3.0  
Solvent B: Acetonitrile + 0.1% formic acid | Waters Alliance 2795 HPLC coupled to a Quatro Premier mass spectrometer | Quantitative                     | LLOQ: 10 ng/ml | (Moreno-Vicente et al., 2015) |
| COC, BZE, CE, HER, 6-AM, MOR     | Breast and bovine milk | Protein precipitation | Purospher star RP-18 (125 x 2.0 mm, 5 µm) | Solvent A: acetonitrile  
Solvent B: Water + 20 mM formic acid/ammonium formate buffer (pH 3.8) | Symbiosis™ Pico HPLC coupled to an AB Sciex 4000 Qtrap mass spectrometer | Quantitative                     | 0.2 - 5 ng/ml  
LOQ: 0.5 - 10 ng/ml | (López-García et al., 2018) |
| BZE, EME, 6-AM, MOR, M3G, M6G    | Urine               | Dilution or enzymatic cleavage | AQUASIL C18 (100 x 2.1 mm, 5 µm) | Solvent A: 10 M ammonium acetate in water  
Solvent B: 10 mM ammonium acetate in 1:1 (v/v) methanol: acetonitrile | Shimadzu Prominence UFLC coupled to an AB Sciex 5500 Qtrap mass spectrometer | Quantitative                     | LLOQ: 5 - 25 ng/ml | (de Jager & Bailey, 2011) |
| COC, BZE, EME, NCCO, CE, MOR, 6-AM | Hair              | SPE                 | Synergi hydro RP column (150 x 2.0 mm, 4 µm) | Solvent A: 10 mM ammonium acetate in water + 0.001% formic acid  
Solvent B: Acetonitrile | Shimadzu LC-20AD HPLC coupled to an MDS Sciex API 3000 triple quadrupole mass spectrometer | Quantitative                     | LOQ: 17 - 83 pg/mg | (Scheidweiler & Huestis, 2004) |

COC: cocaine; BZE: benzoylecgonine; EME: ecgonine methyl ester; CE: cocaethylene; NCCO: norcocaine; HER: heroin; MOR: morphine; 6-AM: 6-acetylmorphine; M3G: morphine-3-glucuronide; M6G: morphine-6-glucuronide
Table 1.2 continued...

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Sample type</th>
<th>Extraction method</th>
<th>Column type</th>
<th>Mobile phase</th>
<th>Instrumentation</th>
<th>Type of measurement</th>
<th>Limit of detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC and BZE</td>
<td>Hair</td>
<td>Molecular imprinted polymers</td>
<td>SymmetryShield RP18 column (150 x 2.1 mm, 3.5 µm)</td>
<td>Isocratic: 80% ammonium formate (5 mM, pH 3.2) and 20% acetonitrile</td>
<td>Agilent 1200 HPLC coupled to a triple quadrupole mass spectrometer</td>
<td>Quantitative</td>
<td>0.01 - 0.02 ng/mg LOQ: 0.04 - 0.07 ng/mg</td>
<td>(Thibert et al., 2012)</td>
</tr>
<tr>
<td>6-AM, MOR including other analytes</td>
<td>Hair</td>
<td>Solvent extraction</td>
<td>Porosell 120 EC-C18 column (50 x 3.0 mm, 2.7 µm)</td>
<td>Solvent A: 5 mM ammonium formate and 0.01% formic acid in water Solvent B: Acetonitrile + 0.01% formic acid</td>
<td>Agilent 1260 Infinity HPLC coupled to a 6460 triple quadrupole mass spectrometer</td>
<td>Quantitative</td>
<td>0.05 - 0.25 ng/10 mg LOQ: 0.05 - 0.5 ng/10 mg</td>
<td>(Kim et al., 2014)</td>
</tr>
<tr>
<td>M3G, MOR, BZE, COC</td>
<td>Urine</td>
<td>SPE</td>
<td>Xterra® MS C18 column (50 x 3.0 mm, 2.5 µm)</td>
<td>Solvent A: 5 mM ammonium acetate with 0.05% acetic acid Solvent B: Acetonitrile</td>
<td>Agilent 1100 HPLC coupled to a API 4000 mass spectrometer</td>
<td>Quantitative</td>
<td>9.00 - 2.29 pg/ml</td>
<td>(Feng et al., 2007)</td>
</tr>
<tr>
<td>COC, BZE, EME, NCOC</td>
<td>Rat brain, peripheral organs and plasma</td>
<td>solvent extraction and centrifugation</td>
<td>LiChropher 60-RP select B (125 x 4.6 mm, 5 µm)</td>
<td>Solvent A: 0.1% formic acid in water Solvent B: 0.1% formic acid in acetonitrile</td>
<td>Agilent 1100 coupled to a API 2000 mass spectrometer</td>
<td>Quantitative</td>
<td>0.05 µg/g</td>
<td>(Bystrowska et al., 2012)</td>
</tr>
<tr>
<td>COC, BZE, NCOC, CE</td>
<td>Hair</td>
<td>Protein precipitation</td>
<td>Kinetex C18 (50 x 3 mm, 2.6 µm)</td>
<td>Solvent A: 2M ammonium acetate in 5% acetonitrile with 0.02% formic acid Solvent B: 2 mM ammonium acetate in 95% acetonitrile</td>
<td>Agilent 1290 coupled to an Sciex 6500 Qtrap mass spectrometer</td>
<td>Quantitative</td>
<td>0.0202 - 0.005 ng/mg LLOQ: 0.01 ng/mg</td>
<td>(Franz et al., 2018)</td>
</tr>
</tbody>
</table>

COC: cocaine; BZE: benzoylecgonine; EME: ecgonine methyl ester; NCOC: norcocaine; CE: cocaethylene; MOR: morphine; 6-AM: 6-acetylmorphine; M3G: morphine-3-glucuronide
Hair is another matrix that has been used to determine drug use. It enables a determination of the history of drug exposure (Negrusz & Cooper, 2013; Caplan & Goldberger, 2001). Hair analysis allows for a longer detection window (weeks to months), depending on the length of hair used for analysis. The collection of a hair sample is easy, non-invasive and not easily substituted (Negrusz & Cooper, 2013). However, the main limitation is that it cannot be used to determine recent drug use (Negrusz & Cooper, 2013; Dasgupta, 2010). Additionally, the influence of cosmetic treatment and potential for external contamination pose a concern for hair drug testing (Negrusz & Cooper, 2013).

1.2.6  Fingertips and drug testing

Fingertip samples have been used for the detection of different drugs of abuse. A few studies have shown the potential use of fingerprints to determine the presence of drugs using liquid chromatography – mass spectrometry (LC-MS) procedures (Zhang et al., 2015; Kuwayama et al., 2014; Kuwayama et al., 2013; Goucher et al., 2009; Jacob et al., 2008). These LC-MS methods have successfully detected methadone, lorazepam, methamphetamine, caffeine, and a cold medicine containing ibuprofen, dihydrocodeine, methylephedrine and chlorpheniramine in fingerprints. A summary of LC-MS methods used for the analysis of drugs in fingerprints, including extraction method and performance characteristics is detailed in Table 1.3. In most studies, the participants administered the drug prior to fingerprint deposition (Kuwayama et al., 2014; Kuwayama et al., 2013; Goucher et al., 2009). The remaining two utilised participants who had a known history of drug abuse to determine the presence of methadone and methamphetamine in fingerprints, respectively (Zhang et al., 2015; Jacob et al., 2008). In this PhD project, fingerprint samples were collected from individuals who had a known history of drug abuse, rather than carrying out an administration study.

Another method has been reported for the detection of cocaine in fingerprints from individuals seeking treatment for drug dependency using paper spray mass spectrometry (Costa et al., 2017). The method has previously been used for the analysis of dried blood spots (Espy et al., 2014; Su et al., 2013; Manicke et al., 2011). Although the method offers similar sensitivity to LC-MS and high throughput analysis, it lacks chromatographic separation which results in lower selectivity and precision than LC-MS.
Table 1.3: LC-MS procedures and performance characteristics for the analysis of drugs in fingerprints.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Extraction method</th>
<th>Type of measurement</th>
<th>Instrumentation</th>
<th>Limit of detection</th>
<th>% Recovery</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine and methamphetamine</td>
<td>Fingermark swabbed with cotton swab (moistened with 50 µl MeOH:H₂O) 60 times. Swab placed in tube with 0.25 ml MeOH and sonicated for 4 min. Mixture was centrifuged for 5 min and 20 µl of supernatant used for analysis</td>
<td>Quantitative</td>
<td>Finnigan Surveyor liquid chromatography and LXQ ion trap mass spectrometer</td>
<td>1.5 ng/fingerprint swab LOQ: 5.0 ng/fingerprint swab</td>
<td>70 - 87</td>
<td>(Zhang et al., 2015)</td>
</tr>
<tr>
<td>Caffeine, theobromine, paraxanthine and theophylline</td>
<td>Paper substrate containing fingerprint was placed in plastic dish with extraction solvent (1.9 ml of MeOH:H₂O, 1:4 (v/v)). Dish was shaking for 3 min and the sample extract transferred to a tube and centrifuged for 1 min, 25 µl of the supernatant was used for analysis.</td>
<td>Quantitative</td>
<td>Shiseido Nanospace SI-2 and Thermo Fisher Scientific TSQ Quantum Ultra mass spectrometer</td>
<td>LLOQ: 0.5 - 5 ng/fingerprint</td>
<td>67 - 81</td>
<td>(Kuwayama et al., 2013)</td>
</tr>
<tr>
<td>Ibuprofen, dihydrocodeine, chlorpheniramine, methylephedrine and ephedrine</td>
<td>Paper substrate containing fingerprint was placed in a tube with 1 ml extraction solution (MeOH:H₂O, 1:4 (v/v)). Tube was shaken for 1 min and centrifuged for 1 min, 50 µl of the supernatant was used for analysis.</td>
<td>Quantitative</td>
<td>Waters AQUITY UPLC I-Class and a Xevo TQ-S LC–mass spectrometer</td>
<td>0.1 - 10 pg/fingerprint LOQ: 0.1 - 100 pg/fingerprint</td>
<td>58 - 95</td>
<td>(Kuwayama et al., 2014)</td>
</tr>
</tbody>
</table>
Table 1.3 continued…

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Extraction method</th>
<th>Type of measurement</th>
<th>Instrumentation</th>
<th>Limit of detection</th>
<th>% Recovery</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methadone and EDDP</td>
<td>Glass cover slip containing fingerprint was placed in a glass vial with a solvent mixture (MeOH:DCM, 2:5 (v/v)). Vial was sonicated for 15 min. The sample extract transferred to an LC-MS vial, evaporated to dryness and reconstituted, 20 µl of the sample was used for analysis.</td>
<td>Quantitative</td>
<td>Waters AQUITY UPLC and Waters Micromass Quattro Premier mass spectrometer</td>
<td>0.01 ng/fingerprint</td>
<td>Not specified</td>
<td>(Jacob et al., 2008)</td>
</tr>
<tr>
<td>Lorazepam and lorazepam- glucuronide</td>
<td>Glass cover slip containing fingerprint was placed in a glass vial with a solvent mixture (MeOH:DCM, 1:1 (v/v)). Vial was sonicated for 1 min and shaken for 15 min. Sample extract was transferred to a clean glass vial, evaporated to dryness and reconstituted before being vortex-mixed and transferred to an LC-MS vial (10 µl used for analysis).</td>
<td>Quantitative</td>
<td>Thermo Scientific Accela LC and Thermo TSQ Quantum Access mass spectrometer</td>
<td>1.5 - 8 pg LOQ: 5 - 30 pg</td>
<td>96 - 100</td>
<td>(Goucher et al., 2009)</td>
</tr>
</tbody>
</table>
Fingerprints offer the advantage that it is easy to collect, non-invasive and can be used for identification purposes. The use of a non-invasive sample collection places a smaller mental and physical burden on the individual (Kuwayama et al., 2014). Additionally, fingerprints can be collected on-site by a non-specialist. Fingerprint sample preparation is simple because there is a smaller amount of matrix component present in fingerprints (Kuwayama et al., 2014). However, the detection of drugs of abuse in fingerprints does require sensitive analytical instrumentation. Although oral fluid drug testing is non-invasive, proper sample storage of the sample matrix is required to prevent drug degradation due to digestive enzymes present (Kuwayama et al., 2014). Furthermore, the use of a buffer solution in oral fluid collection devices, dilutes the oral fluid sample and thus affects sensitivity or requires a sample preparation method.

In the reviewed methods, a fingerprint collection protocol is commonly performed to produce as similar fingerprint depositions as possible. Jacob et al. (2008) and Goucher et al. (2009) performed a grooming procedure after cleaning the hands, which involved rubbing the fingers against the forehead and face prior to deposition. This method results in a sebum-rich fingerprint. Kuwayama et al. (2013) and Kuwayama et al. (2014) utilised a different method, which included a 30 s. waiting period with the hands open prior to fingerprint deposition. This method produces an eccrine-rich fingerprint. Both of these methods do not necessarily represent the actual composition of fingerprints, as this is an unknown proportion of eccrine and sebaceous secretions. However, the sebum-rich fingerprint is possibly more frequently encountered due to human behaviour (touching of the face).

The sample preparation steps for the extraction of fingerprint samples typically use an extraction solution containing either methanol and dichloromethane or water, and an internal standard. After addition of the extraction solution, the sample matrix is normally sonicated, evaporated to dryness and reconstituted in the mobile phase (Goucher et al., 2009; Jacob et al., 2008). However, some studies performed another sample preparation step, which only included shaking and centrifugation of the sample matrix after which the supernatant was injected into the injector system (Zhang et al., 2015; Kuwayama et al., 2014; Kuwayama et al., 2013). In the reviewed methods, the extraction efficiency from glass cover slips produced a better recovery (90 – 100%) (Goucher et al., 2009). The recovery of analytes from wetted filter paper produced recoveries of 57.7 – 95.5% (Kuwayama et al., 2014; Kuwayama et al., 2013).
Zhang et al. (2015) used a cotton swab to collect fingerprints for drug testing purposes (Zhang et al., 2015). Results showed that even without employing a washing procedure prior to fingerprint collection, methamphetamine and amphetamine could be detected in fingerprints (Zhang et al., 2015). The advantage of detecting the main metabolite (amphetamine) in fingerprints is that it shows that the drugs were present due to misuse of the drug. However, the paper does not mention the use of internal standards as a reference for the matrix effects (ion suppression/enhancement) or recovery studies performed.

1.2.6.1 Challenges associated with fingerprint testing

The use of fingerprints for chemical analysis (e.g. drug testing) is a relatively new area of research. Previous research has shown the possibility to detect drugs in fingerprints, but no research has investigated the reliability of the sample matrix itself. Fingerprint testing requires advances in the standardisation of a fingerprint sampling procedure and validation procedures, together with the evaluation of measurement uncertainty and its impact on interpretation criteria (Wille, 2018). The variable nature of a fingerprint sample, including amount of volume deposited and sample size varies between individuals as well as within an individual. This influences the interpretive value of the amount of drug detected in a sample. Additionally, in the absence of a validated fingerprint standard, artificial eccrine sweat has been used in validation procedures to simulate a fingerprint matrix and this is investigated further in Chapter 2. However, at present, quantification has been attempted using nonmatrix-matched calibration curves, which can pose a problem if matrix effects occur in the presence of fingerprints. Reporting results in terms of analyte-to-internal standard ratio also possess a concern as it is non-universal and dependent on the amount of internal standard used.

One of the major concerns and challenges associated with fingerprint testing is the possible presence of contamination either from contact with contaminated surfaces or handling the drug, which are less likely to be encountered in other biological matrices. It is known that traces of cocaine can be found on banknotes (Armenta & de la Guardia, 2008; Carter et al., 2003). Additionally, traces of cocaine (50 ng/swab) have been found on the skin of non-cocaine users or individuals living with drug users (20 µg/swab) (Kidwell et al., 2003). This highlights the importance of the interpretation of results and differentiation between the presence of drugs from external contact residue (non-drug users) and active use (drug users). Advances in hair analysis have led to the use of cut-off levels, which are used as a decision
criterion to determine whether levels are observed above external exposure levels (Cooper et al., 2012). This could potentially be applied to fingerprint testing if environmental exposure poses a problem. Another important factor to consider for fingerprint testing is the detection time of drugs. No research (to our knowledge) has evaluated the detection window of drugs in fingerprint samples and this is an important aspect that should be evaluated to advance fingerprint drug testing.

1.3 Introduction to Tuberculosis

The use of fingerprints for drug testing can have a multitude of applications, including the use of therapeutic drug monitoring. Using fingerprints to monitor compliance has the potential to increase adherence to treatment and improve success rates. This is of particular interest for tuberculosis treatment, where drug resistant tuberculosis can emerge with inadequate adherence or suboptimal adsorption of the antibiotics. Conventional methods to monitor compliance to treatment can include the use of a blood sample, which has associated biohazard risks. An alternative approach using fingerprints is investigated in this PhD project to evaluate treatment adherence to tuberculosis medication.

1.3.1 Tuberculosis and human health

Tuberculosis is an infectious disease caused by the bacteria Mycobacterium tuberculosis (World Health Organisation, 2017). It primarily affects the lungs but can also affect other parts of the body. The disease is easily spread by individuals affected by expelling the bacteria into the air (e.g. by coughing) (World Health Organisation, 2017). Tuberculosis is a curable infectious disease, yet it is one of the top 10 leading cause of death worldwide (World Health Organisation, 2017). In 2016, an estimated 1.7 million deaths were attributed to tuberculosis (World Health Organisation, 2017). Most of these deaths could be prevented by early diagnosis and appropriate treatment. An estimated 10.4 million cases of latent (inactive) tuberculosis were reported in 2016 (World Health Organisation, 2017). The majority of the cases in 2016 occurred in countries in South-East Asia, including India, Indonesia, China, the Philippines and Pakistan (World Health Organisation, 2017).

In the UK, 5,664 cases of tuberculosis were recorded in 2016 of which 59% were male (Public Health England, 2017). Seventy-four percent (4096 cases) of tuberculosis incidences were from non-UK born individuals (Public Health England, 2017). In 2016, the rate of tuberculosis in non-UK born population was 15 times higher than that of the UK born
population (Public Health England, 2017). Additionally, the majority of tuberculosis in the non-UK born population was observed for individuals aged >75 years and older, followed by those aged between 25 – 29 years (Public Health England, 2017).

1.3.2 Treatment and adherence

The World Health Organisation (WHO) has initiated the End of TB strategy 2016 – 2035, with the aim of ending the global tuberculosis epidemic (World Health Organisation, 2017). The recommended treatment for individuals diagnosed with tuberculosis is a combination of four antibiotics for 6 months as the first-line treatment drugs. These include isoniazid, rifampicin, ethambutol and pyrazinamide (World Health Organisation, 2017; Wang et al., 2016; Reynolds & Heysell, 2014). The combination of the four drugs are taken for two months, followed by the use of isoniazid and rifampicin only for the subsequent four months. The dose prescribed for the course of treatment is dependent on the weight of the individual.

Drug resistant tuberculosis is a continuing threat to treatment programmes to eradicate the disease. Drug resistant tuberculosis can emerge because individuals are resistant to a particular antibiotic (primary drug resistance) or can develop resistance (acquired resistance) (Public Health England, 2017; Reynolds & Heysell, 2014). Acquired drug resistant tuberculosis can be attributed to non-adherence as well as suboptimal drug concentrations in treatment regimens (Reynolds & Heysell, 2014). A number of 490,000 million cases emerged worldwide in 2016 with multidrug-resistant tuberculosis (World Health Organisation, 2017; Reynolds & Heysell, 2014; Jnawali & Ryoo, 2013). In the UK, 68 cases of drug-resistant tuberculosis were confirmed (Public Health England, 2017). Treatment for multidrug resistant tuberculosis is longer and requires more expensive and toxic drugs as the second-line drugs (World Health Organisation, 2017; Jnawali & Ryoo, 2013). The second-line drugs often cause severe side effects and thus make adherence for patients more difficult (Parliamentary Office of Science and Technology, 2012). Treatment of multidrug-resistant tuberculosis can last more than 20 months and success rates can be as low as 33% (Reynolds & Heysell, 2014). The cost of treatment for tuberculosis varies between countries, but is around £5,000 per patient in the United Kingdom (Parliamentary Office of Science and Technology, 2012). However, for drug resistant tuberculosis the overall cost can be between £50,000 - £70,000 per patient (Parliamentary Office of Science and Technology, 2012).

There are a number of methods that can be applied to test or monitor for adherence to tuberculosis medication. Directly observed treatment, short-course (DOTS) is the World
Health Organisation tuberculosis control strategy (World Health Organisation, 1999). In DOTS, patients are observed taking their medication to ensure the drugs are taken and for the appropriate duration (World Health Organisation, 1999). DOTS is primarily considered for individuals who have a higher social risk factors (e.g. drug and alcohol related problems, and homelessness) (World Health Organisation, 1999). With the use of DOTS the responsibility to adhere to treatment is not solely reliant on the patient. The use of DOTS therefore improves compliance with tuberculosis treatment and completion rates (London Health Programmes, 2011). However, it has been reported that DOTS is not always available for the patients who would benefit or require the assistance (London Health Programmes, 2011). Other methods rely on clinical improvement, tablet counts and engagement with tuberculosis nursing teams to help assess adherence. Additionally, a urine dipstick test is used on the spot if there are any concerns or doubts about the patient. However, these are not readily used by clinicians due to a limited shelf life (12 months) and cost of the urine dipstick test (£7 per sample), which is not effective in terms of cost-benefit analysis. Furthermore, the urine dipstick test is based on a colorimetric test and cannot provide information on the drug levels present in the urine sample. An alternative strategy to improve tuberculosis treatment completion by monitoring compliance and absorption of the drug would therefore be advantageous.

1.3.3 Isoniazid and chemistry

Isoniazid is the most widely used drug for tuberculosis treatment (Starke, 2012). This is because the drug is effective in killing Mycobacterium tuberculosis, inexpensive and easily administered (Starke, 2012). There are little to no side effects associated with the use of isoniazid (Blomberg, 2009). However, mild elevation in liver enzymes and on rare occasion hepatitis have been reported as side effects from the use of isoniazid in tuberculosis treatment (Blomberg, 2009). The standard treatment dose of isoniazid (adult dose) is 5 mg/kg with a maximum dose of 300 mg taken daily (Van Crevel & Hill, 2017). The duration of treatment is generally 6 months for drug-susceptible tuberculosis (Van Crevel & Hill, 2017). The molecular structure of isoniazid is illustrated in Figure 1.7. It is a small water-soluble molecule and has a molecular weight of 137.14 g/mol.
Once isoniazid is administered orally, it is primarily metabolised through enzymatic pathways (Tostmann et al., 2008). Isoniazid is converted to acetylisoniazid through acetylation and subsequent hydrolysis forms acetylhydrazine and isonicotinic acid (Figure 1.8) (Tostmann et al., 2008; Ellard & Gammon, 1976). Acetylhydrazine is further metabolised into diacetyldihydrazine (through acetylation) or hydrolysed to form hydrazine (Tostmann et al., 2008). The rate of acetylation in humans is genetically determined and can be divided into two groups, namely slow and fast acetylators (Ellard & Gammon, 1976). Approximately half the population of African and Caucasian descent are fast acetylators with elimination half-lives of 0.5 – 1.5 h (Blomberg, 2009).

For slow acetylators, the half-lives of isoniazid is between 2 – 4 h. The majority (80 – 90%) of individuals from Chinese and Japanese descent are fast acetylators (Blomberg, 2009). The acetylation status of an individual has no influence on the medication dose of isoniazid that
is prescribed during tuberculosis treatment (Blomberg, 2009). However, individuals with slow acetylation have an increased risk to certain side effects associated with isoniazid, such as hepatotoxicity (liver damage) and peripheral neuropathy (damage to the peripheral nervous system) (Wang et al., 2016; Blomberg, 2009; Tostmann et al., 2008).

1.3.3.2 Detection of isoniazid in biological matrices

Different methods have been applied for the detection of isoniazid in biological matrices, such as urine and blood (plasma and serum). No study (to our knowledge) has investigated the detection time of isoniazid in biological matrices. However, the use of LC-MS is most commonly used for the analysis of isoniazid, particularly in pharmacokinetic studies as it can provide quantitative information (Want et al., 2003). A number of studies have reported the use of LC-MS analysis for the detection of isoniazid and its respective metabolite acetylisoniazid in urine and blood (plasma and serum) (Seng et al., 2015; Mukherjee et al., 2015; Amlabu et al., 2014; Thee et al., 2011; McIleron et al., 2009). However, the majority of studies utilised a colorimetric test (referred to as the Arkansas method) to determine the presence of isoniazid in urine using a number of reagents which produce a blue colour in the presence of isoniazid metabolites (Nicolaou et al., 2012; Guerra et al., 2010; Schmitz et al., 2010; Hanifa et al., 2007; Eidlitz-Markus et al., 2003). Although this is a low-cost alternative, this method is not as selective compared to mass spectrometry methods. The use of LC-MS analysis for the detection of isoniazid and its respective metabolite acetylisoniazid in fingerprints is investigated in this research, due to the high selectivity associated with the use of mass spectrometry analysis which allows qualitative identification of the analytes of interest.

1.4 Aim and Objectives

The primary aim of this research is to investigate the forensic potential of detecting drugs (in particular cocaine, heroin and their respective metabolites) in human fingerprints. A liquid chromatography – mass spectrometry (LC-MS) method will be developed and validated for the qualitative confirmation/identification of cocaine, benzoylecgonine, heroin and 6-acetylmorphine. The application of this method to determining any substance misuse using fingerprint samples will be explored. Important aspects of using a fingerprint as a new sampling matrix will be investigated, including (i) the impact of a hand cleaning procedure (washing hands or wiping hands with alcohol free wipes), (ii) sampling issues in relation to
environmental contamination (through the collection of fingerprints from a background population of non-drug users), and (iii) the potential of secondary transfer of drugs. The impact of these on the interpretation of results is important to consider in order to allow for a correct application of a fingerprint test. Additionally, the presence of cocaine and heroin after dermal contact with the parent drug will be explored (including the use of hand cleaning procedures) to evaluate whether a fingerprint test is suitable to determine substance misuse. The secondary aim of this research is to explore the potential use of fingerprints in a clinical application, in particular monitoring compliance with tuberculosis medication (isoniazid). The final aim of this research includes a pilot research study to evaluate the use of a portable screening system for drug testing purposes in well-established and conventional matrices, such as, urine and an oral fluid (saliva).

1.4.1 Objectives

The objectives of this study are to:

- review the literature on methods and procedures used for the detection of cocaine and heroin;
- obtain ethical approval from the NHS REC and the University of Surrey for the collection of biological samples from study participants;
- develop and validate an extraction method for the preparation of fingerprint samples for LC-MS analysis according to the Scientific Working Group for Forensic Toxicology guidelines;
- determine the detection rates of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprint samples from (i) individuals seeking treatment for drug dependency at NHS drug and alcohol services, and (ii) a negative control group (background population of non-drug users) using different fingerprint sampling strategies, including (a) natural fingerprints, (b) wiping hands with alcohol free wipes, (c) washing hands with soap and water, and (d) secondary transfer through handshaking;
- determine the detection rates of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprint samples after dermal contact with 2 mg of the parent drug using different fingerprint sampling strategies, including (a) natural fingerprints, (b) wiping hands with alcohol free wipes, (c) washing hands with soap and water, and (d) secondary transfer through handshaking;
• evaluate the detection of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprints and the requirement for a decision level (i.e. to differentiate non-drug user and drug users);
• determine the detection rates of isoniazid and acetylisoniazid in fingerprint samples from individuals (i) receiving treatment for tuberculosis, (ii) who completed treatment (2 – 8 months prior to recruitment) and (iii) a negative control group using different fingerprint sampling strategies, including (a) natural fingerprints and (b) washing hands with soap and water;
• evaluate the suitability of fingerprint samples to monitor compliance with tuberculosis treatment by investigating the elimination profile (T = 0, T = 2 and T = 4 days) after ceasing treatment; and to
• evaluate the suitability of a portable mass spectrometry approach for the detection of cocaine and benzoylecgonine in urine and oral fluid (saliva) samples from individuals receiving treatment for drug dependency at NHS drug and alcohol services.

The potential use of fingerprints for chemical analysis (i.e. drug testing) is increasingly receiving more interest due to its non-invasive approach, ease of sample collection and identification (embedded in the fingerprint ridge detail). Various studies have shown the potential of using fingerprints to determine the presence of drugs, such as methadone, lorazepam, caffeine, cold medicine, cocaine and methamphetamine (Zhang et al., 2015; Kuwayama et al., 2014; Kuwayama et al., 2013; Goucher et al., 2009; Jacob et al., 2008). However, no study (to our knowledge) has evaluated the potential significance of the detection of drugs in fingerprints. The development of an LC-MS method for the analysis of cocaine and heroin in fingerprints, including a fingerprint extraction method will be explored in Chapter 2. The detection of cocaine and heroin use in fingerprints from drug users and a background population of non-drug users is explored in Chapter 3. The presence of cocaine and heroin after dermal contact with the parent drug in fingerprints from non-drug users is investigated in Chapter 4. The application of fingerprints to determine the presence of tuberculosis medication in fingerprints from patients receiving treatment is explored in Chapter 5. Lastly, the potential use of a portable mass spectrometer for the analysis of cocaine use in oral fluid and urine samples is explored in Chapter 6.
Chapter 2 Analytical Methodology

2.0 Introduction

In order to meet the proposed aim and objectives of the research study (as described in Chapter 1 section 1.4) an analytical sequence was set up, as illustrated in Figure 2.1. This chapter describes the procedures applied for the liquid chromatography – mass spectrometry (LC-MS) analysis used in this study at the University of Surrey. The development of a liquid chromatography – mass spectrometry (LC-MS) method for the analysis of cocaine, heroin and their respective metabolites, benzoylecgonine and 6-acetylmorphine in fingerprint samples is described in section 2.1. In order to evaluate the suitability of detecting these compounds in fingerprints, a method was developed for the extraction of the analytes of interest from artificial eccrine perspiration, acetonitrile and fingerprint samples deposited on glass cover slips and paper substrates (section 2.2). The developed extraction method was used for the analysis of all fingerprint samples in the proceeding chapters. The performance characteristics of the developed sample preparation method and LC-MS method are evaluated in section 2.3.

2.1 Liquid Chromatography – Mass Spectrometry (LC-MS)

Chromatographic techniques, such as gas chromatography (GC) and liquid chromatography (LC), are useful for multi-component analysis. Separation of components is based on the partitioning of the solutes between the mobile and stationary phase (Bogusz, 2008; Harris, 2007). Liquid chromatography – mass spectrometry (LC-MS) is an analytical technique that is widely used for the identification of analytes in biological tissues and fluids, due to the extensive range of compounds that can be analysed. This section will detail the principles of LC-MS analysis and the procedures used for the optimisation of instrument parameters.

2.1.1 Principle of chromatography

High performance liquid chromatography (HPLC or LC) uses a high pressure to force a liquid mobile phase through a column containing very fine particles to produce high-resolution separations (Harris, 2007). There are different chromatographic separation modes
available, namely normal-phase and reversed-phase chromatography (McMaster, 2005). In normal phase chromatography, a polar stationary phase is used and a non-polar mobile phase. In contrast, reversed-phase chromatography utilises a non-polar stationary phase and a more polar mobile phase. Reversed-phase chromatography is more commonly used due to the wide variety of compatible mobile phases, allowing greater versatility compared to normal-phase chromatography (McMaster, 2005). In this study, reversed-phase LC-MS was used for the analyses of all samples.

The core of an LC system is the column, as this is where chromatographic separation occurs. The sample is injected and mixed with the mobile phase before it is passed on to the column, where the stationary phase is found. Separation of analytes is determined by the affinity of these chemicals to the stationary phase. The greater the affinity, the longer it takes for the analytes to pass through the column. The time at which the compound elutes is called the retention time. The retention time is characteristic for a given analyte under specific conditions (such as type of column, mobile phase and flow rate). Analytes eluting from the column are then introduced into the mass spectrometer for analysis. LC systems can be run using an isocratic or gradient elution mode. In isocratic elution, the mobile phase composition is kept constant. In contrast, for gradient elution the mobile phase composition is continuously changing during the analysis to increase the eluent strength to elute more strongly retained analytes. In this study gradient elution was applied for the separation of the analytes of interest. Additionally, there are different column phases available to maximise the separation of the analytes of interest. A C18 column is widely used for the analysis of moderately polar to non-polar compounds and was chosen for this study for the analysis of cocaine, heroin and their respective metabolites.

2.1.2 Electrospray interface, ion focussing and ion optics

Electrospray ionisation (ESI) is a soft ionisation technique used to produce ions in a gaseous phase (without fragmentation). The eluate, which is the solvent emerging from the end of the column is pumped through a charged, heated metal capillary. The voltage of the capillary (approximately 3 – 5 kV) causes the liquid spray to be charged as it is nebulised, creating an aerosol of charged droplets (Ho et al., 2003). The droplets evaporate under a stream of nitrogen (carrier gas) and applied heat. As the solvent evaporates, the droplet decreases in size, the analytes are forced closer together, repel each other and break up the droplets forming individually charged analyte ions (Thermo Scientific, 2014; Harris, 2007).
Figure 2.1: Analytical sequence for this study.
In the ion source interface, the analyte ions are extracted into the ion transfer tube and the stacked ring ion guide (called S-lens) region (Thermo Scientific, 2014). Figure 2.2 displays a schematic of the Orbitrap mass spectrometer used in this study. The main function of the S-lens is to focus the ions into a tight beam after exiting the ion transfer tube. The S-lens operates in radio frequency (RF) mode only, to move ions into the ion optics region of the mass spectrometer (Thermo Scientific, 2014). The ion optics transmit the ions from the S-lens to the Orbitrap. The ion optics remove any remaining neutrals or ions of the opposite polarity before entering the quadrupole for mass selection (Thermo Scientific, 2014). The quadrupole mass filter consists of four cylindrical or hyperbolic rods. The mass analyser is positioned between the ion optics and the detector and acts as a mass filter. A combination of a direct current (DC) and radio frequency (RF) voltage is applied to the rods to allow for the transmission of ions of a specific mass or mass range to the detector (Glish & Vachet, 2003). By varying the voltage applied with time, ions with a specified range of mass-to-charge (m/z) ratios will be transmitted to the detector.

After the quadrupole, the ions are introduced into the curved linear trap (C-trap) which is filled with nitrogen and operates at $10^{-6}$ mbar. The C-trap is a RF-only curved quadrupole used to cool down the ions after exiting the quadrupole mass analyser and compress them into a tight ion package before entering the Orbitrap, which is held at $10^{-10}$ mbar (Thermo Scientific, 2014). In addition to the use of voltage differentials, the Orbitrap mass spectrometer also utilises a pressure differential to focus ions through the ion optics before being introduced into the Orbitrap (Thermo Scientific, 2014).

Figure 2.2: Schematic of the Q-Exactive Plus mass spectrometer used for the analysis of all samples in this study (Thermo Scientific, 2014).
2.1.3 Orbitrap analyser

The main purpose of the Orbitrap mass analyser is to separate and detect ions based on their oscillation frequencies. The Orbitrap consists of two electrodes, a central electrode and an outer electrode. The outer electrode is split in half by a ceramic ring. The ions in the Orbitrap are trapped in an electrostatic field by applying voltages to both the outer and central electrode. When the ions enter the Orbitrap, the voltage is increased to both electrodes to confine the ions in the Orbitrap. This forces the ions to move towards the middle of the central electrode and causes axial oscillations. The voltage to the central electrode is then stopped and this allows the ions to spin around the central electrode in a stable trajectory. Different masses will have different frequencies of rotation. Ions of low molecular mass will be kept tighter around the central electrode compared to higher molecular masses, allowing for the separation of ions in the Orbitrap. The outer electrodes are then used to measure the image current observed by the axial oscillations of the ions. Fast Fourier Transform (FFT) is used to convert the oscillation frequency measured by the outer electrodes, a time domain signal, to a frequency domain function and then into a mass spectrum (Zubarev & Makarov, 2013).

2.1.4 Development of a liquid chromatography method

High performance liquid chromatography (HPLC) method development is an important process in an analytical sequence as it will determine the quality of the results obtained. The use of Kinetex XB-C18 column (100 x 2.1 mm, 5 µm) was recommended by Phenomenex for the analysis of cocaine, heroin and their respective metabolites. The Kinetex column utilises a core shell technology which improves the column efficiency, providing narrower peaks and thus improved sensitivity compared to a fully porous column.

The initial conditions for the chromatographic separation used in this study was based on a general method used in the Department of Chemistry at the University of Surrey. This LC method utilised a mobile phase composition containing water (H_{2}O) + 0.1% formic acid and acetonitrile (ACN) + 0.1% formic acid using a gradient elution over a run time of 7 min. The LC method used in this study was optimised on a Waters Acquity ultra high performance liquid chromatography (UHPLC) system combined with a quadrupole – time of flight (Q-ToF) mass spectrometer. A 250 ng/ml standard (in 20% (v/v) ACN in H_{2}O) was used for the optimisation of the chromatographic separation of cocaine, benzoylecgonine, heroin and 6-acetylmorphine. Using the general method, separation was achieved for the
analytes of interest. However, the last compound (cocaine) eluted at 3.35 min. The LC method was therefore modified by creating a steeper gradient to produce a 3 min method with chromatographic separation for cocaine, heroin and respective metabolites benzoylecgonine and 6-acetylmorphine.

Multiple mobile phase compositions have been used for the LC-MS analysis of cocaine and heroin in various biological matrices, including ammonium acetate, water, methanol and acetonitrile (see Table 1.2). Both the retention time and selectivity will change depending on the solvents used due to differences in the chemical properties. In this study, two organic and two aqueous solvents were evaluated, including the use of (A1) water + 0.1% formic acid and (A2) 20 mM ammonium acetate in 5% (v/v) ACN in H2O. For the organic solvent, (B1) ACN + 0.1% formic acid and (B2) MeOH + 0.1% formic acid were evaluated. A combination of A1 + B1, A2 + B1 and A2 + B2 as mobile phase compositions were evaluated at two different initial mobile phase conditions, using a 3 min gradient elution method. This included an initial mobile phase composition starting at (i) 5% organic solvent or (ii) 20% organic solvent. The results obtained for the chromatographic separation of cocaine, benzoylecgonine, heroin and 6-acetylmorphine using these methods are shown in Appendix A. 1 – A. 6. The use of a higher percentage (95%) aqueous solvent in the mobile phase resulted in narrower peaks, especially for 6-acetylmorphine (first eluted compound). Additionally, no significant difference was observed between the use of the different mobile phases (A1, A2, B1, B2), when starting the initial mobile phase composition at 5% organic and 95% aqueous solvent. Overall, the use of a mobile phase including H2O + 0.1% formic acid as the aqueous solvent and ACN + 0.1% formic acid as the organic solvent provided the best results, as the artefact observed for 6-acetylmorphine was minimised using these solvents (see Appendix A. 5).

The LC method developed on the Waters Acquity UHPLC was later transferred to the Dionex Ultimate 3000 HPLC without modification for the analysis of cocaine, benzoylecgonine, heroin and 6-acetylmorphine, where the same separation was achieved for all the analytes. An example of an overlay of extracted ion chromatograms of cocaine, benzoylecgonine, heroin, 6-acetylmorphine extracted from paper (at 10 µL of 50 ng/ml in ACN) and analysed using the developed LC-MS method is shown in Figure 2.3.
Chapter 2: Analytical Methodology

Figure 2.3: An example of an overlay of extracted ion chromatograms of a drug standard at 50 ng/ml containing cocaine (m/z 304.1543), benzoylecgonine (m/z 290.1387), heroin (m/z 370.1649) and 6-acetylmorphine (m/z 328.1543) in 5% (v/v) ACN in H₂O analysed using the LC-MS method developed.

2.1.5 Optimisation of electrospray settings

Most electrospray source conditions, including sheath gas, auxiliary gas, capillary temperature and probe position are dependent on the flow rate used in the chromatographic separation. In this study, Thermo Fisher recommended settings were used for the sheath gas (45 psi), auxiliary gas (10 au), capillary temperature (320 °C) and probe position (C) for a flow rate of 0.250 ml/min. The spray voltage is a more analyte specific parameter and was optimised for the analysis of cocaine, benzoylecgonine, heroin and 6-acetylmorphine. The spray voltage was optimised using a 50 ng/ml standard containing all analytes of interest in 5% (v/v) ACN in H₂O at a flow rate of 0.250 ml/min. The optimised spray voltage was 3 kV.

2.2 Experimental

2.2.1 Chemicals and reagents

Certified reference materials (CRM) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine were used to prepare drug standards (Sigma Aldrich, Dorset, UK). CRMs of cocaine-d₃, benzoylecgonine-d₃, heroin-d₆ and 6-acetylmorphine-d₃ were used as internal standards (Sigma Aldrich, Dorset, UK). Optima grade LC-MS solvents of methanol,
acetonitrile and water were used to prepare solutions and solvent mixtures (Fisher Scientific, Leicestershire, UK). Formic acid was added to the mobile phase solvents at 0.1% (v/v) (Fischer Scientific, Leicestershire, UK). Dichloromethane, chloroform and ethyl acetate were used to prepare solvent extraction mixtures and were analytical or reagent grade (Sigma Aldrich, Dorset, UK). A solution of artificial eccrine perspiration was used to simulate a fingerprint matrix in the experimental design of fingerprint experiments (Pickering Laboratories, Inc., Obertaukirchen, Germany).

### 2.2.2 Instrumentation and conditions

Analysis of samples was carried out using a Dionex Ultimate 3000 HPLC module equipped with a binary solvent manager, column manager and autosampler, coupled to a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK) at the University of Surrey. Chromatographic separation was performed on a Kinetex XB-C18 column (100 x 2.1 mm, 5 µm) operated at 30 ºC with a flow rate of 0.25 ml/min. Gradient elution was performed with an initial mobile phase composition of 95% H₂O (with 0.1% formic acid) and 5% acetonitrile (ACN, with 0.1% formic acid) increased to 80% ACN (0.1% formic acid) and 20% H₂O (0.1% formic acid) over 2 min and kept constant for 0.5 min before returning to the initial mobile phase composition.

Analysis on the Q-Exactive Plus mass spectrometer was performed in full scan mode (mass resolution of 70 000 and 5 ppm mass accuracy) to allow for the identification of the analytes of interest using the molecular ion. Table 2.1 outlines the optimised mass spectrometer (MS) operating conditions used for the analysis of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprint samples.

Table 2.1: Operating conditions of the mass spectrometer used in this research.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Operating condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Spray voltage</td>
<td>3 kV</td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>320 ºC</td>
</tr>
<tr>
<td>S-lens RF level</td>
<td>50</td>
</tr>
<tr>
<td>Sheath gas flow rate</td>
<td>45</td>
</tr>
<tr>
<td>Aux gas flow rate</td>
<td>10</td>
</tr>
<tr>
<td><strong>Scan parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Scan type</td>
<td>Full MS</td>
</tr>
<tr>
<td>Scan range</td>
<td>m/z 50 - 500</td>
</tr>
<tr>
<td>Resolution</td>
<td>70 000 at m/z 200</td>
</tr>
<tr>
<td>Polarity</td>
<td>Positive</td>
</tr>
<tr>
<td>AGC target</td>
<td>10⁶</td>
</tr>
<tr>
<td>Maximum inject time</td>
<td>200</td>
</tr>
</tbody>
</table>
The [M+H] peaks for all analytes of interests and internal standards were monitored. A list of the m/z ratios monitored for the analytes of interests are detailed in Table 2.2.

Table 2.2: Ions (m/z ratio) monitored for the analytes of interest and corresponding deuterated internal standards.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>m/z ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>304.1543</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>290.1387</td>
</tr>
<tr>
<td>Heroin</td>
<td>370.1649</td>
</tr>
<tr>
<td>6-Acetylmorphine</td>
<td>328.1543</td>
</tr>
<tr>
<td>Internal standard</td>
<td>m/z ratio</td>
</tr>
<tr>
<td>Cocaine-d$_3$</td>
<td>307.1732</td>
</tr>
<tr>
<td>Benzoylecgonin-d$_3$</td>
<td>293.1575</td>
</tr>
<tr>
<td>Heroin-d$_9$</td>
<td>379.2214</td>
</tr>
<tr>
<td>6-Acetylmorphine-d$_3$</td>
<td>331.1732</td>
</tr>
</tbody>
</table>

2.2.3 Sample surface and extraction solutions

The preparation of fingerprint samples for LC-MS analysis requires the use of a solvent to extract the fingerprint from the sample surface and into a solution. The surface and extraction solution used for the deposition and extraction of a fingerprint may have a significant effect on the ability to extract and analyse the analytes of interests. Therefore, comparative experiments are recommended using a range of surfaces that are representative of those that the method may be applied to in operational work (Sears et al., 2012). A number of procedures have been reported for the analysis of drugs in fingerprints using LC-MS, including the type of sample substrate used for collection, extraction method and extraction solutions used. These describe different approaches to the collection and extraction of fingerprints. Fingerprint samples have previously been collected on glass cover slips (Goucher et al., 2009; Jacob et al., 2008) or filter paper (Kuwayama et al., 2014; Kuwayama et al., 2013) for the detection of lorazepam, methadone, cold medicine and caffeine, respectively. In this research, two surfaces (chromatography paper and glass cover slips) previously used for fingerprint depositions were investigated in combination with a range of extraction solvents, including methanol, acetonitrile, dichloromethane, chloroform, ethyl acetate, 10% (v/v) methanol in dichloromethane and 10% (v/v) methanol in chloroform. This was to evaluate the suitability of the surfaces and extraction solutions for the analysis of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in an artificial eccrine perspiration solution to simulate a fingerprint deposition.
Ten µl of analyte standard in artificial eccrine perspiration was spiked onto Whatman™ grade-1 chromatography paper (2 x 2 cm) or a glass cover slip (16 mm diameter) and left to dry overnight to produce 5 ng of analyte residue on the surface. The sample (paper or glass cover slip) was placed in a scintillation vial (outer diameter 27 mm) (Fisher Scientific, Leicestershire, UK) with 0.5 ml of one of the extraction solutions mentioned above and sonicated for 15 min. Next, the sample extract was vortex-mixed for 15 sec and transferred to an LC autosampler vial. The sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C) and reconstituted in 100 µl mobile phase solution (5% acetonitrile in water with 0.1% (v/v) formic acid) containing 50 ng/ml internal standard solution and vortex-mixed again for 30 sec prior to LC-MS analysis. The extraction efficiency for the analytes of interest was evaluated in an artificial eccrine perspiration solution to produce a similar sample matrix to eccrine fingerprint samples. These were compared against an analyte standard at 50 ng/ml in 5% (v/v) acetonitrile in water with 0.1% formic acid and 50 ng/ml internal standard solution at the same concentration as the final sample extract.

2.2.3.1 Internal standard

Certified reference material (CRM) of cocaine-d₃, benzoylecgonine-d₃, heroin-d₉ and 6-acetylmorphine-d₃ (100 µg/ml, Sigma Aldrich, Dorset, UK) were used as internal standards. The use of an internal standard can aid quantification of analytes, especially in a multi-step sample preparation method. The internal standard can help to correct for any sample volume loss during extraction as the relative instrument response of the analyte and internal standard would be constant. Five replicate samples were prepared at 5 ng analyte residue on surface by adding 10 µl of 500 ng/ml drug standard (containing cocaine, benzoylecgonine, heroin and 6-acetylmorphine) in acetonitrile on the paper. The stage at which the internal standard was introduced during the sample preparation method was investigated by adding the internal standard (i) before the extraction procedure (500 ng/ml internal standard solution in acetonitrile spiked on paper) and (ii) during reconstitution of the sample extract in the injection solvent (50 ng/ml internal standard solution in 5% (v/v) acetonitrile in water with 0.1% formic acid). The influence of the addition of the internal standard on the percentage recovery of cocaine, benzoylecgonine, heroin and 6-acetylmorphine was investigated using extraction solutions of 10, 20 and 30% (v/v) methanol (MeOH) in dichloromethane (DCM). The extraction was performed by placing the sample in a scintillation vial with 0.5 ml of extraction solution and 15 min sonication. The solvent extract was vortex-mixed for 15 sec,
transferred to an LC autosampler vial and evaporated to dryness under a stream of nitrogen at room temperature (20 °C). The subsequent sample residue was reconstituted in 100 μl mobile phase solution (5% acetonitrile in water with 0.1% (v/v) formic acid containing 50 ng/ml internal standard solution) and vortex-mixed again for 30 sec prior to LC-MS analysis.

2.2.4 Extraction procedures

The extraction efficiency of two different procedures using paper surfaces were evaluated, which included (i) vials and sonication and (ii) microcentrifuge tubes and centrifugation. Both approaches have been used for the extraction of fingerprint material of surfaces using LC-MS analysis (Kuwayama et al., 2014; Kuwayama et al., 2013; Goucher et al., 2009; Jacob et al., 2008). In this case, screw top vials (Smith Scientific, Kent, UK) were evaluated for extraction (outer diameter 27.5 mm) in combination with extraction solutions containing different ratios (v/v) of methanol and dichloromethane (including 10, 20, 30% (v/v) methanol in dichloromethane and 10, 20, 30% (v/v) dichloromethane in methanol). Each extraction solution was evaluated using n = 5 replicate samples at 5 ng of analyte residue in artificial eccrine perspiration on surface. The extraction was performed by placing the sample in a scintillation vial with 0.5 ml of extraction solution and sonicated for 15 min. The solvent extract was vortex-mixed for 15 sec, transferred to an LC autosampler vial and evaporated to dryness under a stream of nitrogen at room temperature (20 °C). The subsequent sample residue was reconstituted in 100 μl mobile phase solution (5% acetonitrile in water with 0.1% (v/v) formic acid containing 50 ng/ml internal standard solution) and vortex-mixed again for 30 sec prior to LC-MS analysis.

An alternative fingerprint extraction method has been reported in the literature (Kuwayama et al., 2014; Kuwayama et al., 2013), which is based on the use of a microcentrifuge tube and centrifugation of the sample, for the extraction of fingerprint material from a paper substrate. For this approach, samples were extracted by placing the paper substrate in a microcentrifuge tube (2 ml) with 1.5 ml of extraction solution (10, 20, 30% (v/v) methanol in dichloromethane and 10, 20, 30% (v/v) dichloromethane in methanol) and centrifuging for 2 min at 9500 x g relative centrifugal force (rcf). The paper was discarded and the sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C). The subsequent sample residue was reconstituted in 100 μl mobile phase solution (5% acetonitrile in water with 0.1% (v/v) formic acid containing 50 ng/ml internal standard solution) and vortex-mixed again for 30 sec prior to LC-MS analysis.
2.2.5 Matrix effects

The experimental design for the fingerprint extraction method development was based on using an artificial eccrine sweat solution to simulate eccrine fingerprint samples in the absence of a validated fingerprint standard. The artificial eccrine perspiration was used in the evaluation of the extraction procedures to determine the suitability of the sample surface and extraction solutions for the analysis of cocaine and heroin use in fingerprints.

2.2.5.1 Participant selection and sampling

The developed extraction method was used to evaluate the effects of the matrix on the extraction efficiency of cocaine, heroin and respective metabolites for (i) acetonitrile, (ii) artificial eccrine perspiration and (iii) fingerprints at three levels, namely 1, 5 and 10 ng of analyte residue on surface. A favourable ethical approval was obtained from the University of Surrey Ethics Committee to collect samples from individuals at the University of Surrey who volunteered to participate. Fingerprint samples were collected from \( n = 5 \) participants without any preparation of the hands (natural fingerprints) and after washing hands with soap and water, prior to wearing nitrile gloves for 10 min to aid secretion of perspiration from the fingertips. Four fingerprints were collected per participant (right thumb, right index, left thumb and left index finger) to prepare the spiked samples and a blank. A minimum of five participants is recommended because this allows to incorporate variations in gender and other factors into the experiment (Sears et al., 2012). However, due to ethical considerations this information is not provided. Five replicate samples were prepared for each matrix at each level. For fingerprints, the analyte standard (10 µl of 100, 500 or 1000 ng/ml) in acetonitrile was pipetted onto the fingerprint samples to produce analyte residues on the sample surface at 1, 5 and 10 ng. Samples were extracted by placing the paper substrate in a microcentrifuge tube (2 ml) with 1.5 ml of extraction solution (10% dichloromethane in methanol) and centrifuging for 2 min at 9500 x g rcf. The paper was discarded and the sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C). The subsequent sample residue was reconstituted in 100 µl mobile phase solution (5% acetonitrile in water with 0.1% (v/v) formic acid containing 50 ng/ml internal standard solution) and vortex-mixed again for 30 sec prior to LC-MS analysis.

2.2.5.2 Ionisation suppression and enhancement

Ionisation suppression or enhancement is commonly observed in LC-MS analysis as ions compete for ionisation in the electrospray (ESI) source. The presence of co-eluting
compounds can interfere with the instrument response of the analyte of interest. This phenomenon is mainly a concern when ionisation suppression occurs as this can negatively affect the limit of detection (Polettini, 2006). The potential of ionisation suppression or enhancement was assessed using the post-extraction addition approach (Scientific Working Group for Forensic Toxicology, 2013).

A quantitative estimate of the ionisation suppression/enhancement can be obtained by addition of drug standards to a blank sample matrix post extraction. Three matrices were evaluated, including acetonitrile, artificial eccrine perspiration and fingerprints at two concentration levels (20 and 80 ng/ml). Two sets of samples were prepared in order to compare the effects of the matrix on the analyte signal. Set one consisted of analytes in standard solution (5% v/v acetonitrile in water with 0.1% formic acid) at 20 ng/ml and 80 ng/ml containing 50 ng/ml internal standard (cocaine-d₃, benzoylecgonine-d₃, heroin-d₉ and 6-acetylmorphine-d₃). Each standard was measured five times to provide an average peak area at both concentration levels. Set two consisted of three different blank matrices (acetonitrile, artificial eccrine perspiration and natural fingerprints) spiked with drug standards in standard solution post extraction. Ten microlitres of blank solvent (acetonitrile) or matrix (artificial eccrine perspiration or natural fingerprint) was added to Whatman 1-Chr grade paper (2 x 2 cm) and left to dry overnight. Five replicate samples were prepared for both acetonitrile and artificial eccrine perspiration at each concentration level. As it is known that the concentration levels of analytes present in fingerprints vary from person to person, natural fingerprints (n = 2 per participant) were collected from 10 individuals to assess the potential matrix effects. Samples were extracted by placing the paper substrate in a microcentrifuge tube (2 ml) with 1.5 ml of extraction solution (10% dichloromethane in methanol) and centrifuging for 2 min at 9500 x g relative centrifugal force (rcf). The paper was discarded and the sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C). The samples were reconstituted with an analyte standard (at 20 or 80 ng/ml) in the mobile phase solution (5% acetonitrile in water with 0.1% (v/v) formic acid containing 50 ng/ml internal standard containing cocaine-d₃, benzoylecgonine-d₃, heroin-d₉ and 6-acetylmorphine-d₃). Each sample was injected five times and the average peak area obtained was compared to those of set one, as shown in Equation 2.1.

\[
\text{Ionisation suppression or enhancement (\%)} = \frac{\text{Average Peak Area of Set 2} - \text{Average Peak Area of Set 1}}{\text{Average Peak Area of Set 1}} \times 100
\]

Eq. 2.1
2.2.6 Spike recovery

The recovery of the developed extraction method was evaluated using analyte standards (containing cocaine, benzoylecgonine, heroin and 6-acetylmorphine) in acetonitrile at 100, 500 and 1000 ng/ml, to produce spiked fingerprints (using n = 5 participants, with 3 fingerprints collected per participant) at 1, 5, and 10 ng of analyte residue on surface. Participant selection and sampling was discussed in section 2.2.5.1. Samples were extracted by placing the paper substrate in a microcentrifuge tube (2 ml) with 1.5 ml of extraction solution (10% dichloromethane in methanol) and centrifuging for 2 min at 9500 x g rcf. The paper was discarded and the sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C). The subsequent sample residue was reconstituted in 100 µl mobile phase solution (5% acetonitrile in water with 0.1% (v/v) formic acid containing 50 ng/ml internal standard solution) and vortex-mixed again for 30 sec prior to LC-MS analysis. The average percentage recovery for cocaine, benzoylecgonine, heroin and 6-acetylmorphine at each level (1, 5 and 10 ng) in fingerprints was calculated based on the original spiked concentration.

2.2.7 Data analysis

The following section details the statistical calculations used in this research including the use of the statistics of repeated measurements by calculating the average, standard deviation and relative standard deviation. Comparison of medians was carried out by using a Mann-Whitney U test or Kruskal-Wallis test. The calculations below are applied throughout Chapters 3 to 6.

2.2.7.1 Average and standard deviation

The average of repeated measurements was calculated for all analytes above the limit of detection using the following equation (Miller & Miller, 2005):

$$\bar{x} = \frac{\Sigma x_i}{n}$$  \hspace{1cm} Eq. 2.2

where $\bar{x}$ = average, $\Sigma x_i$ = sum the individual measurements and $n$ = the number of measurements. The standard deviation ($s$) evaluates the spread of all the individual measurements and was calculated using (Miller & Miller, 2005):

$$s = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{(n-1)}}$$  \hspace{1cm} Eq. 2.3
2.2.7.2 Relative standard deviation (RSD)

The relative standard deviation (or coefficient of variation), used as a measure of the precision of the method, was calculated to evaluate the relative error of the measurements using the following equation (Miller & Miller, 2005):

\[ RSD = \frac{s}{\bar{x}} \times 100 \]  
Eq. 2.4

where \( s \) = standard deviation and \( \bar{x} \) = average.

2.2.7.3 F-test

An F-test was used to determine whether the sample standard deviations of two data sets were significantly different at a specific significance level (i.e. probability \( p = 0.05 \)). In this research a two-sided test was performed to determine whether the sample variances were different in their precision at \( p = 0.05 \) (Miller & Miller, 2005). The \( F \) statistic considers the ratio of the two sample standard deviations and can be calculated using the following information, sample standard deviation \( s_1 \) and sample standard deviation \( s_2 \). Where \( s_1^2 \) is the largest sample variance and \( s_2^2 \) is the smallest sample variance, so that \( F \) is always > 1.

\[ F = \frac{s_1^2}{s_2^2} \]  
Eq. 2.5

The degrees of freedom of the numerator and denominator are \( n_1 - 1 \) and \( n_2 - 2 \). If the \( F_{\text{calc}} \) exceeds \( F_{\text{crit}} \) then the null hypothesis is rejected, and the two sample standard deviations are significantly different at that specific significance level.

2.2.7.4 Comparison of two experimental means

The Student \( t \)-test was used to evaluate the statistical probability of whether the experimental value was significantly different from a second experimental value. The \( t \)-test can be performed using the following information to calculate the statistic \( t \), the average measurement of the sample (\( \bar{x} \)), the known value (\( \mu \)), the sample standard deviation (\( s \)) calculated using Eq. 2.7 and the number of samples (\( n \)). The following equation can be used to calculate \( t \), if the samples have standard deviations which are not significantly different (determined using F-test, where \( F_{\text{calc}} < F_{\text{crit}} \)) (Miller & Miller, 2005):

\[ t = \frac{(\bar{x}_1 - \bar{x}_2)}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \]  
Eq. 2.6

\[ s^2 = \frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{(n_1 + n_2 - 2)} \]  
Eq. 2.7
If the calculated $|t|$ value is above the critical value ($t_{\text{crit}}$) then the difference observed between the two values is significant at that significance level (i.e. $p = 0.05$) for $n_1 + n_2 - 1$ degrees of freedom. If the samples have standard deviations which are significantly different ($F_{\text{calc}}>F_{\text{crit}}$), Eq. 2.8 and Eq. 2.9 are used to calculate the statistic $t$ and degrees of freedom (dof) (Miller & Miller, 2005).

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$$\text{dof} = \frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{(n_1+n_2-2)}$$

Eq. 2.8 and Eq. 2.9

2.2.7.5 Mann-Whitney U-test

The Mann-Whitney U-test is considered to be the equivalent to the Student t-test for data that is not normally distributed (non-parametric) (Mendes, 2008). The Mann-Whitney U-test can be used to compare the median of the dependent variables (e.g. levels of analyte in a fingerprint) of two or more categories of the independent variables (e.g. collection methods, natural, soap or wipe). The null hypothesis for the Mann-Whitney U-test is that the dependent variables (e.g. level of analyte in a fingerprint) are not dependent on the independent variables (e.g. collection methods) and thus have the same median values. The test determines the number of results in one data set that exceeds each of the results in the second data set (Miller & Miller, 2005). The number of values that exceed the data set between the independent variables tested are summed up and compared (Mendes, 2008). The sum of the number of values that are higher is termed the $U$ statistic (Mendes, 2008). The $U$ statistic is compared to a critical $U$ value to determine whether to reject or accept the null hypothesis. If the $U_{\text{calc}}$ is below the critical $U$ value, then the difference observed between the data sets is significant at the specified significance level. In this research a one-sided test was performed to determine whether the level of analyte present in a fingerprint was significantly lower or higher based on independent variables. For a sample size greater than 20, the $U$ distribution approximates a normal distribution and requires the use of a $z$ value (Mendes, 2008). The $z$ statistic can be calculated from the $U$ statistic using the following equation (Miller & Miller, 2005):

$$z = \frac{U - \mu_u}{\sigma_u}$$

Eq. 2.10

where $U =$ the sample statistic; $\mu_u =$ average of the sampling distribution of sample $U$; $\sigma_u =$ standard deviation of the sampling distribution of sample $U$. 46
The average ($\mu_u$) and standard deviation ($\sigma_u$) are calculated using Eq. 2.11 and Eq. 2.12 (Miller & Miller, 2005).

$$
\mu_u = \frac{N_1 N_2}{2} \quad \sigma_u = \sqrt{\frac{N_1 N_2 (N_1 + N_2 + 1)}{12}} \quad \text{Eq. 2.11 and Eq. 2.12}
$$

where $N_1$ and $N_2$ = sample sizes for data set 1 and 2, respectively.

2.2.7.6 Kruskal-Wallis test

The Kruskal-Wallis test is an extension of the Mann-Whitney U test as it allows for the comparison of three or more sample medians (e.g. study populations A, B and C). The individual results from each data set are pooled and ranked (Miller & Miller, 2005). The rank totals are compared by calculating the chi-squared statistic ($\chi^2$) using the following equation (Miller & Miller, 2005):

$$
\chi^2 = \frac{12}{N^2 + N} \left\{ \frac{R_A^2}{n_A} + \frac{R_B^2}{n_B} + \frac{R_C^2}{n_C} + \cdots \right\} - 3(N + 1) \quad \text{Eq. 2.13}
$$

where $n_A$, $n_B$, $n_C$ are the number of measurements, $R_A$, $R_B$ and $R_C$ are the rank totals and $N$ is the sum of numbers in each data set given by $N = n_A + n_B + n_C$, etc.

The calculated chi-squared statistic ($\chi^2$) is then compared to tabulated values, with the number of degrees of freedom given by $k - 1$. If the calculated $\chi^2$ statistic is greater than the tabulated values, the null hypothesis is rejected (e.g. the medians of the samples are not significantly different).

2.3 Results and Discussion

2.3.1 Effect of the sample substrate and extraction solution

The extraction efficiency (expressed as the average percentage recovery) varied greatly depending on the extraction solvent and surface used, as seen for cocaine, heroin and its respective metabolites benzoylecgonine and 6-acetylmorphine in Figure 2.4. This is due to differences in the chemical structure of cocaine, benzoylecgonine, heroin and 6-acetylmorphine, which are fairly non-polar to moderately polar, with the respective metabolites being more polar (Chapter 1, section 1.2.3.2 and 1.2.4.2). A range of extraction solutions of different polarities were therefore evaluated to optimise the extraction efficiency of the analytes of interest. The use of relatively non-polar solvents, such as dichloromethane,
chloroform and ethyl acetate, was not suitable for the extraction of cocaine, benzoylecgonine, heroin and 6-acetylmorphine from both surfaces, as seen in Figure 2.4.

Additionally, the chromatography paper used consisted of cellulose, which contains hydroxyl groups that can interact with the analytes of interest. In order for the analytes of interest to be extracted, the affinity for the extraction solvent must be greater than the paper substrate. Therefore, the use of a polar solvent (such as methanol and acetonitrile) resulted in a higher extraction efficiency particularly for glass cover slips. The presence of a polar protic solvent (such as methanol) allows hydrogen bonding, compared to a polar aprotic solvent (such as acetonitrile). The presence of the amine and carbonyl groups in the analytes of interest can form hydrogen bonding with the hydroxyl present in the extraction solvent (methanol), unlike acetonitrile. Therefore, methanol provides better extraction of cocaine, benzoylecgonine, heroin and 6-acetylmorphine (with the exception of heroin for the glass cover slips). However, a combination of a polar and a semi-polar solvent (e.g. methanol and dichloromethane or chloroform) provided the highest extraction efficiency for all analytes using paper as the sample substrate. The use of a combination of these solvents accounts for interaction with the solvent through a hydrophobic effect (with analyze hydrophobic groups), further increasing the affinity of the analytes of interest for the extraction solution over the paper substrate. The extraction solution containing 10% (v/v) methanol in dichloromethane was chosen for the extraction of cocaine, heroin and respective metabolites. However, the
recoveries for all analytes were generally below 60%. This could be due to the extraction method used, as the sonication caused the paper to rise in the scintillation vial and not stay immersed in the extraction solvent. The use of dichloromethane and methanol has also been reported for the extraction of lorazepam and methadone from fingerprint samples on glass cover slips (Goucher et al., 2009; Jacob et al., 2008).

2.3.2 Internal standard

The point at which the internal standard was added during the extraction procedure was investigated to determine the influence of adding (i) the internal standard before extraction and (ii) in the reconstitution step (Figure 2.5). In quantitative measurements the internal standard is normally added to experience the entire extraction procedure, thus accounting for any loss of signal during the extraction. Addition of the internal standard prior to the extraction procedure resulted in average percentage recoveries of 25 – 29% for cocaine, 28 – 31% for benzoylecgonine, 30 – 33% for heroin and 35 – 41% for 6-acetylmorphine using 10, 20 and 30% methanol in dichloromethane as extraction solutions. In contrast, the addition of the internal standard in the reconstitution step resulted in average percentage recoveries of 53 – 59% for cocaine, 50 – 52% for benzoylecgonine, 52 – 56% for heroin and 49% for 6-acetylmorphine.

Figure 2.5: Evaluation of the addition of the internal standard (IS) during the sample preparation method using 10, 20 and 30% (v/v) methanol (MeOH) in dichloromethane (DCM) as extraction solutions. Internal standard was added before the extraction method or during reconstitution of the sample extract. Data represented as average percentage recovery (± standard deviation, n = 5 measurements).
The results indicate that addition of the internal standard after evaporation to dryness provides higher recoveries for all analytes, for all three extraction solvents evaluated (10, 20 and 30% MeOH in DCM). This is contradictory to what might have been expected. Although this method does not account for any volume loss during the sample preparation steps, better results were obtained adding the internal standard in the reconstitution step. In addition, no significant difference was observed in the extraction efficiency of cocaine, benzoylecgonine, heroin and 6-acetylmorphine for the three extraction solutions evaluated (10, 20 or 30% methanol in dichloromethane).

In light of the results presented in Figure 2.5, a 50 ng/ml internal standard solution (in 5% (v/v) acetonitrile in water with 0.1% (v/v) formic acid) containing cocaine-d₃, benzoylecgonine-d₃, heroin-d₉ and 6-acetylmorphine-d₃ was added in the reconstitution step of the extraction procedure prior to LC-MS analysis in all proceeding experiments to correct for any change in signal observed due to instrument response variance. The average peak area ratio of analyte (A) to internal standard (IS) was calculated for data analysis. As the internal standard does not experience the full extraction procedure, it cannot be used for quantitative measurements as it does not represent the analytes of interest performance during the sample preparation steps.

2.3.3 Extraction procedures

The use of a different vial type for extraction was investigated to improve the recovery for the analytes of interest (at 5 ng analyte residue on surface) using 10, 20 and 30% methanol in dichloromethane and 10, 20 and 30% dichloromethane in methanol as extraction solutions. The use of a different scintillation vial was explored as the vials used previously were small and as a result the paper substrate did not remain immersed in the extraction solution. The use of different screw top vials for extraction improved the percentage recovery to above 60%. In addition, the percentage recovery for each analyte was higher using extraction solutions containing a higher percentage of methanol than dichloromethane, as seen in Figure 2.6, with recoveries close to 90% for all analytes. A higher percentage polar solvent in the extraction mixture is more efficient extracting cocaine, benzoylecgonine, heroin and 6-acetylmorphine as this allows for more hydrogen bonding.
Figure 2.6: Average percentage recovery (± standard deviation, n = 5 measurements) for cocaine, benzoylecgonine, heroin and 6-acetylmorphine in a spiked artificial perspiration standard (n = 5) extracted from paper using scintillation vials using 10, 20, 30% (v/v) methanol (MeOH) in dichloromethane (DCM) and 10, 20, 30% (v/v) dichloromethane (DCM) in methanol (MeOH).

Two different approaches (using scintillation vials and microcentrifuge tubes) for the extraction of the analytes of interest (at 5 ng of cocaine, benzoylecgonine, heroin and 6-acetylmorphine) from paper were investigated to develop an optimised extraction method using 10, 20 and 30% dichloromethane in methanol. Figure 2.7 shows the percentage recoveries obtained for both extraction methods using (i) scintillation vials and (ii) microcentrifuge tubes. The percentage recoveries for all analytes improved with the use of Eppendorf™ microcentrifuge tubes and centrifugation compared to the use of screw top vials (Smith Scientific, Kent, UK) and sonication. During the extraction method development experiments, it was observed that the percentage recoveries obtained in Figure 2.7 were lower than those previously observed in Figure 2.6. This was most likely caused by the ultrasonic bath, as the water was warm and this could have caused evaporation of the sample during sonication as this has been observed in previous experiments. Overall, the extraction method using Eppendorf™ microcentrifuge tubes using 10% (v/v) dichloromethane (DCM) in methanol (MeOH) as the extraction solution, provided the highest percentage recoveries (between 89 – 96%) for all analytes.
Figure 2.7: Average percentage recovery (± standard deviation, n = 5 measurements) for cocaine, benzoylecgonine, heroin and 6-acetylmorphine in a spiked artificial perspiration standard (n = 5) extracted from paper in screw top vials using 10, 20, 30% (v/v) dichloromethane (DCM) in methanol (MeOH).

2.3.4 Extraction method

The developed extraction method was performed based on three steps before LC-MS analysis, namely sample extraction, evaporation to dryness and reconstitution. Figure 2.8 outlines the developed extraction procedure used for the preparation of samples for LC-MS analysis. In order to extract the analytes from the surface, the paper surface was placed in a 2 ml Eppendorf™ microcentrifuge tube with 1.5 ml extraction solution 10% (v/v) dichloromethane (DCM) in methanol (MeOH). The sample was centrifuged for 2 min at 9500 x g relative centrifugal force (rcf). The paper was then removed from the microcentrifuge tube and discarded. The resulting sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C). A 100 µl of mobile phase solution, 5% (v/v) acetonitrile in water with 0.1% formic acid containing 50 ng/ml of internal standard solution (cocaine-d₃, benzoylecgonine-d₃, heroin-d₉ and 6-monoacetylmorphine-d₃) was added to reconstitute the extract. The sample was then centrifuged for 2 min at 9500 x g rcf and transferred to an autosampler vial for LC-MS analysis. This extraction method was applied to all fingerprint samples collected in this research.
2.3.5 Matrix effects

The effects of the matrix on the extraction efficiency of cocaine, heroin and respective metabolites were evaluated for acetonitrile, artificial eccrine perspiration and fingerprints using analyte standards at three concentration levels, 100, 500 and 1000 ng/ml. Five replicate samples were prepared for each matrix at each concentration level, with 10 µl of standard spiked onto paper to produce 1, 5 and 10 ng of analyte residue on surface. For fingerprints, the analyte standard (10 µl of 100, 500 or 1000 ng/ml) in acetonitrile was pipetted onto fingerprint samples collected from non-drug users. Fingerprint samples were collected from n = 5 participants without any preparation of the hands (natural fingerprints) and after washing hands with soap and water, prior to wearing nitrile gloves for 10 min to aid the secretion of perspiration from the fingertips. All samples were extracted using the extraction method detailed in section 2.3.4. Figure 2.9 shows that the average peak area ratio analyte (A) to internal standard (IS) obtained for cocaine and benzoylecgonine was not analyte dependent or matrix dependent, as the ratio A/IS is similar for all concentration levels and matrices assessed. Results for heroin and 6-acetylmorphine also indicate that the extraction of an analyte standard in acetonitrile and a spiked fingerprint sample produce similar effects. However, the use of artificial eccrine perspiration produced matrix effects for heroin and 6-
acetylmorphine as a higher ratio A/IS was consistently observed for both analytes at all three concentration levels compared to acetonitrile or fingerprints. This shows that artificial eccrine perspiration is not representative of a typical fingerprint matrix. Therefore, in the absence of a validated fingerprint standard it was chosen to not produce matrix-matched calibration curves. Although it is possible to report the results for cocaine and benzoylecgonine as an amount of analyte (e.g. in ng) per fingerprint (as no matrix effects are observed), this is not reported throughout the thesis as this is not possible for heroin and 6-acetylmorphine due to differences observed for the analyte signal in the presence of a fingerprint sample compared to a standard in acetonitrile. Results are therefore reported as a ratio of analyte-to-internal standard (ratio A/IS) signal to allow for the comparison between the levels of analytes in fingerprint samples.

Interestingly, the fingerprint samples (natural and after washing hands) from the five donors studied here show that the matrix effects observed between donors do not vary greatly (fingerprint variability (n = 5 participants) <15% for natural fingerprints and <10% after washing hands with soap).

![Graph showing average peak area ratio A/IS](image)

**Figure 2.9:** Average peak area ratio A/IS (± standard deviation, n = 5 measurements) for cocaine, benzoylecgonine, heroin and 6-acetylmorphine for (n = 5) in spiked acetonitrile, artificial eccrine perspiration and fingerprints (natural and after washing hands with soap).

### 2.3.5.1 Ionisation suppression/enhancement

The influence of ionisation suppression/enhancement of the sample matrix on cocaine, benzoylecgonine, heroin and 6-acetylmorphine was evaluated using acetonitrile (n = 5),
artificial eccrine perspiration (n = 5) and natural fingerprints (n = 10). A blank solvent (acetonitrile), blank solution (artificial eccrine perspiration) or natural fingerprints were deposited on paper and extracted using the extraction method detailed in section 2.3.4. The sample matrices were then spiked with 20 and 80 ng/ml drug standard (containing 50 ng/ml internal standard solution) post extraction to assess the influence of the matrix on the analytes of interest. The ionisation suppression/enhancement percentages (%IS/IE) for both concentration levels (20 ng/ml and 80 ng/ml) are provided in Table 2.3 and Table 2.4 for cocaine, benzoylecgonine, heroin and 6-acetylmorphine, respectively. Results show that the percentage ionisation suppression/enhancement (% IS/IE) for cocaine, benzoylecgonine, heroin and 6-acetylmorphine have negative values, which indicates that some ionisation suppression occurred, however this was < 14% for both concentration levels. Additionally, the same effect was observed for the drug and internal standard. As the average ionisation suppression did not exceed 25% (Scientific Working Group for Forensic Toxicology, 2013); this was acceptable. Although the average peak area showed some ionisation suppression/enhancement effects for the analytes of interest, the ratio A/IS was consistent with the reference standards for all analytes at both concentration levels. This is because the same effect was observed for the deuterated internal standards, therefore using the ratio analyte (A) to internal standard (IS) corrected for the ionisation suppression effects observed.

Table 2.3: Ionisation suppression/enhancement (percentage) for the analysis of cocaine, benzoylecgonine, heroin and 6-acetylmorphine at 20 ng/ml in acetonitrile, artificial eccrine sweat and fingerprint samples (participants 1 – 10).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Coc</th>
<th>Coc-d₃</th>
<th>BZE</th>
<th>BZE-d₃</th>
<th>Her</th>
<th>Her-d₉</th>
<th>6-AM</th>
<th>6-AM-d₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>-4</td>
<td>-3</td>
<td>-9</td>
<td>-8</td>
<td>-2</td>
<td>-3</td>
<td>-2</td>
<td>-3</td>
</tr>
<tr>
<td>Artificial eccrine perspiration</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td>-2</td>
<td>2</td>
<td>-2</td>
</tr>
<tr>
<td>Participant 1</td>
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<td>-8</td>
<td>-8</td>
<td>-4</td>
<td>-4</td>
<td>-4</td>
<td>-3</td>
</tr>
<tr>
<td>Participant 2</td>
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<td>-9</td>
<td>-4</td>
<td>-5</td>
<td>-5</td>
<td>-4</td>
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<td>-8</td>
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<td>-6</td>
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<td>-5</td>
</tr>
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<td>-6</td>
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<td>-8</td>
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<td>-7</td>
<td>-3</td>
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<td>-3</td>
<td>-3</td>
</tr>
</tbody>
</table>

Where Coc = cocaine; Coc-d₃ = cocaine-d₃; BZE = benzoylecgonine; BZE-d₃ = benzoylecgonine-d₃; Her = heroin; Her-d₉ = heroin-d₉; 6-AM = 6-acetylmorphine; 6-AM-d₃ = 6-acetylmorphine-d₃
Table 2.4: Ionisation suppression/enhancement (percentage) for the analysis of cocaine, benzoylecgonine, heroin and 6-acetylmorphine at 80 ng/ml in acetonitrile, artificial eccrine sweat and fingerprint samples (participants 1 – 10).

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<th>BZE</th>
<th>BZE-d3</th>
<th>Her</th>
<th>Her-d3</th>
<th>6-AM</th>
<th>6-AM-d3</th>
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<tr>
<td>Acetonitrile</td>
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<td>-8</td>
<td>-8</td>
<td>-2</td>
<td>-3</td>
<td>-2</td>
<td>-2</td>
</tr>
<tr>
<td>Artificial eccrine perspiration</td>
<td>2</td>
<td>2</td>
<td>-5</td>
<td>-5</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
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<td>-5</td>
<td>-6</td>
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<td>0</td>
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<td>-8</td>
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<td>-2</td>
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<td>-2</td>
<td>-2</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
<td>-4</td>
<td>-2</td>
</tr>
<tr>
<td>Participant 10</td>
<td>-4</td>
<td>-4</td>
<td>-5</td>
<td>-5</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
<td>-2</td>
</tr>
</tbody>
</table>

Where Coc = cocaine; Coc-d3 = cocaine-d3; BZE = benzoylecgonine; BZE-d3 = benzoylecgonine-d3; Her = heroin; Her-d3 = heroin -d3; 6-AM = 6-acetylmorphine; 6-AM-d3 = 6-acetylmorphine-d3

2.3.6 Spike recovery

The recovery of cocaine, benzoylecgonine, heroin and 6-acetylmorphine using the developed extraction method was evaluated at 1, 5 and 10 ng of analyte residue on surface in the presence of fingerprints (n = 5 participants). The average percentage recovery for cocaine, benzoylecgonine, heroin and 6-acetylmorphine are outlined in Table 2.5.

Table 2.5: Percentage recovery range for cocaine, benzoylecgonine, heroin and 6-monooacetyl morphine for (n = 5 measurements) spiked fingerprints (natural and after washing hands with soap).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Percentage recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fingerprints (natural)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>85 – 90</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>83 – 91</td>
</tr>
<tr>
<td>Heroin</td>
<td>33 – 37</td>
</tr>
<tr>
<td>6-Acetylmorphine</td>
<td>26 – 30</td>
</tr>
</tbody>
</table>

The initial extraction method was performed using artificial eccrine sweat to simulate a fingerprint sample, however as this resulted in a higher extraction efficiency for heroin and 6-acetylmorphine compared to fingerprints and acetonitrile (as observed in Figure 2.9, section 2.3.5), it was not representative of a fingerprint matrix. Therefore, the recovery was assessed using natural fingerprints and samples collected after washing hands, which were spiked with drug standard in acetonitrile. Good recoveries were obtained for cocaine (85 – 90%) and benzoylecgonine (83 – 91%). However, lower percentage recoveries were
observed for heroin (26 – 30%) and 6-acetylmorphine (23 – 25%) in the presence of fingerprint samples. Overall, the developed fingerprint extraction method was considered to provide reliable and repeatable results for each analyte of interest.

2.3.7 Method performance

A validation study was carried out for qualitative confirmation/identification measurements according to the Scientific Working Group for Forensic Toxicology guidelines to evaluate the analytical performance of the developed method. A set of experiments were performed to determine the reliability of the method for its intended use and evaluate the limitations of the method, including the working range, sensitivity (limit of detection) and sample stability.

2.3.7.1 Definitions

The definitions provided below are according to the guidelines of the Scientific Working Group of Forensic Toxicology.

**Accuracy:**
closeness of agreement between the mean of the result of measurements of a measurand and the true value of a measurand.

**Blank matrix sample (blank sample):**
a biological fluid or tissue (or synthetic substitute) without target analyte or internal standard.

**Calibration model:**
the mathematical model that demonstrates the relationship between the concentration of analyte and the corresponding instrument response.

**Carry-over:**
the appearance of unintended analyte signal in a subsequent sample after the analysis of a positive sample.

**Fortified matrix sample (spiked sample):**
a blank matrix sample spiked with target analyte and/or internal standard using reference materials.
Interferences:
non-target analytes (i.e. matrix components, other drugs and metabolites, internal standard, impurities) which may impact the ability to detect, identify, or quantitate a targeted analyte. Ionisation suppression/enhancement

Limit of detection:
an estimate of the lowest concentration of an analyte in a sample that can be reliably differentiated from blank matrix and identified by the analytical method.

Precision:
the measure of closeness of agreement between a series of measurements obtained from multiple samples of the same homogenous sample.

Repeatability:
expresses the precision under the same operating conditions over a short time interval.

Reproducibility:
expresses the precision under different days, by different analysts, in different laboratories using different instrumentation.

Reference material:
material, sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for intended use in a measurement process.

Stability:
an analyte’s resistance to chemical change in a matrix under specific conditions for given time intervals.

Working range:
the concentration range of that can be adequately determined by an instrument where the instrument provides a useful signal that can be related to the concentration of the analyte.

2.3.7.2 Working range
A linear calibration curve of analytes in a standard solution was prepared with a concentration range of 50 – 1000 ng/ml of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in acetonitrile. Each calibration standard was prepared from a stock solution (at 5000 ng/ml in acetonitrile) containing cocaine, benzoylecgonine, heroin and 6-
acetylmorphine. The stock solution was prepared from certified reference materials of the individual analytes at 1 mg/ml in acetonitrile or methanol. Calibration standards were prepared at 50, 100, 200, 400, 600, 800 and 1000 ng/ml in acetonitrile by appropriate dilution of the stock solution. Ten microlitres of the calibration standard was added to the paper surface (Whatman 1-Chr grade paper, 2 x 2 cm) to produce 0.5, 1, 2, 4, 6, 8, and 10 ng of analyte residue on surface. The samples were subsequently extracted using the method described in section 2.3.4. Each sample was measured five times over five consecutive days to evaluate the repeatability and reproducibility of the method. A blank mobile phase injection was performed after each calibration standard in triplicate to evaluate the potential for carryover at each concentration level. No carryover was observed at any calibration level for cocaine, benzoylecgonine, heroin and 6-acetylmorphine or the internal standards. Appendix A. 7 - A. 11 provides an overview of the calibration curve data obtained for cocaine, benzoylecgonine, heroin and 6-acetylmorphine over five separate runs.

To evaluate the precision of the method for the retention time and peak area ratio A/IS, the relative standard deviation (%RSD) was calculated for the repeated measurements for each standard of the calibration curve. The method provided good peak area ratio A/IS repeatability with RSD values of 0 – 2% for the repeated measurements over five runs (n = 25) and in a single run (n = 5). Additionally, the retention time reproducibility (%RSD) was <1% for all analytes over n = 5 days. The correlation coefficients (R²) were calculated with R² > 0.999. This demonstrates that the method has a satisfactory level of repeatability and reproducibility. A summary of the instrument performance is outlined in Table 2.6.

Table 2.6: Performance data for LC-MS analysis of cocaine, benzoylecgonine, heroin and 6-acetylmorphine from extracted calibration standards from paper.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Retention time (min)</th>
<th>Peak area ratio A/IS reproducibility %RSD</th>
<th>Linearity R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>%RSD&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>2.12</td>
<td>&lt;1</td>
<td>0.999</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>2.01</td>
<td>&lt;1</td>
<td>0.999</td>
</tr>
<tr>
<td>Heroin</td>
<td>2.06</td>
<td>&lt;1</td>
<td>0.999</td>
</tr>
<tr>
<td>6-acetylmorphine</td>
<td>1.85</td>
<td>&lt;1</td>
<td>0.999</td>
</tr>
</tbody>
</table>

<sup>a</sup> for n = 25 injections at each concentration level over 5 days.

2.3.7.3 Limit of detection

The limit of detection was determined by analysis of progressively more dilute extracted drug standards (Armbruster <i>et al.</i>, 1994). This approach was applied, because the blank signal observed for heroin and 6-acetylmorphine was zero and therefore could not be used
to calculate the limit of detection. Standards of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in acetonitrile were spiked on paper to produce 10, 20, 30, 40, 50, 100, 200, 300, 400 and 500 pg of analyte residue on surface, including a blank sample substrate and blank solvent (acetonitrile). Ten microlitres of standard was spiked onto paper (no spike added to the blank paper or blank solvent) and prepared using the extraction method described in section 2.3.4. Using the method by Armbruster et al. (1994), the limit of detection was determined to be the concentration at which reproducible and reliable measurements were obtained for the lowest analyte concentration. The acceptance criteria were based on the retention time within ±2% (n = 5) and repeatability of the peak area ratio A/IS measurements (n = 5) ≤20%. Figure 2.10 shows the trend of the standards extracted from paper used to evaluate the limit of detection. The minimum detectable limit for each analyte of interest was determined to be; cocaine = 10 pg (ratio A/IS 0.002), benzoylecgonine = 30 pg (ratio A/IS 0.004), heroin = 30 pg (ratio A/IS 0.003) and 6-acetylmorphine = 40 pg (ratio A/IS 0.003) extracted from paper.

![Figure 2.10: Evaluation of the limit of detection for cocaine, benzoylecgonine, heroin and 6-acetylmorphine by analysing progressively more dilute standards using extracted drug standards in acetonitrile. Data represented as the average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, n = 5 measurements) with error bars indicating the standard deviation.](image)

2.3.7.4 Sample stability – processed sample

Analyte stability in solution is an important factor to evaluate as it can influence the integrity of the sample for reliable detection and identification of the analytes of interest. Analyte
standards (containing cocaine, benzoylecgonine, heroin and 6-acetylmorphine) in acetonitrile were prepared to produce 0.5, 1, 2, 4, 6, 8, and 10 ng of analyte residue on surface. The sample stability of each analyte in standard solution (5% (v/v) acetonitrile in water with 0.1% formic acid) was evaluated. Each standard was measured \( n = 5 \) times, to provide an average peak area ratio of analyte to internal standard. The samples at each level were analysed after sample preparation to establish the time zero response (run 1). Each subsequent analysis of the samples was performed in 24-hour increments; run 1 = 0 hours, run 2 = 24 hours, run 3 = 48 hours; run 4 = 72 hours and run 5 = 96 hours. Figure 2.11 shows the level of stability maintained for cocaine, benzoylecgonine, heroin and 6-acetylmorphine at each concentration level. The analytes of interest were considered to be stable over 96 hours, with an RSD less than <2% (\( n = 25 \) measurements) for all analytes.

![Graphs showing sample stability](image)

**Figure 2.11:** Evaluation of sample stability (processed sample) for cocaine, benzoylecgonine, heroin and 6-acetylmorphine in standard solution (5% (v/v) acetonitrile in water with 0.1% formic acid and 50 ng/ml internal standard). Data represented as average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, \( n = 5 \) measurements) with error bars indicating the standard deviation.
2.4 Summary

All samples were analysed using liquid chromatography – mass spectrometry (LC-MS) at the University of Surrey. A fingerprint extraction method was developed for the analysis of cocaine, benzoylecgonine, heroin and 6-acetylmorphine. The surface used for the deposition of fingerprints was evaluated using paper (Whatman 1-Chr chromatography paper) and glass cover slips with a range of solvent mixtures for extraction. Both sample surfaces have previously been used for the analysis of drugs in fingerprints (Costa, 2017; Kuwayama et al., 2014; Kuwayama et al., 2013; Goucher et al., 2009; Jacob et al., 2008). Results showed that the use of a paper surface in combination with more polar solvents resulted in higher extraction efficiencies for the analytes of interest (section 2.3.1). The developed extraction method was based on the use of microcentrifuge tubes for the extraction of cocaine, benzoylecgonine, heroin and 6-acetylmorphine from paper using 10% dichloromethane in methanol as the extraction solvent (section 2.3.4). The effect of the matrix on the extraction and analysis of the analytes of interest was explored using fingerprint samples, artificial eccrine perspiration and acetonitrile. Matrix effects were only observed for heroin and 6-acetylmorphine using artificial eccrine sweat compared to the use of fingerprint samples or acetonitrile (section 2.3.5). In light of these results and the absence of a validated fingerprint standard it was not possible to produce matrix-matched calibration curves, results are therefore stated in terms of analyte to internal standard ratio for all analytes. The recovery values based on spiked fingerprints (natural and after washing hands) were 85 – 90% for cocaine, 83 – 91% for benzoylecgonine, 26 – 30% for heroin and 23 – 25% for 6-acetylmorphine (section 2.3.6). The experimental conditions of the LC-MS method are detailed in Table 2.1 (section 2.2.2). A linear response was obtained for all analytes of interest (for 0.5 - 10 ng of analyte residue on surface) with correlation coefficients (R²) > 0.999 (section 2.3.7.2). Retention times and peak area ratio A/IS demonstrated excellent repeatability (n = 25) <1% RSD for all analytes of interest. The limit of detection for cocaine, benzoylecgonine, heroin and 6-acetylmorphine was at 10, 30, 30 and 40 pg of analyte residue on surface (section 2.3.7.3). Additionally, the analytes of interest were stable in solution over 96 hours, with RSD <2% (n = 25) (section 2.3.7.4).
Chapter 3  Investigation into the Suitability of a Fingerprint to Detect Cocaine and Heroin use

3.0  Introduction

One of the objectives of this research was to evaluate the suitability of fingerprints for the analysis of drugs of abuse (namely, cocaine and heroin). The possibility of using fingerprints for drug testing purposes has recently been explored, as described in Chapter 1. It has been shown that an administered substance and its metabolite can be detected in fingerprints and not in negative control subjects (Costa et al., 2017; Zhang et al., 2015; Kuwayama et al., 2014; Kuwayama et al., 2013; Jacob et al., 2008). However, the suitability of a fingerprint as a sampling matrix for drug testing has not yet been explored. Particularly relating to the significance of the detection of drugs from fingerprints, including the prevalence of cocaine and heroin in a background population of non-drug users has not yet been explored. The use of a fingerprint sample for drug testing purposes should be robust enough to provide a good detection rate (>80%), even if a hand cleaning procedure is used prior to sample collection, otherwise the test would be easily falsified. Additionally, the use of a hand cleaning procedure should successfully remove external contamination on the hands from contaminated surfaces or handling the parent drug, which may otherwise yield false positive results. Therefore, different sampling strategies for the collection of fingerprint samples were investigated, which included the collection of fingerprints (i) without any preparation of the hands (natural), (ii) after washing hands with soap and water and (iii) after wiping hands with alcohol free wipes.

The following section will detail the specific participants recruited for the purpose of this study (section 3.1.1) and the sample collection and preparation methods used for fingerprint samples collected from (i) individuals seeking treatment at an NHS Drug and Alcohol Service and (ii) a background population of non-drug users (section 3.2.3). Analysis of fingerprint samples were performed using the procedure outlined in Chapter 2 section 2.3.4. The detection of cocaine, heroin and their respective metabolites (benzoylecgonine and 6-acetylmorphine) in natural fingerprint samples from drug users and a background population of non-drug users are discussed in sections 3.2.2 and 3.2.3. The application of a threshold
level to differentiate substance misuse (drug users) from environmental exposure to drugs (non-drug users) is discussed in section 3.2.4. The influence of a hand cleaning procedure on the detection of cocaine and heroin use in fingerprints from drug users and non-drug users (background population) is discussed in sections 3.2.5 – 3.2.8. The potential transfer of drugs between individuals is outlined in section 3.2.9. The influence of the fingerprint sample collected on the detection rate of cocaine and heroin is outlined in section 3.2.10.

The present chapter investigates important aspects of using a fingerprint as a new sample matrix, including (i) the prevalence of cocaine, heroin and their respective metabolites in a background population of non-drug users and (ii) the use of fingerprint collection procedures to understand the influence of a hand cleaning procedure on the detection of the analytes of interest. Additionally, the presence of contamination from environmental exposure and transfer of drugs between individuals is explored to determine its impact on the interpretation of results.

### 3.1 Experimental

#### 3.1.1 Study populations

Study participants were recruited from different groups to evaluate the significance of the detection of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprints. These included (i) individuals seeking treatment for drug dependency (n = 15) and (ii) a background population of non-drug users (n = 50). Recruitment of individuals seeking treatment for drug dependency was held at The Xchange in Woking in collaboration with Surrey and Borders NHS Foundation Trust. The Xchange is an NHS Drug and Alcohol Service where individuals are in treatment to overcome drug and alcohol dependency. For drug dependency, individuals are prescribed methadone or buprenorphine to ease the transition to a drug free existence. However, most participants recruited in the study admitted to the use of illegal substances (such as cocaine and heroin) alongside the prescribed drugs. In this case, the target of the study was the analysis of cocaine and heroin use from fingerprint samples collected from individuals who admitted to the use of these illegal drugs in the past 24 hours. The inclusion criteria stipulated that participants needed to be enrolled in a drug treatment programme, aged between 18 – 65 years old (male or female), must have the ability to provide consent and must be able to understand enough English to understand the information sheet and consent form. Due to ethical considerations
only, the drugs administered, and the gender of the participant were recorded. Analysis of fingerprint samples for the detection of cocaine and heroin use was carried out using the LC-MS method described in section 2.2.2. Fingerprint samples were also collected from non-drug users \((n = 50)\) to investigate the prevalence of drugs of abuse in fingerprints from the background population. Study participants were recruited from the University of Surrey (Guildford, UK) who admitted to not have taken any drugs of abuse.

Oral fluid samples were also collected from drug users using Alere™ oral fluid collection devices, to help corroborate the fingerprint results obtained from liquid chromatography–mass spectrometry (LC-MS) analysis. The procedure for the collection of oral fluid samples is outlined in section 3.1.3. The oral fluid samples were analysed by Claritest (a drug testing service) in Norwich, England, where screening tests were carried out using immunoassays, and confirmation test were carried out using LC-MS/MS. Additionally, no oral fluid samples were taken from the background population (non-drug users).

A favourable ethical opinion was obtained from the NHS Research Ethics Service (NRES-REC) and the University of Surrey Ethics Committee for the collection of fingerprint and oral fluid samples from individuals enrolled in Surrey and Borders NHS Drug and Alcohol Services who volunteered taking part in this study, as well as fingerprint samples from non-drug users (background population) at the University of Surrey (NRES-REC reference 14/LO/0346). Participants were given information sheets and informed consent forms in order to understand their involvement in the study. Once informed consent was obtained from the participants, samples were collected according to the protocols outlined in sections 3.1.2 and 3.1.3. The standard operating procedures outlining the detailed protocol for the collection of oral fluid and fingerprint samples are included in Appendix B. 1 and B. 2.

### 3.1.2 Fingerprint

Fingerprint samples were collected on chromatography paper (Whatman™ 1-Chr) cut into 2 x 2 cm. Fingerprint collection kits were prepared by taping the paper substrate onto a microscope glass slide labelled with a unique participant identifier, e.g. H0483210 for the participants recruited from NHS Drug and Alcohol Services and e.g. SUB001 for the background population.

Fingerprint samples \((n = 65)\) were collected from individuals seeking treatment for drug dependency under three different conditions: (i) natural; (ii) after washing hands with soap
and water; and (iii) after wiping hands with alcohol free wipes. Samples were only collected from participants who admitted having taken either cocaine or heroin or both within the past 24 h. Fingerprints (n = 5) were collected from all fingers of the right hand for each participant. Natural fingerprint samples collected were deposited upon arrival at the clinic without any preparation of the hands. The fingerprint collection procedure was carried out as follows:

- the fingerprint collection slide was placed on the weighing balance and tared;
- the participant placed the finger displayed on the label onto the collection slide (e.g. right thumb). The fingerprint must be placed within the rectangular guidelines;
- participant must increase the pressure until the balance reads between 800 – 1200 g of pressure and hold for 10 seconds;
- participant must carefully lift the finger upwards, after providing the required pressure for collection; and
- the above steps are repeated until all five fingerprints are collected.

After the collection of natural fingerprints, the participant was asked to wash hands with soap and water or the researcher wiped the fingers and thumbs with an alcohol-free wipe. The participant then put on nitrile gloves for 10 min to induce perspiration on the fingers and thumbs prior to fingerprint deposition. The fingerprint collection procedure described above was repeated to standardise the collection of samples after the hand cleaning procedure.

Each sample collected was labelled with a unique participant identifier; the finger or thumb that was collected and the condition under which the sample was collected (e.g. natural, soap, or wipe). The collected fingerprint sample was then placed into a microscope glass slide storage box for transport to the University of Surrey, UK. In addition, fingerprint samples were collected from volunteers (non-drug users, n = 5) before and after contact (shaking hands) with participants (n = 5) recruited in the study who admitted having taken either cocaine or heroin (or both) to determine the potential of secondary transfer of the drugs between individuals.

Fingerprint samples (n = 100) were collected from n = 50 participants (background population) at the University of Surrey. Fingerprint samples collected from the background population were collected (i) natural and (ii) after washing hands with soap and water to
Chapter 3: Investigation into the Suitability of a Fingerprint to Detect Cocaine and Heroin use

determine the prevalence of cocaine and heroin in the background population. Only the right thumb and right index finger were collected for each participant. A more detailed protocol used for the collection of fingerprint samples is described in the SOP in Appendix B. 2.

3.1.3 Oral fluid

Oral fluid samples were collected from participants at NHS drug and alcohol services only, using Quantisal™ oral fluid collection devices. One oral fluid sample was collected per participant. The collection of oral fluid samples was carried out as follows:

- research personnel took the swab used for the collection of oral fluid from the pre-labelled kit;
- the participant was asked to churn saliva;
- the swab was placed between the teeth and cheek or under the tongue of the participant until the indicator on the swab turned blue. This indicates enough saliva was collected (ca. 1 ml);
- once the indicator turned blue, the swab was removed from the mouth and placed in the collection tube provided in the kit, end first; and
- the lid of the tube was pressed until sealed.

A more detailed description of the standard operating procedure for the collection of oral fluid samples is detailed in Appendix B. 1. Oral fluid samples collected were sent to Claritest (Norwich, UK) for analysis, where screening and confirmation tests were carried out using immunoassay and LC-MS analysis, respectively.

A summary of the study participants and samples collected for each population is provided in Table 3.1.

Table 3.1: Recruitment information for the study populations and type of sample collected.

<table>
<thead>
<tr>
<th>Study population</th>
<th>N</th>
<th>Fingerprint samples collected</th>
<th>Oral fluid sample collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background population</td>
<td>50</td>
<td>Right thumb and index finger</td>
<td>No</td>
</tr>
<tr>
<td>Drug-users</td>
<td>15</td>
<td>Right thumb, index, middle, ring and little finger</td>
<td>Yes</td>
</tr>
<tr>
<td>Secondary transfer</td>
<td>5</td>
<td>Right thumb, index, middle, ring and little finger</td>
<td>No</td>
</tr>
</tbody>
</table>
3.2 Results and Discussion

3.2.1 Comparison between study populations

3.2.1.1 Cocaine

Fingerprint samples were collected from non-drug users (background population, n = 49) and cocaine users (n = 13). Samples were collected using different collection procedures, including (i) natural fingerprints, (ii) after handwashing and (iii) after wiping hands with alcohol free wipes, to determine whether (a) cocaine and benzoylecgonine were prevalent in a background population of non-drug users and (b) the levels observed were different depending on the study population. Figure 3.1 shows the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine in fingerprints collected from cocaine users and non-drug users for the different sample collection procedures (e.g. natural, soap or wipe). Note that for the background population samples were only collected as natural fingerprints and after washing hands with soap and water.

Figure 3.1: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine from a background population (non-drug users) and cocaine users for samples collected as natural fingerprints, after washing hands with soap and water, and after wiping hands with alcohol free wipes.

Results show that cocaine and benzoylecgonine were present in nearly all fingerprint samples collected from cocaine users. However, fingerprint samples from the background population also showed traces of cocaine and benzoylecgonine. One of the main sources of cocaine in fingerprint samples from non-drug users is through environmental exposure (e.g. with banknotes) (Zuccato & Castiglioni, 2009; Armenta & de la Guardia, 2008; Carter et
There is a significant difference in distribution of the level (ratio A/IS) of cocaine (\(U = 0, p < 0.001\)) and benzoylcegonine (\(U = 27, p = 0.001\)) present in fingerprint samples from non-drug users compared to drug users (Appendix E.1). The median cocaine level (ratio A/IS) was 0.004 for non-drug users compared to 0.900 for cocaine users. For benzoylcegonine the median level present in fingerprints from non-drug users was 0.009 compared to 0.028 for cocaine users. The background population admitted not having taken cocaine, suggesting that environmental exposure is the most likely explanation for the presence of these analytes in their fingerprints.

Fingerprints (\(n = 98\)) collected after washing hands with soap and water from non-drug users showed that no traces of cocaine and benzoylcegonine were detected (with one false positive for cocaine). This suggests that the use of a hand cleaning procedure prior to sample collection is advantageous to remove possible presence of cocaine and benzoylcegonine from environmental exposure, which could otherwise lead to false positive tests. Additionally, fingerprints (\(n = 40\)) collected after washing hands from cocaine users showed that the majority of samples remained positive for both cocaine (40 samples) and benzoylcegonine (34 samples). However, a significant difference is observed in the level of cocaine (\(U = 819, p = 0.002\)) and benzoylcegonine (\(U = 461, p < 0.001\)) present in natural fingerprints compared to samples collected after handwashing (Appendix E.1). The median cocaine level (ratio A/IS) was 0.900 for natural fingerprints but reduced to 0.282 after handwashing. Similarly, for benzoylcegonine, the median level (ratio A/IS) present in natural fingerprints was 0.028 compared to 0.009 after handwashing. This suggests that the use of a handwashing procedure did remove some cocaine and benzoylcegonine present on the fingers. Interestingly, no significant difference (at \(p = 0.05\)) was observed for cocaine (\(U = 603, p = 0.059\)) and benzoylcegonine (\(U = 609, p = 0.144\)) between natural fingerprints compared to samples collected after wiping hands with alcohol free wipes (Appendix E.1). The median cocaine level (ratio A/IS) for natural fingerprints was 0.900 compared to 0.513 after wiping hands. For benzoylcegonine the median level (ratio A/IS) was 0.028 compared to 0.023 after wiping hands. This suggests that the use of a hand wiping procedure was most likely not as effective in the removal of cocaine and benzoylcegonine in fingerprints (resulting in similar levels between both sampling methods) compared to a handwashing procedure.
3.2.1.2 Heroin

Comparison between fingerprint samples collected from drug users and non-drug users shows that heroin and 6-acetylmorphine is not prevalent in a background population of non-drug users (see Figure 3.2). In contrast to cocaine, heroin is not detected in any fingerprint sample from the background population (natural or after handwashing). 6-Acetylmorphine was detected in one fingerprint sample (natural) from the background population, however after washing hands all fingerprints were negative. Note that no samples were collected from the background population after wiping hands. Compared to cocaine, heroin is not prevalent on banknotes and is more likely to degrade (Carter et al., 2003; Jenkins, 2001). This suggests that heroin and 6-acetylmorphine are not commonly encountered through environmental exposure and therefore the presence of these analytes in samples most likely arises from handling the parent drug or drug administration.

![Box and whisker plots](image)

**Figure 3.2:** Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine from a background population (non-drug users) and heroin users for samples collected as natural fingerprints, after washing hands with soap and water, and after wiping hands with alcohol free wipes.

Figure 3.2 shows that the level of heroin present in fingerprint samples collected from heroin users varies depending on the sample collection procedure used. A significant difference is observed between the level of heroin present in natural fingerprints compared to those collected after washing hands (U = 0.067, p < 0.001) and after wiping hands (U = 104, p < 0.001) (Appendix E. 1). The median heroin level (ratio A/IS) was 0.067 for natural fingerprints compared to 0.011 after handwashing and 0.016 after wiping hands. In contrast, for 6-acetylmorphine no significant difference (at p = 0.05) was observed between natural
fingerprints compared to fingerprints collected after washing hands with soap and water (U = 807, p = 0.061) or after wiping hands with alcohol free wipes (U = 557, p = 0.633) (Appendix E. 1). The median 6-acetylmorphine level for natural fingerprints was 0.030 compared to 0.021 after hand washing and 0.028 after wiping hands. This suggests that the hand cleaning procedures were more effective in the removal of heroin in fingerprints compared to 6-acetylmorphine.

3.2.2 Drug users

3.2.2.1 Cocaine

A total of 13 participants were recruited who admitted having taken cocaine in the past 24 h. Samples (n = 5 per participant) were collected as natural fingerprints for all participants (41026, 41028 and 41036 – 41046). Cocaine and benzoylecgonine were detected in nearly all fingerprint samples collected, as shown in Figure 3.3. Cocaine was detected in all fingerprint samples for all participants, resulting in a 100% (65 samples) detection rate based on participant testimony. In contrast, 61 samples (94%) were positive for benzoylecgonine in natural fingerprint samples collected. This is because participant 41026 admitted having taken cocaine, but benzoylecgonine was only detected in 1 out of 5 fingerprint samples collected for this participant. The presence of both the parent drug and metabolite is significant. However, a greater significance is attributed to the presence of the metabolite as this indicates that the individual has administered and metabolised the parent drug in the body. In contrast, the sole presence of the parent drug could be due to contamination or contact with the parent drug.

![Figure 3.3](image.png)

Figure 3.3: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) based on n = 5 fingerprint samples for participants who admitted having taken cocaine in the past 24 hours. Solid line represents the limit of detection (LOD).
A similar trend is observed for cocaine and benzoylecgonine in fingerprint samples collected from these participants. A higher level of cocaine present in fingerprint samples also resulted in a higher level of benzoylecgonine, e.g. see participants 41045 and 41040. Interestingly, results for participant 41026 showed the lowest levels of cocaine which was paired with a low detection rate for benzoylecgonine. Overall, the spread in the levels of cocaine and benzoylecgonine observed in fingerprint samples is narrow for each participant, which suggests that although fingerprint variability (sample size and volume) is not accounted for, similar concentrations are present in the samples.

An overview of the oral fluid confirmation and fingerprint test results are shown in Table 3.2. There is a discrepancy observed between the oral fluid and fingerprint screening results for 3 participants, namely participants 41026, 41036 and 41040. In these cases, a negative oral fluid result was obtained, despite a positive fingerprint result for both cocaine and benzoylecgonine in all samples for participants 41036 and 41040. Interestingly, the levels of cocaine and benzoylecgonine observed in fingerprints for participants 41036 and 41040 are similar to those of other participants whose oral fluid samples were positive for cocaine use.

Table 3.2: Oral fluid confirmation and fingerprint results based on n = 5 natural fingerprint samples collected for participants who admitted having taken cocaine in the past 24 hours.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Oral fluid confirmation test (concentration ng/ml)</th>
<th>Fingerprint screening test (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cocaine</td>
<td>Benzoylecgonine</td>
</tr>
<tr>
<td>41026</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>41028</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41036</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>41037</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>41038</td>
<td>Negative</td>
<td>64</td>
</tr>
<tr>
<td>41039</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41040</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>41041</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41042</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41043</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41044</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41045</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41046</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

*LOD = limit of detection (see section 2.3.7.3)
As mentioned previously, for participant 41026, cocaine was present in all fingerprint samples, but benzoylecgonine was only present in one fingerprint sample (right ring finger). Although a high detection rate was observed for cocaine and benzoylecgonine in fingerprint samples, these results should be treated with caution given that these findings are based on natural fingerprint samples collected. The presence of cocaine and benzoylecgonine could arise from external contamination rather than from secretion of drugs from the eccrine glands of the fingertips after ingestion and absorption of the drug. Alternatively, it is possible that the detection window of cocaine and benzoylecgonine in fingerprints is greater than in oral fluid, resulting in negative oral fluid results.

A total of 2 participants admitted having taken heroin only in the past 24 hours. However, fingerprint samples collected from these participants also showed traces of cocaine and benzoylecgonine (see Figure 3.4). All fingerprint samples (natural) collected for participants 41033 and 41035 were positive for cocaine. Additionally, benzoylecgonine was present in all fingerprint samples for participant 41033, but not for participant 41035. The oral fluid results for these participants were negative for both cocaine and benzoylecgonine. This suggests that the detection window of cocaine and benzoylecgonine in fingerprints is >24 h. However, as natural fingerprint samples were collected, contamination present on the hands could also be the source of the presence of these analytes. Overall, the levels of cocaine and benzoylecgonine observed in the fingerprint samples from these participants were lower than those observed for participants who admitted using of cocaine in the past 24 h (see Figure 3.3).

![Figure 3.4: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for cocaine and benzoylecgonine for natural fingerprints collected from individuals admitted having taken heroin only. Solid line represents limit of detection (LOD).](image-url)
3.2.2.2 Heroin

A total of 12 participants were recruited who admitted to the use of heroin in the past 24 h. Natural fingerprint samples (n = 5) were collected for all participants (41033, 41035 – 41039 and 41041 – 41046). Figure 3.5 shows the ratio A/IS obtained for heroin and 6-acetylmorphine in the fingerprint samples collected. Heroin was detected in all fingerprint samples for all participants, with the exception of participant 41036, where heroin was not detected in the right little finger, resulting in a detection rate of 98% (59 samples) based on participant testimony. Additionally, 6-acetylmorphine was detected in all fingerprint samples (n = 60) resulting in a 100% detection rate. The results show that similar levels of heroin and 6-acetylmorphine are present in the fingerprint samples. In contrast, an order magnitude difference was observed between the levels of cocaine and benzoylecgonine in fingerprint samples (section 3.2.2.1). Overall, a similar pattern is observed for the amount of parent drug and metabolite present in each fingerprint. For example, the level of heroin present in fingerprint samples for participant 41033 decreases from right index finger > right thumb > right middle finger > right little finger > right ring finger. A similar pattern is observed for the metabolite 6-acetylmorphine. Additionally, a greater intra-donor variability is observed for this participant for both analytes compared to other participants.

Figure 3.5: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) based on n = 5 natural fingerprint samples collected for participants who admitted having taken cocaine in the past 24 hours. Solid line represents limit of detection (LOD).

Comparison between the oral fluid confirmation and fingerprint results are shown in Table 3.3. There is a disagreement between the oral fluid and fingerprint results obtained for participants 41036 – 41038 and 41046, as the oral fluid results do not confirm opiate use for these participants. In contrast, the fingerprint results conform to the participant testimony.
Interestingly, the highest levels of heroin were present in fingerprint samples from participant 41033 and the oral fluid sample for this participant also resulted in the highest morphine concentration. It is possible that this is observed due to recent heroin use. However, a limitation of monitoring morphine is that it is not solely indicative of heroin use. Morphine could be present if the individual has administered morphine itself or codeine or heroin (as metabolism of these substances forms morphine) (Bogusz, 2008).

Table 3.3: Oral fluid confirmation and fingerprint results based on n = 5 natural fingerprint samples collected from participants who admitted having taken heroin in the past 24 hours.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Oral fluid confirmation test (concentration ng/ml)</th>
<th>Fingerprint screening test (n = 5) Number of values &gt;LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morphine 6-Acetylmorphine</td>
<td>Heroin 6-Acetylmorphine</td>
</tr>
<tr>
<td>41033</td>
<td>&gt;240 32</td>
<td>5 5</td>
</tr>
<tr>
<td>41035</td>
<td>138 32</td>
<td>5 5</td>
</tr>
<tr>
<td>41036</td>
<td>Negative Negative</td>
<td>4 5</td>
</tr>
<tr>
<td>41037</td>
<td>Negative Negative</td>
<td>5 5</td>
</tr>
<tr>
<td>41038</td>
<td>Negative Negative</td>
<td>5 5</td>
</tr>
<tr>
<td>41039</td>
<td>Negative &gt;32</td>
<td>5 5</td>
</tr>
<tr>
<td>41041</td>
<td>69 &gt;32</td>
<td>5 5</td>
</tr>
<tr>
<td>41042</td>
<td>&gt;189 &gt;32</td>
<td>5 5</td>
</tr>
<tr>
<td>41043</td>
<td>60 &gt;32</td>
<td>5 5</td>
</tr>
<tr>
<td>41044</td>
<td>Negative 22.6</td>
<td>5 5</td>
</tr>
<tr>
<td>41045</td>
<td>90 Negative</td>
<td>5 5</td>
</tr>
<tr>
<td>41046</td>
<td>Negative Negative</td>
<td>5 5</td>
</tr>
</tbody>
</table>

*LOD = limit of detection (see section 2.3.7.3)

A total of 3 participants (41026, 41028 and 41040) admitted to the sole use of cocaine in the past 24 h. Furthermore, the oral fluid samples for these participants were negative for opiate use. No heroin or 6-acetylmorphine were detected in the fingerprint samples for participant 41026. However, the results showed that the fingerprint samples for participant 41028 were positive for heroin in all natural samples collected (see Figure 3.6). 6-Acetylmorphine was also present in all fingerprint samples for this participant, except for the right thumb. Additionally, for participant 41040, heroin was detected in the right middle finger only, whereas the right index, middle and ring finger were positive for 6-acetylmorphine. It is possible that these traces of heroin and 6-acetylmorphine were present due to heroin use >24 h, which suggests that the detection window is longer than 24 h.
Figure 3.6: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for heroin and 6-acetylmorphine for natural fingerprints collected from individuals admitted having taken cocaine only. Solid line represents limit of detection (LOD).

### 3.2.2.3 Relationship between the parent drug and metabolite

The relationship between the parent drug and metabolite was evaluated in natural fingerprint samples by comparing the average peak area A/IS (n = 5) as a ratio of parent drug-to-metabolite. Figure 3.7 shows the ratio of cocaine-to-benzoylcegonine for cocaine users and heroin users in natural fingerprint samples. The results show that the ratio of cocaine-to-benzoylcegonine varies within a participant based on the different fingerprint samples collected as well as between participants. The difference between participants is not surprising as the dose and time of drug administration is likely to vary from participant to participant. However, the difference observed within a participant suggests that there are different levels of cocaine and benzoylcegonine present in each of the fingerprint samples. This can be a result of the different sample size between fingerprints and the amount of sample collected, which were not controlled.

For participants 41026, 41036 and 41040, the oral fluid tests were negative for cocaine. However, cocaine and benzoylcegonine were present in the natural fingerprint samples collected. The ratio cocaine-to-benzoylcegonine for participant 41036 is lower than those observed for the other participants. However, for participants 41026 and 41040, the ratio of cocaine-to-benzoylcegonine falls within the range of the participants whose oral fluid samples were positive for cocaine. This demonstrates that it is not possible to use the relationship between cocaine and benzoylcegonine to make a distinction between participants whose oral fluid were positive and negative for cocaine in natural fingerprint samples.
Participants 41033 and 41035 admitted having taken heroin only. However, for participant 41033, both cocaine and benzoylecgonine were detected in all natural fingerprint samples collected. The ratio cocaine-to-benzoylecgonine observed for participant 41033 is in the lower range of the ratios observed for cocaine users. For participant 41035, the ratio of cocaine-to-benzoylecgonine could not be determined, as benzoylecgonine was not detected in any of the fingerprint samples.

Figure 3.7: Average peak area ratio of cocaine-to-benzoylecgonine for cocaine and heroin users in natural fingerprint samples collected.

The relationship between the presence of heroin and 6-acetylmorphine for individuals who admitted having taken heroin in the past 24 h is shown in Figure 3.8. The results show that, similarly to cocaine, variability is observed in the natural fingerprint samples collected from the same participant and between participants. For participant 41026, heroin and 6-acetylmorphine were not detected in any fingerprint sample, therefore the ratio heroin-to-6-acetylmorphine could not be calculated. Similarly, for participant 41040, the ratio heroin-to-6-acetylmorphine could only be calculated for the right ring finger. However, this fingerprint sample still falls within the heroin-to-6-acetylmorphine range observed for heroin users. For participants 41036, 41037, 41038 and 41046, the oral fluid test was negative for opiates. This despite the presence of both heroin and 6-acetylmorphine in all (natural) fingerprints collected for these participants. The ratio heroin-to-6-acetylmorphine observed for these participants is similar to those observed in fingerprint samples from participants whose oral fluid samples were positive. No clear distinction can be made using
the relationship between heroin and 6-acetylmorphine between these participants based on the collection of natural fingerprints. The ratio heroin-to-6-acetylmorphine for the participants (41026, 41028 and 41040) who admitted having taken cocaine only shows that no distinction can be made between fingerprint samples from cocaine users and heroin users in natural fingerprints (see Figure 3.8).

Figure 3.8: Average peak area ratio of heroin-to-6-acetylmorphine (n = 5 measurements) for heroin and cocaine users in natural fingerprint samples collected.

3.2.3 Background population (non-drug users)

To evaluate the significance of the detection of trace cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprint samples from individuals seeking treatment for drug dependency, fingerprint samples were also collected from a background population (non-drug users). This should help to determine the prevalence of the analytes of interest in fingerprint samples from the background population. In a multiple donor study, fingerprints should be collected from as many different people (preferably >40) to represent the study population (Sears et al., 2012). In this study, natural fingerprint samples (n = 2 per participant, right thumb and right index finger) were collected from 50 participants (SUB003-SUB053). For one participant SUB016, the right index finger failed during LC-MS analysis. Therefore, it was decided not to include the data for SUB016 due to an incomplete set of fingerprint samples.
3.2.3.1 Cocaine

For the majority of the fingerprint samples collected (natural) from the non-drug users, cocaine and benzoylecgonine were not detected. Figure 3.9 shows the fingerprint for cocaine and benzoylecgonine in the background population. Thirteen fingerprint samples (13%) collected from non-drug users (49 participants), were positive for cocaine. In contrast, benzoylecgonine was detected in 5 fingerprint samples (5%) of the background population. A combination of cocaine and benzoylecgonine in fingerprint samples was only detected in 4% (4 samples) of the background population.

![Cocaine Fingerprint Results](chart1.png)

**Figure 3.9:** Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for cocaine and benzoylecgonine from the background population for natural fingerprints collected (n = 2 fingerprint per participant). Solid line represents the limit of detection (LOD).

For participants SUB013 and SUB014, both cocaine and benzoylecgonine were detected in the right thumb, whereas the right index finger was negative for both analytes. In contrast, for participant SUB037, cocaine and benzoylecgonine were detected in the right index finger.
but not the right thumb. For participant SUB043, both the right thumb and right index were positive for cocaine. However, benzoylecgonine was only detected in the right index finger for this participant. Overall, the level of cocaine present in the fingerprint samples from the background population (ratio A/IS 0.004 – 0.061) were lower than those observed for drug users (ratio A/IS 0.069 – 22.981). In contrast, similar levels of benzoylecgonine were observed in the fingerprints of non-drug users (ratio A/IS 0.005 – 0.015) compared to those found in fingerprints from drug users (ratio A/IS 0.004 – 0.426). These results suggest that the collection of natural fingerprint samples can lead to false positive results for non-drug users, due to the prevalence of cocaine in the environment if no threshold level is applied. This demonstrates that the choice of sampling regime is an important consideration for fingerprint testing.

3.2.3.2 Heroin

The fingerprint results from the background population were all negative for the presence of heroin. 6-Acetylmorphine was present in 1% (1 sample) of the background population (for SUB037 right index finger). However, no heroin was present in the right index finger for this participant. Additionally, the right thumb was negative for both heroin and 6-acetylmorphine. All fingerprint samples (n = 98) from the background population were negative for the combination of heroin and 6-acetylmorphine. This is contrary to the prevalence of cocaine in the background population, where 4% (4 samples) was positive for both cocaine and benzoylecgonine. Compared to cocaine, heroin is not prevalent on banknotes and is more likely to degrade (Carter et al., 2003; Jenkins, 2001). The possible presence of heroin in fingerprint samples due to environmental exposure is therefore less likely to occur.

3.2.4 Threshold level

3.2.4.1 Cocaine

The results obtained for natural fingerprint samples collected (n = 98) from the background population showed that cocaine can readily be detected in fingerprint samples from non-drug users (13%), whereas benzoylecgonine was detected in 5% of the background population. This indicates that there is a requirement to establish a threshold level for natural fingerprint samples to differentiate non-drug users from drug users. A key concept in drug testing is the use of a cut-off level. A threshold level is chosen which will maximise the detection rate, but also minimise the number of false positive results obtained in drug testing.
Chapter 3: Investigation into the Suitability of a Fingerprint to Detect Cocaine and Heroin use

The use of this concept (threshold level) should help to differentiate between fingerprint samples from individuals who admitted taking a substance and the background population (non-drug users). It must be noted that with the use of a threshold level, a negative screening result does not necessarily indicate no substance was taken. It might contain drugs which are below the proposed threshold level.

The influence of various threshold levels on the detection rate of cocaine and benzoylecgonine in natural fingerprint samples collected from drug users and non-drug users were evaluated per analyte at the limit of detection (ratio A/IS 0.002 for cocaine and 0.004 for benzoylecgonine) and ratio A/IS of 0.005 – 0.030, in increments of 0.005. The threshold levels were proposed to lower the false positive detection rate of cocaine and benzoylecgonine in fingerprint samples from the background population, which could otherwise produce false positive fingerprint results. For the background population, only the right thumb and index finger (n = 2) were collected for the purpose of this study. In contrast, n = 5 fingerprint samples (right thumb to right little finger) per participant were collected from the drug users.

The detection rates for cocaine and benzoylecgonine based on the proposed threshold levels for both groups, drug users and non-drug users (background population) are shown in Table 3.4 (cocaine) and Table 3.5 (benzoylecgonine). In order for all fingerprint samples from the background population to be negative (0% positive detection rate), a threshold level at ratio A/IS 0.65 is required for cocaine. This is because a high level of cocaine was present in the right thumb for participant SUB014 (non-drug user). In contrast, a threshold level at ratio A/IS 0.020 results in a 0% false positive detection rate for benzoylecgonine in the background population. As the level of cocaine in fingerprint samples collected from drug users are significantly higher than those of the background population (non-drug users), a 100% positive detection rate is still observed in all fingerprints at this threshold level. In contrast, a threshold level (ratio A/IS) at 0.020 for benzoylecgonine results in a 63% positive detection rate for drug users (based on all 5 fingerprints), but 77% based on n = 2 fingerprints (right thumb and index). Therefore, caution should be taken when proposing a high threshold level as this can negatively affect the detection rate of the analyte in fingerprint samples from drug users.
Table 3.4: Comparison of the detection rate of cocaine in fingerprint samples collected from participants admitted having taken cocaine in the past 24 h and the background population based on the proposed threshold levels.

<table>
<thead>
<tr>
<th>Threshold level</th>
<th>Sample</th>
<th>Cocaine detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinic samples (n = 13)</td>
<td>Background samples (n = 98)</td>
</tr>
<tr>
<td></td>
<td>Right thumb</td>
<td>Right index finger</td>
</tr>
<tr>
<td>0.002</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.005</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.010</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.015</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.020</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.025</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.030</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.065</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The use of threshold levels has a greater influence on the detection rate of benzoylecgonine compared to cocaine, due to the fact that similar levels of benzoylecgonine were present in fingerprints from drug users and non-drug users. The differences in the levels present in fingerprints correlates with previous findings by Jacob et al. (2008), who found that fingerprints taken from the same individual could vary up to 3-fold in the amount of substance detected from each finger (Jacob et al., 2008). This suggests that a threshold level cannot be applied to all fingerprint samples but has to be evaluated separately for each fingerprint sample and per analyte, unless a standardised fingerprint sampling procedure has been used to account for fingerprint variability (sample size and volume).

Table 3.5: Comparison of the detection rate of benzoylecgonine in fingerprint samples collected from participants admitted having taken cocaine in the past 24 h and the background population based on the proposed threshold levels.

<table>
<thead>
<tr>
<th>Threshold level</th>
<th>Sample</th>
<th>Benzoylecgonine detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinic samples (n = 13)</td>
<td>Background samples (n = 98)</td>
</tr>
<tr>
<td></td>
<td>Right thumb</td>
<td>Right index finger</td>
</tr>
<tr>
<td>0.004</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>0.005</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>0.010</td>
<td>92</td>
<td>85</td>
</tr>
<tr>
<td>0.015</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>0.020</td>
<td>85</td>
<td>69</td>
</tr>
<tr>
<td>0.025</td>
<td>77</td>
<td>54</td>
</tr>
<tr>
<td>0.030</td>
<td>46</td>
<td>54</td>
</tr>
</tbody>
</table>

In drug testing an emphasis is put on the detection of drug metabolites, because this is indicative of administration of a substance rather than contact with a contaminated surface. A combination of cocaine and benzoylecgonine was only detected in 4% (4 samples) of
fingerprint samples from the background population. However, by applying a threshold level at ratio A/IS 0.005 for both cocaine and benzoylecgonine and the requirement for the presence of both cocaine and benzoylecgonine in fingerprint samples from the background population, only results in a false positive rate of 1% (1 sample). At this threshold level, a 92% detection rate is obtained in fingerprints from drug users (based on the right thumb and index finger) which relates to an 8% false negative detection rate.

3.2.4.2 Heroin
The results obtained for natural fingerprint samples collected from the background population showed that there is no significant environmental exposure to heroin. From n = 98 fingerprint samples, only 1% (1 sample) was positive for 6-acetylmorphine, whereas none were positive for heroin. In contrast to cocaine, this shows that there is no requirement to impose a threshold level for natural fingerprint samples for the analysis of heroin. The detection limit can be used as the decision level instead, as the detection of heroin and 6-acetylmorphine above the detection limit would be significant. However, there is a limitation to the evaluation of natural fingerprint samples, as mentioned previously. Although there is no false positive rate observed for the background population, the presence of heroin and 6-acetylmorphine in natural fingerprints collected might still provide false positive results if the participant touched a contaminated surface or handled the parent drug. In this instance, the presence of heroin and 6-acetylmorphine would not necessarily indicate administration of a drug.

3.2.5 Effect of wiping hands: drug users
The fingerprint sampling strategy was evaluated by comparing the collection of natural fingerprints to samples collected after wiping hands with alcohol free wipes. This is particularly important, as external contamination of drugs on the hands can result in false positive results if natural fingerprints are collected. Comparison in the distribution of cocaine and benzoylecgonine between natural fingerprints and samples collected after wiping hands with alcohol free wipes is shown in Figure 3.10. No significant difference (at \( p = 0.05 \)) is obtained for cocaine (\( U = 231, p = 0.114 \)) and benzoylecgonine (\( U = 258, p = 290 \)) between natural fingerprints and samples collected after wiping hands with alcohol free wipes for same participants (Appendix E. 2). The median cocaine level (ratio A/IS) was 0.850 for natural fingerprints and 0.513 after wiping hands. For benzoylecgonine, the median benzoylecgonine level in natural fingerprints was 0.030 compared to 0.023 after
wiping hands. This could mean that the use of a hand wiping procedure was not effective in the removal of cocaine and benzoylecgonine, therefore resulting in similar levels for both sample collection procedures.

Figure 3.10: Box and whisker plots (median, 25 – 75\textsuperscript{th} percentiles, whiskers 5 – 95\textsuperscript{th} percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine between natural fingerprints and samples collected after wiping hands with alcohol free wipes for the same participants.

Comparison in the distribution of the levels of heroin and 6-acetylmorphine present in natural fingerprints and after handwashing for the same participants is shown in Figure 3.11. The results show that a similar distribution is observed for heroin in natural fingerprints compared to samples collected of wiping hands. However, the two hand cleaning procedures result in a difference in the median level of heroin present.

Figure 3.11: Box and whisker plots (median, 25 – 75\textsuperscript{th} percentiles, whiskers 5 – 95\textsuperscript{th} percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine between natural fingerprints and samples collected after wiping hands with alcohol free wipes for the same participants.
In comparison to natural fingerprint samples, the heroin levels are significantly lower after wiping hands with alcohol-free wipes based on a Mann-Whitney U test (U = 42, p < 0.001). The median heroin level (ratio A/IS) present in natural fingerprints was 0.050 compared to 0.016 after wiping hands. This suggests that the alcohol-free wipes removed heroin present on the hands, resulting in lower analyte levels. For 6-acetylmorphine, a closer distribution is observed after wiping hands compared to natural fingerprints. However, no significant difference is observed in the levels present based on the two sample collection procedures. The median 6-acetylmorphine level (ratio A/IS) was 0.019 in natural fingerprints compared to 0.028 after wiping hands for the same participants. A higher median level is observed after wiping hands, which could be due to new sweat secretion.

3.2.5.1 Cocaine

A number of participants (n = 5) were recruited who admitted having taken cocaine in the past 24 h and whose fingerprint samples (n = 5) were collected as natural fingerprints and after wiping hands with alcohol-free wipes. Figure 3.12 shows the fingerprint results (ratio A/IS) for cocaine and benzoylecgonine after wiping hands for each participant. Both cocaine and benzoylecgonine were detected in all fingerprints for all participants after wiping hands with alcohol-free wipes (100% detection rate). The results show a narrow spread in the levels of cocaine and benzoylecgonine present in the fingerprint samples, which suggests that the inter- and intra-donor variability is small, similar to the natural fingerprints collected from these participants.

Figure 3.12: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) based on n = 5 fingerprint samples collected after wiping hands with alcohol-free wipes for participants who admitted having taken cocaine in the last 24 h. Solid line represents limit of detection (LOD).
An overview of the oral fluid confirmation and fingerprint results after wiping hands with alcohol free wipes is shown in Table 3.6. A disagreement is observed between the fingerprint screening results and oral fluid results for participant 41040. A negative oral fluid confirmation result was obtained, but both cocaine and benzoylecgonine were detected in all fingerprint samples after wiping hands with alcohol free wipes. This is consistent with the results obtained from natural fingerprints collected for this participant. It is possible that the use of alcohol free wipes was not effective in the removal of external contamination of the fingers and thumbs or that the discrepancy is observed due to a difference in detection time window between the two matrices. The detection window for cocaine in fingerprints is unknown. This has been evaluated for sweat using sweat patches, which showed that cocaine use can be determined using two-week sweat patches (Liberty & Johnson, 2004). However, it must be noted that this is not representative of a fingerprint sample as the patches are used to accumulate sweat over a number of days. It has been reported that sweat (patch) has a longer detection window than oral fluid (United Nations Office on Drug and Crime, 2014).

Cocaine can be detected in oral fluid for 5 – 12 h, whereas benzoylecgonine can be detected for 12 – 24 h after a single dose, but this can be up to 10 days for chronic users (Verstraete, 2004).

Table 3.6: Oral fluid confirmation and fingerprint results based on n = 5 fingerprint samples collected after wiping hands with alcohol free wipes from participants who admitted having taken cocaine in the past 24 hours.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Oral fluid confirmation test (concentration ng/ml)</th>
<th>Fingerprint screening test (n = 5)</th>
<th>Number of values &gt; LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cocaine</td>
<td>Benzoylecgonine</td>
<td>Cocaine</td>
</tr>
<tr>
<td>41039</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>5</td>
</tr>
<tr>
<td>41040</td>
<td>Negative</td>
<td>Negative</td>
<td>5</td>
</tr>
<tr>
<td>41041</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>5</td>
</tr>
<tr>
<td>41042</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>5</td>
</tr>
<tr>
<td>41043</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>5</td>
</tr>
</tbody>
</table>

*LOD = limit of detection (see section 2.3.7.3)

3.2.5.2 Heroin

A number of participants (n = 4) were recruited who admitted having taken heroin in the past 24 h and whose fingerprint samples were collected (i) natural and (ii) after wiping hands with alcohol free wipes. Figure 3.13 shows the fingerprint results (ratio A/IS) for heroin and 6-acetylmorphine after wiping hands with alcohol free wipes for each participant. Both heroin and 6-acetylmorphine were detected in all fingerprints for all participants after wiping.
hands with alcohol free wipes, which is consistent with the natural fingerprint samples collected for these participants (100% detection rate). Similar to the results of cocaine, a narrow spread is observed in the levels of heroin and 6-acetylmorphine in fingerprint samples after wiping hands. Overall, the results show that the levels of analytes present between participants after wiping hands is more similar compared to natural fingerprints from these participants (see Figure 3.5). The use of alcohol free wipes has reduced the inter-donor variability.

Figure 3.13: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) based on n = 5 fingerprint samples collected after wiping hands with alcohol free wipes for participants who admitted having taken heroin in the last 24 h. Solid line represents limit of detection (LOD).

The fingerprint results after wiping hands with alcohol free wipes are compared to the oral fluid confirmation results in Table 3.7. There is an excellent agreement between the oral fluid and fingerprint results obtained as both matrices were consistent with the participant testimony, indicating heroin use.

Table 3.7: Oral fluid confirmation and fingerprint results based on n = 5 fingerprint samples collected after wiping hands with alcohol free wipes from participants who admitted having taken heroin in the past 24 hours.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Oral fluid confirmation test (concentration ng/ml)</th>
<th>Fingerprint screening test (n = 5) Number of values &gt;LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morphine</td>
<td>6-Acetylmorphine</td>
</tr>
<tr>
<td>41039</td>
<td>Negative</td>
<td>&gt;32</td>
</tr>
<tr>
<td>41041</td>
<td>69</td>
<td>&gt;32</td>
</tr>
<tr>
<td>41042</td>
<td>&gt;189</td>
<td>&gt;32</td>
</tr>
<tr>
<td>41043</td>
<td>60</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

*LOD = limit of detection (see section 2.3.7.3)
Participant 41040 admitted to the sole use of cocaine in the past 24 hours, however traces of heroin and 6-acetylmorphine were present in the natural fingerprints collected for this participant. Although the levels of heroin and 6-acetylmorphine present in the natural fingerprint samples were similar to those of heroin users, after wiping hands with alcohol free wipes, no heroin or 6-acetylmorphine was present in any fingerprint sample for this participant. It is possible that these traces were present due to external contamination present on the hands, which were removed with the hand cleaning procedure. It is also possible that the levels of analytes were present due to heroin use >24 h and after wiping hands were below the limit of detection and therefore produced negative fingerprint results. The oral fluid sample for this participant was negative for opiates.

3.2.5.3 Relationship between parent drug and metabolite

The distribution in the level of parent drug-to-metabolite for natural fingerprints compared to samples collected after wiping hands with soap and water for the same participants is shown in Figure 3.14. The results show that a close distribution is observed between the cocaine-to-benzoylcegonine ratio between participants, especially for natural fingerprints.

---

![Box and whisker plots](image)

**Figure 3.14:** Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of cocaine-to-benzoylcegonine ratio for cocaine users for samples collected as natural fingerprints and samples collected after wiping hands with alcohol free wipes.

No significant difference was observed in the level of cocaine and benzoylcegonine between natural fingerprints and samples collected after wiping hands. Similarly, the cocaine-to-benzoylcegonine ratio showed no significant difference (at $p = 0.05$) between the two sample
collection procedures \((U = 295, p = 0.734)\) (Appendix E. 3). The median level of cocaine-to-benzoylecgonine ratio in natural fingerprints was 37.621 compared to 32.834 after wiping hands.

The individual ratio of cocaine-to-benzoylecgonine for each participant for natural fingerprints and samples collected after wiping hands with alcohol free wipes from participants admitted having taken cocaine in the past 24 h is shown in Figure 3.15. Although a negative oral fluid result was obtained for participant 41040, the cocaine-to-benzoylecgonine ratio is similar to those for participants whose oral fluid samples were positive. The relationship between cocaine and benzoylecgonine could not be used to differentiate between those who had positive and negative oral fluid results.

![Figure 3.15: Average peak area ratio of cocaine-to-benzoylecgonine (n = 5 measurements) for cocaine and heroin users in natural fingerprint samples and samples collected after wiping hands.](image)

Comparison of the relationship between heroin and 6-acetylmorphine present in natural fingerprints and samples collected after wiping hands for the same participants is shown in Figure 3.16. Initial results already showed that the use of alcohol free wipes resulted in significantly lower heroin levels for samples collected after wiping hands compared to natural fingerprints, but not for 6-acetylmorphine (see section 3.2.5.2). This translates to a significant difference in the heroin-to-6-acetylmorphine ratio present in fingerprints between the two sample collection procedure \((U = 40, p < 0.001)\), resulting in lower levels after wiping hands (Appendix E. 3).
Figure 3.16: Box and whisker plots (median, 25 – 75\textsuperscript{th} percentiles, whiskers 5 – 95\textsuperscript{th} percentiles) showing the distribution of heroin-to-6-acetylmorphine ratio for heroin users for samples collected as natural fingerprints and samples collected after wiping hands with alcohol free wipes.

The individual ratio of heroin-to-6-acetylmorphine for natural fingerprints and samples collected after wiping hands with alcohol free wipes for the same participants is shown in Figure 3.17. The intra-donor variability between fingerprint samples is smaller for participants 41042 and 41043 compared to the remaining participants. Compared to the natural fingerprint samples collected from these participants, the ratio heroin-to-6-acetylmorphine decreased for nearly all participants after wiping hands with alcohol free wipes, due to a decreased signal for heroin.

Figure 3.17: Average peak area ratio of heroin-to-6-acetylmorphine (n = 5 measurements) for heroin and cocaine users in natural fingerprint samples and samples collected after wiping hands.
3.2.6 Effect of washing hands: drug users

In addition to the use of a hand wiping procedure (with alcohol free wipes), the influence of a handwashing procedure on the detection rate of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprints was also evaluated. Comparison of the levels of cocaine and benzoylecgonine in natural fingerprints and after handwashing for the same participants is shown Figure 3.18. A Mann-Whitney U test showed that a significant difference was observed for cocaine (\(U = 516, p = 0.006\)) and benzoylecgonine (\(U = 260, p < 0.001\)) between the two sample collection procedures (Appendix E.4). The median cocaine level (ratio A/IS) in natural fingerprints was 1.030 compared to 0.292 after handwashing. For benzoylecgonine, the median level (ratio A/IS) in natural fingerprints was 0.029 compared to 0.009 after handwashing. This suggests that the use of a handwashing procedure removed cocaine and benzoylecgonine present on the hands, resulting in lower levels.

The influence of a handwashing procedure on the level of heroin and 6-acetylmorphine present in fingerprint samples for the same participants is shown in Figure 3.19. The results show that a greater variability is observed in the level of heroin in fingerprints after handwashing, resulting in an uneven box plot size. Based on a Mann-Whitney U test, a significant difference is observed in the level of heroin (\(U = 290, p < 0.001\)) and 6-acetylmorphine (\(U = 450, p = 0.008\)) present in natural fingerprints compared to samples collected after wiping hands (Appendix E.4). The median heroin level (ratio A/IS) in natural fingerprints was 0.118 compared to 0.011 after handwashing. For 6-acetylmorphine, the
median (ratio A/IS) was 0.037 in natural fingerprints compared to 0.021 after handwashing. This suggests that the use of a handwashing procedure removed heroin and 6-acetylmorphine present on the hands, resulting in lower analyte levels.

![Box and whisker plots showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine between natural fingerprints and samples collected after wiping hands with alcohol free wipes for the same participants.]

### 3.2.6.1 Cocaine

A total of 8 participants were recruited who admitted having taken cocaine and whose fingerprint samples were collected after washing hands with soap and water. Natural fingerprint samples \((n = 5)\) were collected from each participant \(41026, 41028, 41036 – 41038\) and \(41044 – 41046\) and after hand washing. The fingerprint results obtained for cocaine and benzoylecgonine after handwashing are shown in Figure 3.20. Cocaine was detected in all fingerprint samples for each participant after handwashing, resulting in a 100% detection rate based on participant testimony. In contrast, benzoylecgonine was detected in 87% (35 samples) after washing hands with soap and water. This is because benzoylecgonine was not detected in any fingerprint samples for participant \(41026\) after handwashing. This is consistent with results obtained from natural fingerprint samples collected for this participant. Additionally, the lowest level of cocaine in fingerprint samples was observed for this participant. Furthermore, the oral fluid result for participant \(41026\) was negative for cocaine use. It is possible that cocaine was not removed completely after the handwashing procedure or that not enough sweat had been secreted for benzoylecgonine.
to be detected, or the drug administration was too recent for it to produce a positive oral
fluid or fingerprint test.

Figure 3.20: Fingerprint results (average peak area ratio analyte (A) to internal standard
(IS), ± standard deviation n = 5 measurements) based on n = 5 fingerprint
samples collected after handwashing for participants who admitted having
taken cocaine in the last 24 h. Solid line represents limit of detection (LOD).

A comparison between the oral fluid and fingerprint screening results (after handwashing)
is shown in Table 3.8. It must be noted that a discrepancy is still observed between the
fingerprint and oral fluid results for participant 41036. A negative oral fluid confirmation
result was obtained, but cocaine and benzoylecgonine were detected in all fingerprints after
washing hands with soap and water. This could possibly be observed due to a difference in
detection window between the matrices or the handwashing procedure was not sufficient in
the removal of external contamination from the fingers.

Table 3.8: Oral fluid confirmation and fingerprint results based on n = 5 fingerprint
samples collected after handwashing from participants who admitted having
taken cocaine in the past 24.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Oral fluid confirmation test (concentration ng/ml)</th>
<th>Fingerprint screening test (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cocaine</td>
<td>Benzoylecgonine</td>
</tr>
<tr>
<td>41026</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>41028</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41036</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>41037</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>41038</td>
<td>Negative</td>
<td>64</td>
</tr>
<tr>
<td>41044</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41045</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>41046</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

*LOD = limit of detection (see section 2.3.7.3)
Chapter 3: Investigation into the Suitability of a Fingerprint to Detect Cocaine and Heroin use

Fingerprint samples collected from participants 41033 and 41035 who admitted to the sole use of heroin, showed traces of cocaine and benzoylecgonine in natural fingerprints (see Figure 3.4). It is possible that these participants had taken cocaine >24 h and therefore resulted in positive fingerprint samples or that these traces were present from external contamination. Figure 3.21 shows that after washing hands with soap and water, cocaine was still detected in all fingerprint samples for both participants. In contrast to the natural fingerprint samples collected for participant 41033, benzoylecgonine was not detected in any fingerprint sample after handwashing. For participant 41035, one fingerprint sample (right little finger) was positive for benzoylecgonine, whereas none were positive in natural fingerprint samples collected. The oral fluid results for these participants were negative for both cocaine and benzoylecgonine. Comparison of the cocaine levels detected in natural fingerprint samples collected and fingerprints collected after handwashing showed a decrease in signal, which suggests that the hand washing procedure removed some external contamination that was present on the hands or that not enough sweat was secreted to allow for similar levels to be detected (<LOD).

Figure 3.21: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for cocaine and benzoylecgonine from fingerprints collected after handwashing from individuals admitted having taken heroin only. Solid line represents limit of detection (LOD).

3.2.6.2 Heroin

A total of n = 8 participants were recruited who admitted having taken heroin the past 24 h. Fingerprint samples (n = 5) were collected from participants 41036 – 41038 and 41044 – 41046 (i) natural and (ii) after washing hands with soap and water. The fingerprint results obtained for heroin and 6-acetylmorphine after washing hands are shown in Figure 3.22. Eighty-five percent (34 samples) of the fingerprint samples were positive for heroin,
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whereas 90% (36 samples) were positive for 6-acetylmorphine based on participant testimony. For participant 41036, both heroin and 6-acetylmorphine were not detected in the right middle, ring or little finger. For participant 41038, no heroin was detected in the right middle finger and no 6-acetylmorphine in the right little finger. Additionally, the right ring finger was negative for both analytes for participant 41037, whereas no heroin was detected in the right middle finger for participant 41035 after washing hands. This could be due to lower concentrations of analytes present in these fingers compared to the right thumb and index finger. The natural fingerprint samples collected for these participants, however were all positive for heroin and 6-acetylmorphine. In contrast to the use alcohol free wipes, the detection rate of heroin and 6-acetylmorphine is decreased by using this handwashing procedure from 98% for heroin and 100% for 6-acetylmorphine to 85% and 90%, respectively.

Figure 3.22: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) based on n = 5 fingerprint samples collected after handwashing for participants who admitted having taken heroin in the past 24 h. Solid line represents limit of detection (LOD).

The oral fluid confirmation and fingerprint results after handwashing are shown in Table 3.9. There still is a discrepancy between the oral fluid and fingerprint results. In particular for participants 41037, 41038 and 41046 as heroin and 6-acetylmorphine were still detected in nearly all fingerprints even after washing hands with soap and water. It is possible that the participants did not thoroughly wash their hands and as a result the external contamination of heroin was not effectively removed. Another possibility is that a difference in detection window between the matrices can result in these discrepancies observed. The results obtained for participant 41036 suggest that the detection of heroin and 6-acetylmorphine was due to external contamination. Especially compared to the natural
fingerprint samples collected for this participant which were all positive, however after handwashing only the right thumb and index finger remained positive for heroin and 6-acetylmorphine.

Table 3.9: Oral fluid confirmation and fingerprint results based on n = 5 fingerprint samples collected after washing hands with soap and water from participants who admitted having taken heroin in the past 24 h.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Oral fluid confirmation test (concentration ng/ml)</th>
<th>Fingerprint screening test (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morphine</td>
<td>6-Acetylmorphine</td>
</tr>
<tr>
<td>41033</td>
<td>&gt;240</td>
<td>32</td>
</tr>
<tr>
<td>41035</td>
<td>138</td>
<td>32</td>
</tr>
<tr>
<td>41036</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>41037</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>41038</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>41044</td>
<td>Negative</td>
<td>22.6</td>
</tr>
<tr>
<td>41045</td>
<td>90</td>
<td>Negative</td>
</tr>
<tr>
<td>41046</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*LOD = limit of detection (see section 2.3.7.3)

Fingerprint samples collected from participants (41026 and 41028) who admitted to the sole use of cocaine showed that traces of heroin and 6-acetylmorphine were present for participant 41028 in natural fingerprints (as seen in Figure 3.23). For participant 41026, no heroin or 6-acetylmorphine was detected in any natural fingerprint sample or after handwashing.

Figure 3.23: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for heroin and 6-acetylmorphine for fingerprints collected after handwashing or wiping hands from individuals admitted having taken cocaine only. Solid line represents limit of detection (LOD).
For participant 41028, heroin was still detected in all fingerprint samples after handwashing. In contrast, only 2 out of 5 fingerprints were positive for 6-acetylmorphine after handwashing, which could be due to the low levels of analytes present (<LOD). Although the oral fluid samples were negative for opiate use, it is possible that traces of these analytes could be present from heroin use >24 h or possible external contamination on the hands which was not removed by the handwashing procedure.

3.2.6.3 Relationship between parent drug and metabolite

Comparison of the cocaine-to-benzoylcegonine ratio for natural fingerprints and samples collected after washing hands with soap and water is shown in Figure 3.24. The results show a similar distribution of the cocaine-to-benzoylcegonine ratio for both sample collection procedures. No significant difference (at \( p = 0.05 \)) is observed between the cocaine-to-benzoylcegonine ratio after washing hands compared to natural fingerprints (\( U = 588, p = 0.778 \)) (Appendix E. 5). The median level for the cocaine-to-benzoylcegonine ratio in natural fingerprints was 37.621 compared to 32.834 after handwashing. This suggests that although the handwashing procedure resulted in significantly lower results for cocaine and benzoylcegonine compared to natural fingerprints (section 3.2.6.1), the cocaine-to-benzoylcegonine ratio remained similar.

![Figure 3.24](image)

Figure 3.24: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of cocaine-to-benzoylcegonine ratio for cocaine users for samples collected as natural fingerprints and samples collected after washing hands with soap and water.
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The individual ratio cocaine-to-benzoylecgonine for natural fingerprints and samples collected after washing hands for each participant is shown in Figure 3.25. For participants 41026, 41036 and 41040 (whose oral fluid samples were negative for cocaine), the handwashing procedure still resulted in a ratio cocaine-to-benzoylecgonine similar to those of participants whose oral fluid samples were positive. This suggests that it is not possible to differentiate these participants by using the ratio cocaine-to-benzoylecgonine. It is possible that a difference in detection window can result in these differences observed, or that external contamination remaining on the hands can produce similar results between the presence of cocaine and benzoylecgonine.

In contrast to the cocaine-to-benzoylecgonine ratio, the heroin-to-6-acetylmorphine ratio is significantly lower for samples collected after handwashing ($U = 353, p = 0.001$) compared to natural fingerprints as seen in Figure 3.26 (Appendix E. 5). The use of the handwashing procedure resulted in significantly lower levels for heroin and 6-acetylmorphine (section 3.2.6.2). The median level of heroin-to-6-acetylmorphine ratio in natural fingerprints was 2.854 compared to 1.317 after handwashing. In contrast to cocaine, this suggests that the amount of heroin and 6-acetylmorphine removed by washing hands varied, resulting in a lower heroin-to-6-acetylmorphine ratio after handwashing.
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Figure 3.26: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of heroin-to-6-acetylmorphine ratio for heroin users for samples collected as natural fingerprints and samples collected after washing hands with soap and water.

The individual ratio of heroin-to-6-acetylmorphine for natural fingerprints compared to samples collected after washing hands with soap and water is shown in Figure 3.27. The use of a hand cleaning procedure has not reduced the variability observed between fingerprints within a participant and between participants.

Figure 3.27: Average peak area ratio of heroin-to-6-acetylmorphine (n = 5 measurements) for heroin and cocaine users in natural fingerprints and samples collected after washing hands with soap and water.

For participants 41037, 41038 and 41046, negative oral fluid results were obtained. However, the heroin-to-6-acetylmorphine ratio is similar to those for participants with positive oral fluid samples. This indicates that the relationship between the parent drug and
metabolite could not be used to provide a clear distinction between participants whose oral fluid samples were positive or negative for heroin use.

3.2.7 Effect of washing hands: background population (non-drug users)

3.2.7.1 Cocaine

The collection of natural fingerprint samples from the background population resulted in the presence of cocaine and benzoylecgonine in 13% and 5% of samples, respectively. To reduce the detection of cocaine and benzoylecgonine from environmental exposure, a different sampling strategy was evaluated, which included washing the hands thoroughly with soap and water prior to sample collection. Fingerprint samples (n = 2 per participant, right thumb and index finger) were collected from 50 participants (SUB057 – SUB106) after washing hands with soap and water and wearing nitrile gloves for 10 min to promote the secretion of eccrine sweat from the fingertips. Results showed that cocaine was only detected in one fingerprint sample (SUB082 right thumb) and all samples were negative for benzoylecgonine. However, based on the presence of both the parent drug and metabolite in fingerprint samples, a 0% false positive rate would be obtained after handwashing, compared to 4% false positive rate if natural fingerprints are collected. The introduction of a handwashing step prior to sample collection results in a lower detection rate for both cocaine and benzoylecgonine from environmental contamination in the background population.

3.2.7.2 Heroin

The prevalence of heroin and 6-acetylmorphine in natural fingerprints from the background population was insignificant, as only 1% was positive for 6-acetylmorphine, whereas none were positive for heroin. The influence of the use of a handwashing procedure on the detection of heroin and 6-acetylmorphine in the background population was also evaluated. Fingerprint samples (n = 2 per participant, right thumb and index) were collected from 50 participants (SUB057 – SUB106) after washing hands with soap and water and wearing nitrile gloves for 10 min to promote the secretion of eccrine sweat from the fingertips. From the 100 fingerprint samples, none were positive for heroin and 6-acetylmorphine. Similar to the results obtained from the background population for natural fingerprint samples collected, heroin is not likely observed due to environmental contamination. This suggests that the detection of heroin and 6-acetylmorphine in fingerprint samples is significant above the detection limit as no false positive results would be observed in a background population of non-drug users.
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3.2.8 Effect of a hand cleaning procedure

3.2.8.1 Cocaine

The fingerprint sampling strategy was evaluated by comparing the collection of fingerprint samples after wiping hands with alcohol free wipes or washing hands with soap and water. It is important to show that (i) a fingerprint test cannot be falsified by the use of a hand cleaning procedure and (ii) the use of a hand cleaning procedure reduces the possibility of external contamination on the fingers and thumbs. Comparison between the two hand cleaning procedures (alcohol free wipes and handwashing) showed that the same fingerprint results were obtained as the collection of natural fingerprints for those participants.

The results obtained for fingerprint samples collected after handwashing (n = 100) from the background population showed that cocaine and benzoylecgonine were not present (with 1 false positive for cocaine), in contrast to the results obtained for natural samples collected. This shows that the use of a handwashing procedure has reduced the prevalence of cocaine in fingerprint samples from the background population of non-drug users. This whilst maintaining the same detection rate for cocaine (100%) and benzoylecgonine (87%) for cocaine users after handwashing compared to natural fingerprint samples collected. This suggests that there is no requirement to impose a threshold level for fingerprints if samples are collected after washing hands, implying that the detection limit can then be used as the decision level, with a 1% false positive rate for cocaine in the background population (non-drug users).

3.2.8.2 Heroin

The majority of the natural fingerprint samples collected from individuals admitted having used heroin in the past 24 h were positive for heroin (85% detection rate) and 6-acetylmorphine (100% detection rate). However, comparison between the two hand cleaning procedures (wiping hands with alcohol free wipes and handwashing) showed that there is a difference observed between the two approaches, as 100% detection rate is observed using alcohol free wipes for both heroin and 6-acetylmorphine. The collection of fingerprint samples after handwashing indicates that it is possible that the detection of heroin and 6-acetylmorphine could have been due to contamination of heroin on the hands of the participants. This is suggested by the results obtained after handwashing, which reduced the overall detection rate to 85% and 90% for heroin and 6-acetylmorphine, respectively and additionally were inconsistent with corresponding oral fluid samples. However, as the
detection window for heroin in fingerprints is unknown, it is possible that this could also be a result of a difference in detection window for drugs of abuse between oral fluid and fingerprints. Another possibility is that not enough sweat was secreted for these samples to produce a positive result or the levels were below the limit of detection. These results highlight the challenges associated with the use of fingerprints for drug testing purposes and the need of a standardised fingerprint collection procedure. It is possible that external contamination present on the hands even after a hand cleaning procedure can result in false positive tests. Furthermore, it is also possible that the hand cleaning procedure used was not effective in the removal of external contamination, which would still result in a false positive fingerprint result.

The results obtained for heroin and 6-acetylmorphine from the background population in natural fingerprints and after handwashing suggest that these analytes are not commonly observed due to environmental exposure. This implies that the detection of heroin and 6-acetylmorphine is significant above the detection limit and there is no requirement to impose a threshold level for fingerprint analysis. However, the use of a hand cleaning procedure is advantageous as this could help to reduce the possibility of the presence of analytes on the hands from contamination.

3.2.9 Secondary transfer of drugs
The potential transfer of drugs between individuals was investigated between non-drug users and drug users through shaking hands. This was investigated because it is important to understand if drugs can be present in fingerprints from non-drug users through secondary transfer and what the influence of this is on the interpretation of results. Comparison between the distribution of cocaine and benzoylecgonine in samples collected from non-drug users before and after shaking hands, with those from cocaine users is shown in Figure 3.28. A significant difference was observed between the levels of cocaine ($\chi^2 = 46.29, p < 0.001$) and benzoylecgonine ($\chi^2 = 12.95, p = 0.002$) in non-drug users (before and after shaking hands) compared to natural fingerprints from cocaine users based on a Kruskal-Wallis test (Appendix E. 6). The median cocaine level (ratio A/IS) was 0.802 for cocaine users in natural fingerprints compared to 0.003 for non-drug users. The median cocaine level (ratio A/IS) for non-drug increased to 0.023 after shaking hands. This suggests that by shaking hands, cocaine was transferred between the individuals resulting in an increase in the median cocaine level detected. However, no significant difference (at $p = 0.05$) was observed
between the samples collected from non-drug users before and after secondary transfer based on a Mann-Whitney U test \((U = 147, p = 0.005)\) (Appendix E. 7). For benzoylecgonine, the median level (ratio \(A/IS\)) in natural fingerprints was 0.026 for cocaine users compared to 0.006 for non-drug users. After shaking hands, the median benzoylecgonine level (ratio \(A/IS\)) for non-drug users remained 0.006. No significant difference (at \(p = 0.05\)) was observed between the benzoylecgonine levels before and after shaking hands for non-drug users \((U = 6, p = 1)\) (Appendix E. 7). This suggests that although benzoylecgonine was more prevalent in the fingerprints of non-drug users after shaking hands, the amount of analyte transferred was similar to those present in natural fingerprints for those participants (possibly from environmental exposure).

![Box and whisker plots](image)

**Figure 3.28:** Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine between cocaine users and non-drug users before and after shaking hands.

The distribution of heroin and 6-acetylmorphine in fingerprints samples collected from non-drug users before and after secondary transfer compared to samples collected from heroin users is shown in Figure 3.29. The results show that no heroin or 6-acetylmorphine was present in natural fingerprints from non-drug users. This is consistent with the results obtained from a background population of non-drug users (section 3.2.3.2). A significant difference is observed between the levels of heroin \(\chi^2 = 17.57, p < 0.001\) and 6-acetylmorphine \(\chi^2 = 11.12, p < 0.001\) from non-drug users (natural and after shaking hands) compared to natural fingerprints from heroin users (Appendix E. 6). This suggests that although the act of shaking hands has resulted in secondary transfer of heroin and 6-
acetylmorphine, the analyte levels present were lower than those normally observed for heroin users.

Figure 3.29: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine between cocaine users and non-drug users before and after shaking hands.

3.2.9.1 Cocaine

Natural fingerprint samples (n = 5 per participant) were collected from non-drug users (SUB001, SUB002, and SUB054 – 56) to determine the presence of drugs prior to contact with drug users (at an NHS Drug and Alcohol Service). Additional fingerprint samples were collected from the volunteers (non-drug users) after shaking hands once with participants who admitted to the use of cocaine in the past 24 h. Details of the secondary transfer experiment are outlined in Table 3.10.

Table 3.10: Details of the secondary transfer experiment carried out between non-drug users (volunteers) and cocaine users.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Hands shaken with participant</th>
<th>Participant testimony</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUB001</td>
<td>41026</td>
<td>Cocaine</td>
</tr>
<tr>
<td>SUB002</td>
<td>41028</td>
<td>Cocaine</td>
</tr>
<tr>
<td>SUB054</td>
<td>41044</td>
<td>Cocaine and heroin</td>
</tr>
<tr>
<td>SUB055</td>
<td>41045</td>
<td>Cocaine and heroin</td>
</tr>
<tr>
<td>SUB056</td>
<td>41046</td>
<td>Cocaine and heroin</td>
</tr>
</tbody>
</table>

Figure 3.30 shows the fingerprint results for samples collected from the volunteers (i) natural and (ii) after secondary transfer with participants admitted to cocaine use in the past 24 h. Cocaine was detected in 7 out of 25 natural fingerprint samples collected from the volunteers (non-drug users). This is not surprising as it is known that traces of cocaine can be found in the environment (Zuccato & Castiglioni, 2009; Armenta & de la Guardia, 2008; Carter et
Additionally, the results obtained from natural fingerprint samples collected from the background population (section 3.2.3.1) showed that cocaine was present in 13% of the background population. In contrast, benzoylecgonine, was only detected in 2 out of 25 fingerprint samples collected (natural) from the non-drug users. After shaking hands with a participant from the Xchange (drug users), all 25 fingerprint samples (100%) from the non-drug users were positive for cocaine and 5 fingerprint samples (20%) for benzoylecgonine. This demonstrates that cocaine can readily be transferred between individuals through contact and to a lesser extent for benzoylecgonine. These findings demonstrate that the collection of natural fingerprint samples can result in a false positive test, as secondary transfer of cocaine can be a potential source for the presence of these analytes. This also further highlights the importance of the sampling strategy for fingerprint testing and the importance of careful interpretation of the results obtained.

Figure 3.30: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for volunteers before and after secondary transfer with participants admitted having taken cocaine in the past 24 hours. Solid line represents limit of detection (LOD).

A threshold level was required to differentiate non-drug users from drug users for natural fingerprint samples (section 3.2.4.1). The influence of the same proposed threshold levels on the detection rate of cocaine and benzoylecgonine before and after secondary transfer was explored. Threshold levels were evaluated per analyte at the limit of detection (ratio A/IS 0.002 for cocaine and 0.004 for benzoylecgonine) and ratio A/IS of 0.005 – 0.030 in increments of 0.005. Figure 3.31 shows the detection rate for cocaine and benzoylecgonine in fingerprint samples from (n = 5) non-drug users before and after secondary transfer based
on the proposed threshold levels. The results show that the detection rate for cocaine before secondary transfer with a drug user can be reduced to 0% for the right index, middle, ring and little finger, by imposing a cut-off level at ratio A/IS 0.005. However, for the right thumb the detection rate remains 20% at all proposed cut-off levels. This is a result of participant SUB056 where cocaine was present at ratio A/IS 0.075 in the right thumb collected (natural fingerprint). In contrast, the detection rate for benzoylecgonine in all natural fingerprint samples collected from non-drug users, is readily reduced to 0% by imposing a cut-off level at ratio A/IS 0.010.

Figure 3.31: Detection rate (percentage) of cocaine and benzoylecgonine in natural fingerprint samples collected from (n = 5) non-drug users before and after shaking hands with a cocaine user (based on n = 5 fingerprint samples per participant) for each proposed cut-off level.

The collection of natural fingerprint samples from non-drug users after contact with a participant who admitted having taken cocaine, resulted in a 100% detection rate for cocaine based on the limit of detection. Although the use of various threshold levels was explored, it was not possible to obtain a 0% detection rate for cocaine for non-drug users after hands
were shaken with a cocaine user. This is due to the higher levels of cocaine transferred between the participants. However, the levels of benzoylecgonine transferred between the participants were lower and therefore the use of a threshold level at ratio A/IS 0.010 results in a 0% detection rate for all fingerprint samples. If both analytes were required to be present and a threshold level of ratio A/IS 0.010 was imposed for benzoylecgonine, no false positive tests would be obtained even after secondary transfer.

3.2.9.2 Heroin

Natural fingerprint samples (n = 5 per participant) were collected from non-drug users (SUB054 – 56) to determine the presence of heroin in fingerprint samples prior to contact with participants recruited from the Xchange (NHS Drug and Alcohol Service) who admitted having taken heroin. An additional set of fingerprint samples were collected from the non-drug users after shaking hands. Details of the secondary transfer experiment are outlined in Table 3.11.

Table 3.11: Details of the secondary transfer experiment carried out between non-drug users (volunteers) and heroin users.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Hands shaken with participant</th>
<th>Participant testimony</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUB054</td>
<td>41044</td>
<td>Cocaine and heroin</td>
</tr>
<tr>
<td>SUB055</td>
<td>41045</td>
<td>Cocaine and heroin</td>
</tr>
<tr>
<td>SUB056</td>
<td>41046</td>
<td>Cocaine and heroin</td>
</tr>
</tbody>
</table>

Figure 3.32 shows that no heroin and 6-acetylmorphine were detected in natural fingerprint samples collected from the volunteers (non-drug users), which is consistent with the results obtained for the background population in section 3.2.3.2. However, after shaking hands with participants who admitted to the use of heroin in the past 24 h, both heroin and 6-acetylmorphine were detected in 7 fingerprint samples (28%) of the volunteers (n = 3 participants).

It is important to determine whether after contact with an individual who administered a drug, these traces can be transferred onto the hands of a non-drug user. More importantly because this will result in a positive result if natural fingerprint samples are collected. This also further highlights the importance of a hand cleaning procedure prior to fingerprint collection to remove external contamination which could be present. As the results from the background population of non-drug users showed that heroin and 6-acetylmorphine were not prevalent, there was no requirement to impose threshold levels for heroin and 6-acetylmorphine for natural fingerprint samples collected. However, if the same principle is
applied here, the results indicate that a false positive fingerprint test could be obtained due to secondary transfer between individuals. This highlights that the sampling strategy for a fingerprint test is particularly important, as it is possible that the source of the presence of the analytes could come from contact with an individual rather than administration of the drug.

Figure 3.32: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for volunteers before and after secondary transfer with participants admitted having taken heroin in the past 24 hours. Solid line represents limit of detection (LOD).

3.2.9.3 Relationship between parent drug and metabolite

Comparison of the distribution of the cocaine-to-benzylecgonine ratio in fingerprint samples from non-drug users (before and after shaking hands with cocaine user) and cocaine users is shown in Figure 3.33. The results show a significant difference is observed in the distribution of the cocaine-to-benzylecgonine ratio between the non-drug users (before and after shaking hands) and cocaine users based on a Kruskal-Wallis test ($\chi^2 = 8.46, p = 0.015$) (Appendix E. 8). The median cocaine-to-benzylecgonine ratio in natural fingerprints for cocaine users was 29.901 compared to 6.007 for non-drug users. After shaking hands with a cocaine user, the median cocaine-to-benzylecgonine ratio for non-drug users increased to 17.25. Additionally, the results show a close distribution of the cocaine-to-benzylecgonine ratio for non-drug users after shaking hands, suggesting similar levels of drugs were transferred between the individuals.
**Figure 3.33:** Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of cocaine-to-benzoylecgonine ratio for natural fingerprints collected from cocaine users and non-drug users before and after shaking hands with cocaine users.

The individual cocaine-to-benzoylecgonine ratio for fingerprint samples collected from cocaine users (n = 13) and non-drug users (n = 3) before and after shaking hands with cocaine users is shown in Figure 3.34. For participants SUB054 and SUB056, the right thumbs were positive for cocaine and benzoylecgonine before hands were shaken with the participant. In contrast, for the remaining participants, no benzoylecgonine was present, and therefore the ratio cocaine-to-benzoylecgonine could not be calculated.

**Figure 3.34:** Average peak area ratio of cocaine-to-benzoylecgonine (n = 5 measurements) for fingerprint samples collected from non-drug users after shaking hands with participants admitted having taken cocaine.
After shaking hands with a cocaine user, the prevalence of cocaine and benzoylecgonine in fingerprint samples from non-drug users increased. Although a significant difference is observed in the distribution of the cocaine-to-benzoylecgonine ratio between non-drug users (before and after shaking hands) and cocaine users (Figure 3.33), the individual values observed are in the range of those of natural fingerprint samples collected for participants admitted having taken cocaine (Figure 3.34). This suggests that the ratio cocaine-to-benzoylecgonine in natural fingerprint samples is not able to differentiate between participants who have taken cocaine and participants who have come into contact with a cocaine user.

A comparison of the distribution of the heroin-to-6-acetylmorphine ratio in fingerprints from heroin users and non-drug users (before and after shaking hands with heroin users) is shown in Figure 3.35. A significant difference is observed in the distribution of the heroin-to-6-acetylmorphine ratio between heroin users and non-drug users (before and after handshaking) based on a Kruskal-Wallis test ($\chi^2 = 6.01, p = 0.014$) (Appendix E. 8). The median heroin-to-6-acetylmorphine ratio for heroin users was 2.691 compared to 0.871 for non-drug users after shaking hands.

Figure 3.35: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of heroin-to-6-acetylmorphine ratio for natural fingerprints collected from heroin users and non-drug users before and after shaking hands with heroin users.
Chapter 3: Investigation into the Suitability of a Fingerprint to Detect Cocaine and Heroin use

The individual ratio heroin-to-6-acetylmorphine for fingerprint samples collected from heroin users (n = 12) and non-drug users (n = 3) before and after shaking hands with participants admitted having taken heroin in the past 24 h is shown in Figure 3.36. The results show that heroin and 6-acetylmorphine are not present in fingerprint samples collected before contact with a heroin user, and therefore the ratio heroin-to-6-acetylmorphine could not be calculated. This is consistent with the results obtained from the background population in section 3.2.3.2, which showed that heroin was not prevalent in non-drug users. However, after contact with a heroin user, fingerprint samples for SUB055 (right middle and ring finger) and SUB056 (right middle and little finger) were positive for heroin and 6-acetylmorphine. The individual ratio heroin-to-6-acetylmorphine observed for non-drug users after contact with a heroin user, were similar to the levels observed for than heroin users. Therefore, is not possible to determine the difference between a participant who has taken heroin and a participant who has been in contact with a heroin user based on the ratio heroin-to-6-acetylmorphine.

Figure 3.36: Average peak area ratio of cocaine-to-benzoylecgonine (n = 5 measurements) for fingerprint samples collected from non-drug users after shaking hands with participants admitted having taken cocaine.

3.2.10 Effect of the finger on the detection rate

3.2.10.1 Cocaine

The level of cocaine and benzoylecgonine present in fingerprint samples collected from drug users varied depending on the fingerprint sample collected (right thumb to right little finger). However, in natural fingerprint samples collected the same detection rate (100%) was observed for cocaine in all fingers (see Table 3.12). Therefore, the use of the finger to collect...
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a sample from for drug testing purposes did not influence the interpretation of the results. However, for benzoylecgonine the highest detection rate was observed for the right ring finger (100% detection rate), compared to the other fingerprint samples (92% detection rate). This is caused by participant 41026, whose natural fingerprint samples (were negative for benzoylecgonine in all samples, except for the right ring finger. In light of the different results obtained, it would therefore be good practice to collect more than one fingerprint sample to confirm the results obtained. In addition, the detection rates of cocaine and benzoylecgonine after using a hand cleaning procedure (e.g. washing hands or wiping hands with alcohol free wipes) were consistent for all fingerprints. This shows that the finger used to test for illicit substances is not dependent on the fingerprint sample collected.

Table 3.12: Positive detection rate (percentage) of cocaine and benzoylecgonine (BZE) above the detection limit from participants admitted to the use of cocaine in the past 24 h, per fingerprint sample based on participant testimony and collection method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collection method</th>
<th>Detection rate (%)</th>
<th>Natural (n = 13)</th>
<th>Soap (n = 8)</th>
<th>Wipe (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cocaine</td>
<td>BZE</td>
<td>Cocaine</td>
<td>BZE</td>
</tr>
<tr>
<td>Right thumb</td>
<td>Natural</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Soap</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Wipe</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td>Right index finger</td>
<td>Natural</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Soap</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Wipe</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td>Right middle finger</td>
<td>Natural</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Soap</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Wipe</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td>Right ring finger</td>
<td>Natural</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Soap</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Wipe</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td>Right little finger</td>
<td>Natural</td>
<td>100</td>
<td>92</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Soap</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Wipe</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87</td>
</tr>
</tbody>
</table>

3.2.10.2 Heroin

The level of heroin and 6-acetylmorphine detected in fingerprint samples from heroin users varied for each finger. An overview of the detection rates per finger for heroin and 6-acetylmorphine based on participant testimony and fingerprint collection method is shown in Table 3.13. The detection rates of the analytes were not dependent on the finger collected for natural fingerprint and fingerprints collected after wiping hands as the same detection rates were observed (with one false positive due to participant 41036, where heroin was not detected in the right little finger).

Interestingly, the results varied for fingerprints collected after washing hands with soap and water. The highest detection rates were observed for the right thumb and index finger and reduced for the middle, ring and little finger. This suggests that lower levels of heroin and 6-acetylmorphine were present after handwashing for these fingers which resulted in
negative results. The results indicate that the use of the right thumb and right index finger for fingerprint drug testing would provide the highest detection rates.

Table 3.13: Positive detection rate (percentage) of heroin and 6-acetylmorphine (6-AM) above the detection limit from participants who admitted to the use of heroin in the past 24 h, per fingerprint sample based on participant testimony and collection method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collection method</th>
<th>Detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Natural (n = 12)</td>
<td>Soap (n = 8)</td>
</tr>
<tr>
<td></td>
<td>Heroin 6-AM</td>
<td>Heroin 6-AM</td>
</tr>
<tr>
<td>Right thumb</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Right index finger</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Right middle finger</td>
<td>100</td>
<td>62</td>
</tr>
<tr>
<td>Right ring finger</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Right little finger</td>
<td>92</td>
<td>87</td>
</tr>
</tbody>
</table>

3.3 Summary

Cocaine, benzoylecgonine, heroin and 6-acetylmorphine can be detected in natural fingerprints from cocaine and heroin users (section 3.2.1.1). However, traces of cocaine and benzoylecgonine were also detected in natural fingerprints from a background population of non-drug users (section 3.2.3). With the use of a threshold level (at ratio A/IS 0.005), the false positive detection rate of cocaine and benzoylecgonine can be reduced to 1% for non-drug users (based on the presence of both analytes), with an 8% false negative detection rate for cocaine users (section 3.2.4.1). No threshold level was required for heroin and 6-acetylmorphine, as these analytes were not prevalent in fingerprints from non-drug users (section 3.2.4.2). Additionally, threshold levels are not required if a hand washing procedure is used prior to sample collection, as cocaine and benzoylecgonine were not commonly observed in fingerprints from non-drug users after handwashing (1% false positive detection rate for cocaine) (section 3.2.7). The hand washing procedure did result in significantly lower levels (ratio A/IS) for cocaine, benzoylecgonine and heroin compared to natural fingerprints (but not for 6-acetylmorphine). Compared to washing hands with soap and water, the use of alcohol free wipes (as an alternative hand cleaning procedure) did not result in significantly lower analyte levels (sections 3.2.5 and 3.2.6). This suggests that washing hands is more effective in removal of analytes from the fingers compared to wiping hands with alcohol free wipes. In addition, traces of cocaine, benzoylecgonine, heroin and 6-acetylmorphine can be transferred between non-drug users and drug users by shaking hands...
(section 3.2.9). However, the trace levels are significantly lower compared to those of drug users. With the use of a threshold level (at ratio A/IS 0.010) for cocaine and benzoylecgonine, only a 1% false positive detection rate for cocaine is observed. The use of a hand washing procedure prior to sample collection would be a good approach to reduce the possible presence of drugs in fingerprints from non-drug users from secondary transfer. The finger used for analysis resulted in different analyte levels, but the detection rate for cocaine, benzoylecgonine, heroin and 6-acetylmorphine was not dependent on the finger (section 3.2.10). However, for testing purposes the collection of two samples would be favourable to allow for confirmation.
Chapter 4 Investigation into the Presence of Cocaine and Heroin in Fingerprints from Contact Residue

4.0 Introduction

The main objective of this chapter was to explore the possible presence of cocaine and heroin and their respective metabolites in fingerprint samples after physical contact with the parent drug. In the Chapter 3, the presence of cocaine, heroin and their respective metabolites (benzoylecgonine and 6-acetylmorphine) in fingerprint samples from participants who admitted having taken the substance in the past 24 h was evaluated. This showed that if a decision level is set at an appropriate value, it is possible to distinguish between drug use and environmental exposure, as well as secondary transfer from shaking hands. This then rules out an accidental contamination of the hands with cocaine or heroin. For a fingerprint test to be useful in certain operational scenarios, it is also beneficial to determine whether the test can distinguish between drug administration and drug contact. Whilst contact with a reasonable quantity of cocaine or heroin may imply direct involvement with illicit drugs rather than environmental exposure, this has a different legal implication to consuming a drug and being under its influence. No study (to our knowledge) has explored the influence of the possible presence of drugs from contact residue in relation to that of drug users. In collaboration with Forensic Science Ireland (FSI), the possibility to distinguish the presence of drugs in fingerprints from drug use and drug contact is investigated in this chapter. To evaluate the presence of cocaine or heroin due to physical contact with the parent drug, fingerprint samples were collected under four different conditions:

- scenario 1: after physical contact with the parent drug;
- scenario 2: after wiping hands with alcohol free wipes;
- scenario 3: after washing hands with soap and water; and
- scenario 4: after secondary transfer of drugs between participants (non-drug users).

Fingerprint samples were collected from individuals (non-drug users) who volunteered to participate in the study. Four fingerprints were collected for each participant (right thumb, index finger, middle finger and ring finger). The presence of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprint samples collected after contact with the parent
drug was determined by liquid chromatography – mass spectrometry (LC-MS, section 2.2.2). The fingerprint samples were extracted using the previously described sample preparation method (section 2.3.4). The sample collection method for each scenario is described in section 4.1. The presence of cocaine and heroin in fingerprint samples after each scenario is discussed in section 4.2.

The presence of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprints after drug administration has been explored in Chapter 3. In this chapter, the presence of cocaine and heroin after dermal contact with the parent drug is evaluated using different sampling strategies to determine its impact on the suitability of a fingerprint test to determine drug use. This will help to understand whether a fingerprint test can differentiate contact residue from drug administration.

### 4.1 Experimental

Fingerprint samples were collected after contact with seized street drugs under four different scenarios. The seized drugs were analysed at FSI using their standard analysis protocol using gas chromatography – mass spectrometry (GC-MS). The cocaine and heroin used were determined to have a purity of 41% and 11%, respectively. Results obtained for the GC-MS analysis of cocaine and heroin are provided in Appendix C.1 and Appendix C.2.

Figure 4.1 illustrates the conditions under which fingerprint samples were collected for scenarios 1 to 4. Three participants (non-drug users) volunteered to participate in the study. Four fingerprint samples were collected per participant, namely the right thumb, index, middle and ring finger. Each step details the preparation for the collection of fingerprint samples after dermal contact with 2 mg of drug (cocaine or heroin). Scenario 2 and 3 utilised a hand cleaning procedure to explore whether dermal contact with the parent drug could be removed by washing hands or wiping hands with alcohol free wipes. In contrast, scenario 4 investigated the potential secondary transfer of drugs between individuals after dermal contact with the parent drug. Each sample collected was labelled with a unique participant identifier; the finger or thumb that was collected and the condition under which the sample was collected. All samples were prepared for LC-MS analysis according to the protocol developed in Chapter 2, section 2.3.4.
Figure 4.1: Fingerprint collection procedure used at Forensic Science Ireland to determine the presence of drugs (cocaine and heroin) after contact, washing hands with soap and water, wiping hands with alcohol free wipes and secondary transfer (through shaking hands).

4.2 Results and Discussion

4.2.1 Comparison between scenarios 1 – 4

4.2.1.1 Cocaine

The collection of fingerprint samples (n = 12) after dermal contact with 2 mg of cocaine (scenario 1) resulted in the presence of both cocaine and benzoylecgonine in all samples. The presence of benzoylecgonine in fingerprint samples was due to the cocaine used in this experiment, which was confirmed by GC-MS analysis at Forensic Science Ireland (see Appendix C.1). Even after using a hand cleaning procedure (i) wiping hands with alcohol free wipes (scenario 2) and (ii) washing hands with soap and water (scenario 3), all fingerprint remained positive for cocaine and benzoylecgonine. Figure 4.2 shows the distribution of the level (ratio A/IS) of cocaine and benzoylecgonine in fingerprints for each scenario. A significant difference is observed in the level of cocaine ($\chi^2 = 25.61, p < 0.001$) and benzoylecgonine ($\chi^2 = 26.67, p < 0.001$) between the scenarios based on a Kruskal-Wallis test (Appendix E. 9). The median level (ratio A/IS) was 72.790 for cocaine and 5.714 for benzoylecgonine after direct dermal contact with 2 mg of cocaine.
Figure 4.2: Box and whisker plots (median, 25 – 75\textsuperscript{th} percentiles, whiskers 5 – 95\textsuperscript{th} percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine in non-drug users after dermal contact with 2 mg of cocaine (scenario 1), after wiping hands with alcohol free wipes (scenario 2) and after washing hands with soap and water (scenario 3). Dots represent outliers.

Additionally, after washing hands the median cocaine level (ratio A/IS) was 0.431 compared to 2.516 after wiping hands with alcohol free wipes. For benzoylecgonine, the median level (ratio A/IS) after handwashing was 0.022 compared to 0.101 after wiping hands. This suggests that the use of a handwashing procedure is more effective in the removal of external contamination on the hands. However, the results indicate that both analytes can be present from external contamination on the hands if a large quantity (2 mg) of drug has been handled and cannot be removed by the hand cleaning procedures evaluated here. A more thorough hand cleaning procedure needs to be implemented (including more participants) to help exclude external contamination as a possible source for a positive fingerprint test. In addition to the presence of cocaine from handling the parent drug (dermal contact), the influence of secondary transfer of cocaine after dermal contact was evaluated.

Figure 4.3 shows the distribution of cocaine and benzoylecgonine after shaking hands and after washing hands with soap and water. A Mann-Whitney U test showed that a significant difference is observed between the level of cocaine (U = 1, \( p < 0.001 \)) and benzoylecgonine (U = 0, \( p < 0.001 \)) present in samples after shaking hands and washing hands (Appendix E. 10). The median cocaine level (ratio A/IS) was 0.761 after shaking hands compared to 0.030 after washing hands with soap and water. For benzoylecgonine the median level (ratio A/IS) was 0.055 after shaking hands and 0.007 after handwashing. This shows that after shaking
hands with an individual who has handled 2 mg of cocaine, the levels are decreased by washing hands with soap and water, but not completely removed.

Figure 4.3: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine in non-drug users after shaking hands with a participant with dermal contact of cocaine and after washing hands with soap and water.

4.2.1.2 Heroin

Fingerprints collected in scenario 1 after dermal contact with 2 mg of heroin showed that both heroin and 6-acetylmorphine were detected in all fingerprint samples (n = 12). The GC-MS results from FSI confirmed that 6-acetylmorphine was already present in the heroin used in this experiment (see Appendix C.2). After using a hand cleaning procedure, such as wiping hands with alcohol free wipes (scenario 2) or washing hands with soap and water (scenario 3), both heroin and 6-acetylmorphine remained present in all fingerprints. Figure 4.4 shows the distribution of the level of heroin and 6-acetylmorphine for each scenario. A significant difference is observed in the level of heroin ($\chi^2 = 28.76, p < 0.001$) and 6-acetylmorphine ($\chi^2 = 30.71, p < 0.001$) between the three scenarios (Appendix E. 9). The median level (ratio A/IS) was 48.312 for heroin and 10.405 for 6-acetylmorphine after dermal contact with 2 mg of heroin. The use of alcohol free wipes lowered the median levels of heroin and 6-acetylmorphine present in the fingerprint samples to 0.360 and 0.137, respectively. Compared to wiping hands with alcohol free wipes, the use of a handwashing procedure was more effective in the removal of heroin and 6-acetylmorphine as lower median levels (ratio A/IS) were observed (heroin 0.028 and 6-acetylmorphine 0.009).
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Figure 4.4: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine in non-drug users after dermal contact with 2 mg of heroin (scenario 1), after wiping hands with alcohol-free wipes (scenario 2) and after washing hands with soap and water (scenario 3). Dots represent outliers.

The act of shaking hands with a participant who has handled 2 mg of heroin resulted in secondary transfer of both heroin (7 out of 8 samples) and 6-acetylmorphine (7 out of 8 samples) as seen in Figure 4.5. Interestingly, no heroin and 6-acetylmorphine are present after washing hands with soap and water. This suggests that although dermal contact with 2 mg of heroin cannot be removed by washing hands, it is possible to remove secondary transfer of heroin and 6-acetylmorphine.

Figure 4.5: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine in non-drug users after shaking hands with a participant with dermal contact of heroin and after washing hands with soap and water. Dots represent outliers.
4.2.2 Comparison of natural fingerprints: drug administration and drug contact

4.2.2.1 Cocaine

Fingerprint samples (n = 12) were collected from non-drug users (n = 3) after dermal contact with 2 mg of cocaine. Fingerprint samples (n = 65) were also collected from cocaine users (n = 13) (Chapter 3, section 3.2.2.1). All fingerprint samples collected after dermal contact with 2 mg of cocaine were positive for cocaine (12/12 samples) and benzoylecgonine (12/12 samples). Figure 4.6 shows the distribution of cocaine and benzoylecgonine in fingerprints from non-drug users after dermal contact with cocaine compared to cocaine users. A significantly higher level (ratio A/IS) is observed for cocaine (U = 777, p < 0.001) and benzoylecgonine (U = 732, p < 0.001) after dermal contact with 2 mg of cocaine compared to cocaine users (Appendix E. 11). The median cocaine level (ratio A/IS) was 0.900 for cocaine users compared to 72.790 for non-drug users after dermal contact with cocaine.

![Box and whisker plots showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine in fingerprints from non-drug users after dermal contact with 2 mg of cocaine (scenario 1) and cocaine users. Dots represent outliers.](image)

The relationship between the presence of cocaine and benzoylecgonine after drug contact and drug administration was assessed by comparison of the parent drug-to-metabolite ratio. Figure 4.7 shows the ratio of cocaine-to-benzoylecgonine for participants after contact with 2 mg of cocaine (scenario 1). Significantly lower cocaine-to-benzoylecgonine ratios are observed for non-drug users after dermal contact with cocaine compared to cocaine users (U = 143, p = 0.001) (Appendix E. 12). The median cocaine-to-benzoylecgonine ratio for non-drug users after dermal contact with cocaine was 10.296 compared to 29.901 for cocaine users. Although a significant difference is observed between the median levels (cocaine-to-
benzoylcegonine ratio), no differentiation between the two groups is possible based on individual levels.

Figure 4.7: Box and whisker plots (median, 25–75th percentiles, whiskers 5–95th percentiles) showing the distribution of the average peak area ratio cocaine-to-benzoylcegonine in fingerprints from non-drug users after dermal contact with 2 mg of cocaine (scenario 1) and cocaine users. Dots represent outliers.

4.2.2.2 Heroin

Fingerprint samples (n = 12) were collected from non-drug users (n = 3) after dermal contact with 2 mg of heroin. Fingerprint samples (n = 60) were also collected from heroin users (n = 12) (Chapter 3, section 3.2.2.2). All fingerprint samples collected after dermal contact with 2 mg of the parent drug were positive for heroin (12/12 samples) and 6-acetylmorphine (12/12 samples). Figure 4.8 shows distribution of heroin and 6-acetylmorphine for heroin users and non-drug users after dermal contact with heroin. A significantly higher level (ratio A/IS) is observed for heroin after dermal contact with the parent drug in comparison to fingerprints collected from heroin users, based on a Mann-Whitney U-test (U = 707 p < 0.001, Appendix E. 11). The median level (ratio A/IS) for heroin was 0.067 for heroin users compared to 48.312 for non-drug users after dermal contact with heroin. In contrast, no significant difference (at p = 0.05) is observed for 6-acetylmorphine between drug users and non-drug users after dermal contact with 2 mg of heroin (U = 242, p = 0.073, Appendix E. 11). The median level for 6-acetylmorphine was 0.030 for heroin users and 0.016 for non-drug users (after dermal contact with heroin).
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Figure 4.8: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine in fingerprints from non-drug users after dermal contact with 2 mg of heroin (scenario 1) and heroin users. Dots represent outliers.

Based on the relationship between heroin and 6-acetylmorphine (Figure 4.9), a significantly lower heroin-to-6-acetylmorphine ratio is observed for heroin users compared to non-drug users after dermal contact with 2 mg of heroin ($U = 543, p = 0.004$) (Appendix E. 12). The median heroin-to-6-acetylmorphine ratio was 2.691 for heroin users compared to 4.625 after dermal contact with 2 mg of heroin.

Figure 4.9: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio heroin-to-6-acetylmorphine in fingerprints from non-drug users after dermal contact with 2 mg of heroin (scenario 1) and heroin users. Dots represent outliers.
Additionally, a closer distribution is observed for the ratio heroin-to-6-acetylmorphine after dermal contact compared to heroin users. This is most likely attributed to the same amount of drug handled and distributed between fingers. Although a significant difference is observed in the median level (heroin-to-6-acetylmorphine ratio), it is not possible to differentiate heroin users and participants who have handled the parent drug based on individual levels observed for each participant.

4.2.2.3 Threshold level

The previous chapter explored the significance of the detection of cocaine, benzoylecgonine heroin and 6-acetylmorphine in natural fingerprint samples based on the prevalence of these analytes in a background population of non-drug users (section 3.2.3). As cocaine and benzoylecgonine were present in 13% (13 samples) and 5% (5 samples) of fingerprint samples collected from the background population, there was a requirement to impose a threshold level to differentiate non-drug users from drug users (section 3.2.4). In this chapter the influence of those same proposed threshold levels on the detection rate of cocaine and benzoylecgonine after dermal contact with the drug are evaluated. Threshold levels were evaluated per analyte at the limit of detection (ratio A/IS cocaine 0.002 and benzoylecgonine 0.004, see section 2.3.7.3) and ratio A/IS of 0.005 – 0.030, in increments of 0.005 for fingerprint samples collected after dermal contact with 2 mg of cocaine (scenario 1). The results showed that due to the large amount of cocaine handled (2 mg), the levels of cocaine and benzoylecgonine present in the fingerprint samples were higher than the proposed threshold levels. Therefore, all samples remained positive for cocaine (12/12 samples) and benzoylecgonine (12/12 samples), independent of the threshold level proposed. This means that a threshold level cannot be successfully applied to natural fingerprints collected in order to distinguish drug administration from drug contact. In contrast, heroin and 6-acetylmorphine were not prevalent in samples from a background population of non-drug users therefore threshold levels were not required to differentiate non-drug users from drug users. However, if this same criterion is applied here, all fingerprint samples would return a positive test after handling the parent drug. Depending on the application of the fingerprint test, demonstrating whether someone has taken a substance or handled a substance, this could be significant already.
4.2.3 Effect of wiping hands: drug contact and drug administration

In scenario 2, fingerprint samples were collected after dermal contact with 2 mg of parent drug (cocaine or heroin) and subsequently wiping hands with alcohol free wipes. The use of a hand wiping procedure was evaluated to determine whether external contamination from handling the parent drug could be removed.

4.2.3.1 Cocaine

Fingerprints collected after dermal contact with cocaine and wiping hands with alcohol free wipes showed that cocaine (12/12 samples) and benzoylecgonine (12/12 samples) were still present in all fingerprint samples for the three participants, after the hand cleaning procedure. The use of a hand cleaning procedure did lower the level (ratio A/IS) of analytes in fingerprints compared to samples collected directly after dermal contact with 2 mg of cocaine. Figure 4.10 shows the distribution in the level of cocaine and benzoylecgonine for cocaine users and non-drug users (after dermal contact), after wiping hands with alcohol free wipes. Overall, the levels of cocaine and benzoylecgonine present in non-drug users from dermal contact were significantly higher compared to levels present after cocaine use, even after the hand cleaning procedure (U = 269, \( p < 0.001 \) for cocaine and \( U = 292, p < 0.001 \) for benzoylecgonine) (Appendix E. 13). The median cocaine level (ratio A/IS) present in fingerprints from non-drug users was 2.516 compared to 0.512 for cocaine users.

Figure 4.10: Box and whisker plots (median, 25 – 75\textsuperscript{th} percentiles, whiskers 5 – 95\textsuperscript{th} percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine in fingerprints collected after wiping hands with alcohol free wipes from non-drug users after dermal contact with 2 mg of cocaine (scenario 2) and cocaine users. Dots represent outliers.
For benzoylecgonine the median level (ratio A/IS) was 0.101 after dermal contact and 0.023 for cocaine users. The results show that the presence of cocaine and benzoylecgonine after dermal contact with the parent drug cannot be completely removed from the hands by using alcohol free wipes. A more thorough hand cleaning procedure needs to be implemented to help exclude external contamination as a possible source for a positive fingerprint test.

Comparison of the relationship between cocaine and benzoylecgonine in fingerprint samples collected after wiping hands with alcohol free wipes from cocaine users and non-drug users (after dermal contact with 2 mg of cocaine) is shown in Figure 4.11. Although a significant difference was observed between the level (ratio A/IS) of cocaine and benzoylecgonine between the two groups, no significant difference (at \( p = 0.05 \)) is observed for the cocaine-to-benzoylecgonine ratio (\( U = 99, p = 0.102 \)) (Appendix E. 14). This suggests that it is not possible to differentiate drug contact from drug administration by evaluating the cocaine-to-benzoylecgonine ratio.

![Figure 4.11](image)

Figure 4.11: Box and whisker plots (median, 25 – 75\(^{th}\) percentiles, whiskers 5 – 95\(^{th}\) percentiles) showing the distribution of the average peak area ratio cocaine-to-benzoylecgonine in fingerprints collected after wiping hands with alcohol free wipes from non-drug users after dermal contact with 2 mg of cocaine (scenario 2) and cocaine users.

4.2.3.2 Heroin

Fingerprints collected from non-drug users after dermal contact with heroin and subsequently wiping hands with alcohol free wipes still resulted in the presence of heroin and 6-acetylmorphine in all samples (12/12 for both analytes). Compared to the levels of
heroin and 6-acetylmorphine observed in scenario 1, the hand cleaning procedure has removed some external contamination on the hands. The distribution of heroin and 6-acetylmorphine level in fingerprints collected after wiping hands with alcohol free wipes from non-drug users (after dermal contact) and heroin users is shown in Figure 4.12. No significant difference (at $p = 0.05$) is observed in the level (ratio $A/IS$) of heroin in fingerprints from non-drug users compared to heroin users ($U = 88, p = 0.0224$) (Appendix E. 13). The median heroin level was 0.592 for drug users compared to 0.360 for non-drug users. In contrast, a significant difference ($U = 212, p < 0.001$) is observed in the level of 6-acetylmorphine present in fingerprints between the two groups (Appendix E. 13). The median 6-acetylmorphine level (ratio $A/IS$) in non-drug users was 0.137 compared to 0.028 for heroin users.

![Box and whisker plots](image)

Figure 4.12:  Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine in fingerprints collected after wiping hands with alcohol free wipes from non-drug users after dermal contact with 2 mg of heroin (scenario 2) and cocaine users. Dots represent outliers.

Although all fingerprints samples were positive, these results demonstrate that the presence of these analytes does not necessarily indicate drug administration. Comparison of the distribution of the heroin-to-6-acetylmorphine ratio present in fingerprints after drug contact and drug administration, and subsequently wiping hands is shown in Figure 4.13. The relationship between heroin and 6-acetylmorphine shows that there is a significant difference ($U = 222, p < 0.001$) in the ratio of heroin-to-6-acetylmorphine between the two groups (Appendix E. 14). The median heroin-to-6-acetylmorphine ratio was 2.056 for non-drug users (from dermal contact) and 0.582 for heroin users. Overall, the results suggest that
there is a difference in the relationship between heroin and 6-acetylmorphine from drug contact and drug administration. However, based on individual participant levels, differentiation between the two groups would not be possible as there is some overlap in the levels observed.

![Box and whisker plots](image)

Figure 4.13: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio heroin-to-6-acetylmorphine in fingerprints collected after wiping hands with alcohol free wipes from non-drug users after dermal contact with 2 mg of heroin (scenario 2) and heroin users.

4.2.4 Effect of washing hands: drug contact and drug administration

In scenario 3, a handwashing procedure was implemented after dermal contact with the parent drug. This was to explore whether the use of a handwashing procedure could remove cocaine and heroin present on the hands after handling 2 mg of the parent drug.

4.2.4.1 Cocaine

Fingerprints collected after washing hands with soap and water from non-drug users after dermal contact with 2 mg of cocaine still resulted in the presence of cocaine (12/12 samples) and benzoylecgonine (12/12 samples). Comparison of the distribution of cocaine and benzoylecgonine in fingerprints collected after handwashing from cocaine users and non-drug users (after dermal contact with the parent drug) is shown in Figure 4.14. No significant difference is observed in the level (ratio A/IS) of cocaine (U = 305, p = 0.158) present in fingerprints from non-drug users and cocaine users (Appendix E. 15). In contrast, a
significant difference is observed for benzoylecgonine (U = 393, p < 0.001) (Appendix E. 15).

Figure 4.14: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine in fingerprints collected after washing hands with soap and water from non-drug users after dermal contact with 2 mg of cocaine (scenario 3) and cocaine users. Dots represent outliers.

The median cocaine level was 0.292 for drug users compared to 0.431 for non-drug users after handling 2 mg of cocaine. For benzoylecgonine the median level was 0.009 for drug users compared to 0.101 for non-drug users. Overall, this suggests that there is a difference between the two groups. However, when evaluating individual participant levels, differentiation between drug contact and administration is not possible.

The distribution of the cocaine-to-benzoylecgonine ratio between non-drug users (after dermal contact) and cocaine users is shown in Figure 4.15. Although a significant difference is observed in the median levels (ratio A/IS) of cocaine and benzoylecgonine in fingerprints between non-drug users (after dermal contact) and cocaine users, individual levels could not be used to differentiate drug contact and drug administration. Additionally, comparison of the cocaine-to-benzoylecgonine ratio shows a significant difference is observed for fingerprints collected after handwashing between drug administration and drug contact (U = 172, p = 0.438) (Appendix E. 16). The median cocaine-to-benzoylecgonine ratio was 32.834 for cocaine users and 24.318 for non-drug users after dermal contact with the parent drug. This suggests that the presence of cocaine and benzoylecgonine in fingerprints is not solely indicative of cocaine administration and the relationship between the parent drug and metabolite cannot differentiate the two.
4.2.4.2 Heroin

Fingerprints collected after washing hands with soap and water from non-drug users (after dermal contact with the parent drug) showed that all samples (12/12) were positive for both heroin and 6-acetylmorphine. The handwashing procedure was not able to remove the external contamination present on the hands from handling the parent drug. Comparison of the level (ratio A/IS) of heroin and 6-acetylmorphine in samples from non-drug users and heroin users after washing hands is shown in Figure 4.16. No significant difference (at $p = 0.05$) is observed in the levels (ratio A/IS) of heroin ($U = 267, p = 0.115$) and 6-acetylmorphine ($U = 155, p = 0.180$) between the two groups (Appendix E. 15). The median heroin level was 0.011 for heroin users compared to 0.028 for non-drug users after dermal contact with heroin. This suggests that similar levels of heroin and 6-acetylmorphine can be present (after a handwashing procedure) from drug administration and drug contact.

Although the level of analyte present (after handwashing) from drug contact and drug administration was not significantly different between non-drug users (after dermal contact) and heroin users, a difference is observed for heroin-to-6-acetylmorphine ratio between the two groups.
Figure 4.16: Box and whisker plots (median, 25 – 75\textsuperscript{th} percentiles, whiskers 5 – 95\textsuperscript{th} percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine in fingerprints collected after washing hands with soap and water from non-drug users after dermal contact with 2 mg of cocaine (scenario 3) and heroin users. Dots represent outliers.

Figure 4.17 shows the distribution of the heroin-to-6-acetylmorphine ratio after washing hands for non-drug users (after dermal contact) and heroin users. The heroin-to-6-acetylmorphine ratio is significantly higher ($U = 329, p < 0.001$) for non-drug users (after dermal contact) compared to heroin users (Appendix E. 16). The median heroin-to-6-acetylmorphine ratio was 1.317 for heroin users after handwashing compared to 2.581 for non-drug users.

Figure 4.17: Box and whisker plots (median, 25 – 75\textsuperscript{th} percentiles, whiskers 5 – 95\textsuperscript{th} percentiles) showing the distribution of the average peak area ratio heroin-to-6-acetylmorphine in fingerprints collected after washing hands with soap and water from non-drug users after dermal contact with 2 mg of cocaine (scenario 3) and heroin users.
Based on individual participant levels, overlap is observed between the low heroin-to-6-acetylmorphine ratio for non-drug users and high heroin-to-6-acetylmorphine ratio for heroin users. This shows that it is not possible to differentiate the two groups based on the relationship between the parent drug and metabolite.

### 4.2.5 Secondary transfer of drugs

The possible transfer of drugs between a participant who has handled cocaine and a non-drug user was evaluated, similar to the secondary transfer experiment carried out in Chapter 3, section 3.2.9. Fingerprint samples (n = 4) were collected from 2 participants (S4P1 and S4P2) after shaking hands with an individual who touched 2 mg of cocaine. An additional set of fingerprint samples were collected for these two participants after washing hands with soap and water (S4P1-1 and S4P2-2) to try and remove the possible presence of cocaine after shaking hands.

#### 4.2.5.1 Cocaine

The results show that it is possible to transfer cocaine and benzoylecgonine through shaking hands with an individual who has handled 2 mg of the parent drug. Comparison in the level of cocaine and benzoylecgonine present in fingerprints after secondary transfer for non-drug users and cocaine users is shown in Figure 4.18. A significant difference is observed in the distribution of the level (ratio A/IS) of cocaine ($\chi^2 = 21.24, p < 0.001$) and benzoylecgonine ($\chi^2 = 18.87, p < 0.001$) between the data sets (Appendix E. 17).

![Figure 4.18: Box and whisker plots (median, 25–75th percentiles, whiskers 5–95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for cocaine and benzoylecgonine in fingerprints collected after handshaking and handwashing for non-drug users and cocaine users. Dots represent outliers.](image-url)
However, based on individual data for each participant, it is difficult to differentiate secondary transfer (after handshaking) and drug administration. The median cocaine level (ratio A/IS) was 0.761 after handshaking and 0.030 after handwashing for non-drug users, compared to 0.900 for cocaine users.

Comparison of the distribution of the cocaine-to-benzoylecgonine ratio in fingerprint samples from non-drug users (after shaking hands with a participant who handled 2 mg of cocaine and after washing hands) and cocaine users is shown in Figure 4.19. A significant difference is observed in the level (cocaine-to-benzoylecgonine ratio) present from secondary transfer compared to cocaine use ($\chi^2 = 29.97, p < 0.001$) (Appendix E. 18). The median cocaine-to-benzoylecgonine ratio present from secondary transfer was 13.403 after shaking hands and 4.495 after handwashing. In contrast, for cocaine users the cocaine-to-benzoylecgonine ratio was 31.804. This suggests that there is an overall difference between the groups, however based on individual levels no differentiation is possible.

![Box and whisker plots showing the distribution of the average peak area ratio cocaine-to-benzoylecgonine in fingerprints collected after handshaking and handwashing for non-drug users and cocaine users. Dots represent outliers.](image)

Figure 4.19: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio cocaine-to-benzoylecgonine in fingerprints collected after handshaking and handwashing for non-drug users and cocaine users. Dots represent outliers.

4.2.5.2 Heroin

Similar to the results obtained for cocaine, heroin and 6-acetylmorphine were transferred through shaking hands with a participant who handled 2 mg of heroin. However, after washing hands with soap and water no heroin and 6-acetylmorphine were present. Compared to the level (ratio A/IS) of heroin and 6-acetylmorphine present in fingerprints from heroin
users, a significant difference is observed in the distribution of the level (ratio A/IS) of heroin ($\chi^2 = 8.05, p = 0.018$) present from secondary transfer (see Figure 4.20) (Appendix E. 17). The median heroin level (ratio A/IS) present in fingerprints was 0.020 for non-drug users after shaking hands compared to 0.067 for heroin users. No significant difference (at $p = 0.05$) was observed for 6-acetylmorphine ($\chi^2 = 3.81, p = 0.148$) between the two (Appendix E. 17). The median 6-acetylmorphine level (ratio A/IS) present in fingerprints from non-drug users (after shaking hands) was 0.015 compared to 0.030 for heroin users. Although a significant difference is observed in the distribution of the analytes between the groups, no differentiation is possible based on individual data as there is overlap in the levels observed.

Figure 4.20: Box and whisker plots (median, 25 – 75th percentiles, whiskers 5 – 95th percentiles) showing the distribution of the average peak area ratio analyte (A) to internal standard (IS) for heroin and 6-acetylmorphine in fingerprints collected after handshaking and handwashing for non-drug users and heroin users. Dots represent outliers.

Comparison of the heroin-to-6-acetylmorphine ratio between fingerprints collected after secondary transfer (from non-drug users) and heroin use (from heroin users) is shown in Figure 4.21. The results show that no significant difference is observed (at $p = 0.05$) between the heroin-to-6-acetylmorphine ratio ($\chi^2 = 3.35, p = 0.188$) observed after drug administration and secondary transfer of drugs (Appendix E. 18). The median heroin-to-6-acetylmorphine ratio present after secondary transfer was 1.905 compared to 2.655 for heroin use. This suggests that a fingerprint test is not possible to differentiate the two groups based on the heroin-to-6-acetylmorphine ratio.
Chapter 4: Investigation into the Presence of Cocaine and Heroin in Fingerprints from Contact Residue

4.3 Summary

This research evaluated the presence of cocaine and heroin in fingerprint samples from dermal contact with the parent drug (2 mg). The results showed that cocaine, benzoylecgonine, heroin and 6-acetylmorphine can be present in fingerprints from non-drug users after dermal contact with the drug, even after a hand cleaning procedure (washing hands and wiping hands with alcohol free wipes) (section 4.2.1). The presence of benzoylecgonine and 6-acetylmorphine in fingerprint samples was attributed to the cocaine and heroin used, which was confirmed by GC-MS analysis carried out by FSI. The use of both hand cleaning procedures (scenario 2: wiping hands with alcohol free wipes and scenario 3: washing hands with soap and water) reduced the level of cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprint samples (section 4.2.1). The use of a handwashing procedure was more effective in the removal of contact residue compared to wiping hands with alcohol free wipes. However, all fingerprints remained positive. Secondary transfer of drugs (through shaking hands) is another possible source for the presence of these analytes in fingerprints (section 4.2.5). In contrast to cocaine and benzoylecgonine, secondary transfer of heroin and 6-acetylmorphine can be removed from the fingers by washing hands with soap and water. Overall, this chapter highlights that the presence of these analytes in fingerprints does not necessarily indicate drug administration, as these can also be present from handling the parent drug even after hand cleaning.
procedures. Although some differences were observed in the level of analytes present from drug contact and drug administration, based on individual results no differentiation is possible (sections 4.2.2, 4.2.3 and 4.2.4). In light of these findings, a fingerprint test would not be able to differentiate drug contact from drug administration.
Chapter 5  Monitoring Compliance with Tuberculosis Medication from a Fingerprint

5.0  Introduction

One of the objectives of this research was to evaluate the use of fingerprints to monitor compliance with medication to treat tuberculosis (in particular the first line drug, isoniazid). The detection of drugs of abuse has been explored in Chapter 3 for cocaine, heroin and their respective metabolites. The results obtained highlighted the challenges associated with the attributing presence of these compounds from administration (Chapter 3) or handling of the parent drug (Chapter 4). In this chapter, the use of fingerprint samples for a clinical application is investigated. Tuberculosis is one of the top ten causes of death globally and treatment of the infectious disease is confounded by the formation of drug resistant bacteria, which can appear if patients do not adhere to their treatment (World Health Organisation, 2017).

Various strategies have been employed to increase adherence to treatment and improve treatment success rates. Current methods to monitor adherence include regular engagement with medical and nursing teams, tablet counts and assessment of clinical improvement (Nackers et al., 2012; Osterberg & Blaschke, 2005). A urine dipstick test (based on a colorimetric test) can also be used on the spot if there are concerns or doubts (Guerra et al., 2010; Hanifa et al., 2007). However, each of these approaches have their limitations: tablet count is ineffective as it is easily altered by the patient (Nackers et al., 2012; Osterberg & Blaschke, 2005) engagement with the patient through direct observation therapy (DOTS) is expensive to administer (Steffen et al., 2010; Osterberg & Blaschke, 2005) and a urine dipstick test requires clinic access. A fingerprint may offer a simple route to confirming adherence to treatment and this is explored in this chapter.

This chapter investigates the detection of tuberculosis medication (isoniazid and its respective metabolite acetylisoniazid) in fingerprint samples from individuals receiving treatment (section 5.2.5) and individuals who have completed treatment (section 5.2.6). Information on the chemistry and metabolism of isoniazid and acetylisoniazid is described in Chapter 1, section 1.3.3. The significance of the detection of these analytes of interest in fingerprint samples was determined by comparison with a negative control group (section 5.2.7). Different sampling strategies were evaluated, including the collection of natural
fingertips and after washing hands with soap and water (section 5.2.8). The use of a fingerprint sample should be robust enough to detect the analytes of interest even after a hand cleaning procedure is used prior to sample collection, otherwise a high false negative rate would be observed unless patients were instructed to avoid handwashing. Additionally, the elimination profile of isoniazid and acetylisoniazid in fingerprint samples was explored by collecting samples from a patient who was coming off treatment (section 5.2.9). This was to investigate the detection window of the analytes of interest in fingerprint samples after their treatment was completed. Some individuals will cease to take their medication once they start to feel better. Therefore, it is particularly important to demonstrate whether fingerprints can be used to determine if an individual is not compliant with their treatment to improve patient outcome.

The following section details the specific participants recruited for the purpose of this study (section 5.1.1) and the sample collection and preparation methods used for fingerprint samples collected from participants. All samples were analysed on a Thermo Ultimate 3000 HPLC coupled to an Orbitrap mass spectrometer. The instrumentation and optimisation for isoniazid and acetylisoniazid are described in section 5.1.4. The fingerprint extraction method developed for the analytes of interest is described in section 5.2.1. The performance characteristics of the developed LC-MS method are evaluated in section 5.2.4.

The present chapter investigates whether a fingerprint can be used to detect tuberculosis medication to monitor treatment adherence. In addition, the detection window of isoniazid and acetylisoniazid in fingerprint is explored, to demonstrate whether a fingerprint test is suitable to determine if a patient is no longer compliant with their treatment.

5.1 Experimental

5.1.1 Study populations

Study participants were recruited to evaluate whether isoniazid and acetylisoniazid could be detected in fingerprint samples from individuals prescribed tuberculosis medication. This was to explore the possibility of using fingerprint samples to monitor compliance with tuberculosis treatment. In collaboration with Frimley Health NHS Foundation Trust, participants who (i) were on treatment (n = 27) and (ii) completed treatment (n = 6) were recruited from the chest clinic at King Edward VII Hospital, to show if a distinction could be made between the two groups. Additionally, as part of the study, a participant who was
completing their treatment was recruited to monitor the elimination profile of isoniazid and acetylisoniazid over a number of days (T = 0, T = 2 and T = 4 days). It is important to test the detection window of these analytes in fingerprint samples to show if an individual is not complying with the treatment, a negative test result will be obtained. Furthermore, fingerprint samples were collected from a negative control group (n = 10) to show whether these specific analytes were detected in individuals not taking medication and that there are no interfering compounds present with the same mass and retention time.

5.1.2 Fingerprints

Fingerprint samples from participants were collected on chromatography paper (Whatman™ 1-Chr) cut into 2 x 2 cm. Fingerprint collection kits were prepared by taping the paper substrate onto a microscope glass slide labelled with a unique participant identifier, e.g. TB-2017-FP001 for participants from the chest clinic who were on treatment or completed treatment. For participants coming off treatment to monitor the elimination profile, samples were labelled as TB-2017-E001. Whereas, samples from the negative control group were labelled as TB-2017-N001. For each participant, fingerprint samples were collected from all fingers and thumbs of the right hand as natural fingerprints and after washing hands with soap and water. This was to evaluate whether the analytes of interest could still be detected in fingerprint samples after a handwashing procedure.

All fingerprint samples were collected as described in the fingerprint collection procedure outlined in Chapter 3, section 3.1.2. Natural fingerprints were collected from tuberculosis patients upon arrival at the chest clinic without any preparation of the hands. Afterwards the participant was asked to wash their hands with soap and water. The participant then put on nitrile gloves for 10 min to induce perspiration on the fingers and thumbs prior to fingerprint deposition. Each sample collected was labelled with a unique participant identifier; the finger or thumb that was collected and the condition under which the sample was collected (e.g. natural or soap). The collected fingerprint sample was then placed into a microscope glass slide storage box for transport to the University of Surrey, UK.

Fingerprint samples were collected from n = 10 volunteers (as negative controls) at the University of Surrey. Fingerprint samples collected from the negative control group were collected as natural fingerprints and after washing hands with soap and water to determine whether the analytes of interest or any interfering compounds would be present. Fingerprint
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samples (n = 5) were collected from the negative control group as described in Chapter 3, section 3.1.2.

A favourable ethical opinion was obtained from the NHS Research Ethics Service (NRES-REC) and the University of Surrey Ethics Committee for the collection of fingerprint samples from individuals receiving treatment for tuberculosis at Wexham Park Hospital (Frimley Health NHS Foundation Trust) who volunteered taking part in this study. Additionally, fingerprint samples were collected from a negative control group (background population) at the University of Surrey (NRES-REC reference 16/LO/1663). Participants were given information sheets and informed consent forms in order to understand their involvement in the study. Once informed consent was obtained from the participants, samples were collected according to the standard operating procedures outlined in Appendix B. 2 (fingerprints).

5.1.3 Chemicals and reagents
Isoniazid, acetylisoniazid and isoniazid-d₄ (used as internal standard) were purchased from Toronto Research Chemicals (North York, Canada). Optima grade LC-MS solvents of methanol, acetonitrile and water were used to prepare solutions and solvent mixtures (Fisher Scientific, Leicestershire, UK). Formic acid was added to the mobile phase solvents at 0.1% (v/v) (Fischer Scientific, Leicestershire, UK). Dichloromethane, chloroform and ethyl acetate were used to prepare solvent extraction mixtures and were analytical or reagent grade (Sigma Aldrich, Dorset, UK). A solution of artificial eccrine perspiration was used to simulate a fingerprint matrix in the experimental design of fingerprint experiments (Pickering Laboratories, Inc., Obertaufkirchen, Germany).

5.1.4 Instrumentation and conditions
Analysis of samples was performed using the Dionex Ultimate 3000 HPLC coupled to a Q-Exactive Plus mass spectrometer as detailed in Chapter 2, section 2.2.2. The electrospray source parameters (polarity, probe position, auxiliary gas flow rate and spray voltage) were optimised for isoniazid and acetylisoniazid using an infusion of a 100 ng/ml standard of the analytes of interest in 50:50 (v/v) acetonitrile (ACN) in water (H₂O) + 0.1% formic acid. The optimised mass spectrometer parameters for the analysis of isoniazid and acetylisoniazid are outlined in Table 5.1.
Table 5.1: Operating conditions of the LC-MS for the analysis of isoniazid and acetylisoniazid in fingerprint samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Operating condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Spray voltage</td>
<td>4 kV</td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>320 ºC</td>
</tr>
<tr>
<td>S-lens RF level</td>
<td>50</td>
</tr>
<tr>
<td>Sheath gas flow rate</td>
<td>35</td>
</tr>
<tr>
<td>Aux gas flow rate</td>
<td>8</td>
</tr>
<tr>
<td><strong>Scan parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Scan type</td>
<td>Full MS</td>
</tr>
<tr>
<td>Scan range</td>
<td>m/z 120 - 1000</td>
</tr>
<tr>
<td>Resolution</td>
<td>70 000 at m/z 200</td>
</tr>
<tr>
<td>Polarity</td>
<td>Positive</td>
</tr>
<tr>
<td>AGC target</td>
<td>$10^6$</td>
</tr>
<tr>
<td>Maximum inject time</td>
<td>200</td>
</tr>
</tbody>
</table>

The previously developed chromatography method for the analysis of cocaine, heroin and their respective metabolites (benzoylcegonine and 6-acetylmorphine) (section 2.2.2) was applied to isoniazid and acetylisoniazid. A Kinetex C$_{18}$ column (100 x 2.1 mm, 5 µm) was used for the LC-MS analysis of all samples. Methanol and acetonitrile were evaluated as organic mobile phase solvents for the chromatographic separation of the analytes of interest in combination with water as the aqueous solvent. Interestingly, broader peak shapes were observed when using the initial mobile phase composition (5% (v/v) ACN in H$_2$O + 0.1% formic acid) as the sample solution. However, sharper peaks were obtained using 50% (v/v) ACN in H$_2$O + 0.1% formic acid. As the peaks eluted early in the chromatogram, the higher organic content in the mobile phase composition minimised peak broadening, resulting in sharper peaks. Therefore, all samples were reconstituted in that solvent mixture containing 50 ng/ml isoniazid-d$_4$ as the internal standard.

Different gradients and run times were evaluated for the separation of isoniazid and acetylisoniazid, however none of the methods explored achieved baseline separation. An example of an overlaid extracted ion chromatogram for isoniazid and acetylisoniazid using the developed method is shown in Figure 5.1. Although baseline separation was not achieved for isoniazid and acetylisoniazid, the use of a high resolution mass spectrometer still allowed for the identification of these analytes based on their m/z ratio. The m/z ratio for isoniazid (138.0661), acetylisoniazid (180.0768) and isoniazid-d$_4$ were within 2 ppm at 70,000 mass resolution.
5.1.5 Extraction method

A range of solvents were evaluated for the extraction of isoniazid and acetylisoniazid from paper (Whatman™ grade-1 chromatography paper 2 x 2 cm square). The fingerprint extraction method was adopted from the previously developed method for the analysis of cocaine, heroin and their respective metabolites (section 2.3.4). The use of methanol, acetonitrile, dichloromethane, chloroform, ethyl acetate and 10% (v/v) methanol in dichloromethane and 10% (v/v) methanol in chloroform as extraction solvents were evaluated to determine which solvent would provide the best extraction.

Ten microlitres of the analyte standard in methanol (at 500 ng/ml) was spiked onto Whatman™ grade-1 chromatography paper (2 x 2 cm square) and left to dry overnight to produce 5 ng of analyte residue on surface. The sample was placed in a 2 ml Eppendorf microcentrifuge tube with 1.5 ml extraction solvent. The sample was centrifuged for 5 min at 10000 x g relative centrifugal force (rcf) and the paper was subsequently removed and discarded. The sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 ºC), reconstituted in 100 µl mobile phase solution containing 50 ng/ml internal standard solution and vortexed-mixed for 30 sec prior to LC-MS analysis. The extracted sample was compared against an analyte standard (at 50 ng/ml) in 50% (v/v) ACN.
in H₂O with 0.1% formic acid and 50 ng/ml internal standard solution at the same concentration as the final sample extract.

5.1.6 Matrix effects

5.1.6.1 Participant selection and sampling

The influence of the matrix on the extraction efficiency of isoniazid and acetylisoniazid was evaluated for methanol, artificial eccrine perspiration and fingerprints. Analyte standards were prepared at three concentration levels, 100, 500 and 1000 ng/ml, with five replicate samples per concentration level. Ten microlitres of analyte standard was spiked onto paper to produce 1, 5 and 10 ng of analyte residue on surface and extracted using the method described in section 5.2.1. A favourable ethical approval was obtained from the University of Surrey Ethics Committee to collect samples from individuals at the University of Surrey who volunteered to participate. Fingerprint samples were collected from n = 5 participants without any preparation of the hands (natural fingerprints) and after washing hands with soap and water, prior to wearing nitrile gloves for 10 min to aid secretion of perspiration from the fingertips. Four fingerprints were collected per participant (right thumb, right index, left thumb and left index finger) to prepare the spiked samples and a blank. A minimum of five participants is recommended because this allows to incorporate variations in gender and other factors into the experiment (Sears et al., 2012). However, due to ethical considerations this information is not provided. Five replicate samples were prepared for each matrix at each level. For fingerprints, the analyte standard (10 µl of 100, 500 or 1000 ng/ml) in methanol was pipetted onto the fingerprint samples to produce 1, 5, and 10 ng of analyte residue on the sample surface. Samples were extracted by placing the paper substrate in a microcentrifuge tube (2 ml) with 1.5 ml of extraction solution (10% dichloromethane in methanol) and centrifuging for 5 min at 10000 x g rcf. The paper was discarded and the sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C). The subsequent sample residue was reconstituted in 100 µl mobile phase solution (50% acetonitrile in water with 0.1% (v/v) formic acid containing 50 ng/ml isoniazid-d₄ as internal standard) and vortex-mixed again for 30 sec prior to LC-MS analysis.

5.1.6.2 Ionisation suppression and enhancement

The presence of matrix effects in the form of ionisation suppression/enhancement was investigated to determine whether the sample matrix contained interferences that co-eluted with the analytes of interest. These effects can attribute to a change in instrument response
of the analyte compared to a standard solution of the same concentration. The presence of ion suppression/enhancement was evaluated using the post-extraction addition approach as used in Chapter 2 section 2.2.5.2 for cocaine, heroin and their respective metabolites (Scientific Working Group for Forensic Toxicology, 2013). Two matrices were assessed, including methanol (blank solvent) and fingerprint samples collected without any preparation of the hands (natural) and after washing hands with soap and water. Two sets of samples were prepared in order to compare the effects of the matrix on the analyte signal. Set one consisted of a standard solution (50:50 (v/v) acetonitrile in water + 0.1% formic acid) of isoniazid and acetylisoniazid at 20 ng/ml and 80 ng/ml, both containing 50 ng/ml internal standard (isoniazid-d₄). Set two consisted of the different blank matrices (methanol, fingerprint samples natural and after handwashing) spiked with a standard solution of isoniazid and acetylisoniazid post extraction at 20 ng/ml and 80 ng/ml. The effect of the sample matrix on ionisation suppression/enhancement was evaluated by comparing the average peak area of samples from set two with that of the standard solution (set one) of the same concentration.

Ten microlitres of blank solvent (methanol) or matrix (fingerprint) was added to the paper substrate (Whatman 1-Chr grade paper 2 x 2 cm) and left to dry overnight. The right thumb (blanks) and index finger (spiked) were collected from n = 5 individuals, natural and after handwashing to assess the potential matrix effects between participants. Each sample was extracted using the extraction method described in section 5.2.1. After the extraction was performed and the sample was evaporated to dryness, the samples were reconstituted with an analyte standard (at 20 ng/ml or 80 ng/ml) in 50:50 (v/v) acetonitrile in water with 0.1% formic acid containing 50 ng/ml isoniazid-d₄ as the internal standard. Each sample was measured five times and the average peak area obtained was compared to that of set one.

5.1.7 Method recovery

The recovery of the developed extraction method was evaluated using analyte standards (containing isoniazid and acetylisoniazid) in methanol at 100, 500 and 1000 ng/ml, to produce spiked fingerprints (using n = 5 participants, with 3 fingerprints collected per participant) at 1, 5, and 10 ng of analyte residue on surface. Participant selection and sampling is discussed in section 5.1.6.1. Samples were extracted by placing the paper substrate in a microcentrifuge tube (2 ml) with 1.5 ml of extraction solution (10% dichloromethane in methanol) and centrifuging for 5 min at 10000 x g rcf. The paper was
discarded and the sample extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C). The subsequent sample residue was reconstituted in 100 µl mobile phase solution (50% acetonitrile in water with 0.1% (v/v) formic acid containing 50 ng/ml internal standard solution) and vortex-mixed again for 30 sec prior to LC-MS analysis. The average percentage recovery for isoniazid and acetylisoniazid at each level (1, 5 and 10 ng) in fingerprints was calculated based on the original spiked concentration.

5.2 Results and Discussion

5.2.1 Extraction method

The extraction efficiency (calculated by % recovery) for isoniazid and acetylisoniazid using methanol, acetonitrile, dichloromethane, chloroform, ethyl acetate and 10% (v/v) methanol in dichloromethane and 10% (v/v) methanol in chloroform as extraction solvents is shown in Figure 5.2.

![Figure 5.2](image)

Figure 5.2: Average percentage recovery (± standard deviation n = 5 measurements) for isoniazid and acetylisoniazid (n = 1 sample) extracted from paper (at 5 ng) with methanol, acetonitrile, dichloromethane (DCM), ethyl acetate, chloroform (CHCl₃), 10% (v/v) methanol (MeOH) in chloroform, 10% (v/v) dichloromethane (DCM) in methanol (MeOH).

A higher percentage recovery for isoniazid and acetylisoniazid is observed using a polar protic solvent, such as, methanol compared to the other solvents evaluated. This is a result of the hydrogen bonding between methanol and the analytes of interest which increases the
affinity for the extraction from the paper substrate. A relatively high percentage recovery (127 – 140%) was observed for acetylisoniazid using (i) 10% (v/v) DCM in MeOH or (ii) MeOH. For isoniazid, the corresponding recoveries were 78 and 75%, respectively. The use of 10% (v/v) DCM in MeOH was chosen as the solvent for the extraction of isoniazid and acetylisoniazid from paper substrates as this provided the best % recovery.

5.2.2 Matrix effects
The effect of the matrix on the extraction of isoniazid and acetylisoniazid at each concentration level (1, 5 and 10 ng) is shown in Figure 5.3. For isoniazid, the methanol standard produced matrix enhancement compared to fingerprint samples (natural and after washing hands) and artificial eccrine perspiration. However, these results were consistent for each concentration level. The results show that there are sample matrix effects observed for acetylisoniazid in the presence of artificial eccrine sweat, as a higher ratio A/IS was consistently observed at each concentration level. Due to the differences observed between the matrix effects for isoniazid and acetylisoniazid and the absence of a validated fingerprint standard, it was decided not to produce matrix-matched calibration curves. Additionally, due to differences observed for the analyte signal in the presence of a fingerprint sample compared to standard in methanol, results are reported as a ratio of analyte-to-internal standard (ratio A/IS) signal.

Figure 5.3: Average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, n = 5 measurements) for isoniazid and acetylisoniazid for spiked acetonitrile, artificial eccrine perspiration and fingerprints (natural and after washing hands with soap).
The fingerprint samples (natural or after washing hands with soap) from the five participants studied here show that the matrix effects observed between participants does not vary greatly. The variability (n = 5 participants) between samples for isoniazid was ≤9 and ≤7% for natural fingerprints and after handwashing, respectively. For acetylisoniazid, the corresponding variability was ≤13 and ≤14% for natural fingerprint samples and after handwashing, respectively (see Figure 5.3).

5.2.2.1 Ionisation suppression/enhancement

Figure 5.4 shows the effect of the blank solvent (methanol) extracted from paper on the signal of isoniazid, acetylisoniazid and isoniazid-d₄ at 20 ng/ml (A1) and 80 ng/ml (B1) compared to a standard solution of the same concentration. The results show that the signal of all analytes were lower in the presence of extracted compounds from the paper compared to the reference standard of the same concentration for both concentration levels. This indicates that there are matrix effects observed from the paper substrate in the form of ionisation suppression. The variability (%RSD) in ionisation suppression for n = 5 samples at 20 ng/ml was 9% for isoniazid, 5% for isoniazid-d₄, and 4% acetylisoniazid. In contrast, at 80 ng/ml, the variability was slightly higher for all analytes (13% for isoniazid and isoniazid-d₄ and 10% for acetylisoniazid).

Figure 5.4: Average peak area (n = 5 measurements) for isoniazid, acetylisoniazid and isoniazid-d₄ for blank solvent (methanol) extracted and spiked with (A1) 20 ng/ml and (B1) 80 ng/ml of standard solution containing 50 ng/ml isoniazid-d₄ as internal standard.

The effect of ionisation suppression on the analytes of interest in the presence of natural fingerprints at both concentration levels (20 ng/ml and 80 ng/ml) was also evaluated as seen in Figure 5.5 for natural fingerprints and Figure 5.6 for fingerprints collected after washing hands.
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Figure 5.5: Average peak area (n = 5 measurements) for isoniazid, acetylisoniazid and isoniazid-d₄ in natural fingerprint samples extracted and spiked with (A2) 20 ng/ml and (B2) 80 ng/ml of standard solution containing 50 ng/ml isoniazid-d₄ as internal standard.

Compared to the reference standard, lower signals were observed for all analytes in the presence of a fingerprint (natural and after handwashing) at both concentration levels. Interestingly, the results show that the degree of ionisation suppression for the analytes of interest varies greatly between participants at both concentration levels. The variability (%RSD) observed between the response from isoniazid, isoniazid-d₄ and acetylisoniazid for natural fingerprint samples at 20 ng/ml was 54, 53 and 37%, respectively. At the higher concentration level (80 ng/ml), the variability observed was 47, 49 and 27% for isoniazid, isoniazid-d₄ and acetylisoniazid, respectively for n = 5 participants.

Figure 5.6 shows the effect of ion suppression in fingerprints after washing hands with soap and water. The results show that the ionisation suppression effects are still observed. However, the variability in ionisation suppression effects between (n = 5) participants for isoniazid, isoniazid-d₄ and acetylisoniazid at 20 and 80 ng/ml were lower compared to natural fingerprint samples. At 20 ng/ml the variability was 25% for isoniazid and isoniazid-d₄, and 17% for acetylisoniazid. In addition, at 80 ng/ml the variability observed in ionisation suppression for n = 5 participants was 27% for isoniazid and isoniazid-d₄, and 20% for acetylisoniazid. The lower variability observed after handwashing could be a result of the removal of fingerprint constituents by using a hand cleaning procedure, which may result in a lower degree of matrix effects compared to natural fingerprints. An estimation of the degree of ionisation suppression for isoniazid, acetylisoniazid and isoniazid-d₄ was calculated using Equation 2.1. The negative values in Table 5.2 and Table 5.3 indicate that ionisation suppression is observed for the analytes of interest in blank solvent (methanol).
and fingerprint samples (natural and after handwashing for n = 5 participants) extracted from paper at both concentration levels (20 and 80 ng/ml).

Figure 5.6: Average peak area (n = 5 measurements) for isoniazid, acetylisoniazid and isoniazid-d₄ in fingerprint samples collected after handwashing extracted and spiked with (A3) 20 ng/ml and (B3) 80 ng/ml of standard solution containing 50 ng/ml isoniazid-d₄ as internal standard.

The degree of ionisation suppression observed for isoniazid and acetylisoniazid ranged from -31 to -47% in the presence of paper substrates and -32 to -93% in the presence of fingerprint samples. For the internal standard, the ionisation suppression effects observed for isoniazid and acetylisoniazid, ranged from -43 to -50% in the presence of paper and -46 to -92% in the presence of fingerprints. The results highlight that the ionisation suppression effects observed varies significantly between participants using both fingerprint collection methods.

Table 5.2: Ionisation suppression or enhancement (percentage) for the analysis of isoniazid, acetylisoniazid and isoniazid-d₄ at 20 ng/ml in methanol and fingerprint samples natural and after washing hands with soap and water (participants 1 – 5).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Isoniazid</th>
<th>Acetylisoniazid</th>
<th>Isoniazid-d₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank solvent</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sample 1</td>
<td>-44</td>
<td>-35</td>
<td>-46</td>
</tr>
<tr>
<td>Sample 2</td>
<td>-41</td>
<td>-34</td>
<td>-43</td>
</tr>
<tr>
<td>Sample 3</td>
<td>-33</td>
<td>-33</td>
<td>-44</td>
</tr>
<tr>
<td>Sample 4</td>
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<td>-31</td>
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</tr>
<tr>
<td>Sample 5</td>
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<td>-50</td>
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<tr>
<td>Fingerprint</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>natural</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Participant 1</td>
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<tr>
<td>Participant 2</td>
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<td>Participant 3</td>
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</tr>
<tr>
<td>Participant 4</td>
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<td>-32</td>
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<tr>
<td>Participant 5</td>
<td>-58</td>
<td>-41</td>
<td>-59</td>
</tr>
<tr>
<td>Fingerprint</td>
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<td></td>
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<tr>
<td>soap</td>
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<td></td>
<td></td>
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<tr>
<td>Participant 1</td>
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<td>-64</td>
</tr>
<tr>
<td>Participant 2</td>
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<td>-46</td>
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</tr>
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<td>Participant 3</td>
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<tr>
<td>Participant 4</td>
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<td>-47</td>
</tr>
<tr>
<td>Participant 5</td>
<td>-56</td>
<td>-40</td>
<td>-57</td>
</tr>
</tbody>
</table>
Table 5.3: Ionisation suppression or enhancement (percentage) for the analysis of isoniazid, acetylisoniazid and isoniazid-d₄ at 80 ng/ml in methanol and fingerprint samples natural and after washing hands with soap and water (participants 1 – 5).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ionisation suppression or enhancement (IS/IE) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isoniazid</td>
</tr>
<tr>
<td>Blank solvent (methanol)</td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>-58</td>
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<tr>
<td>Sample 2</td>
<td>-62</td>
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<td>Sample 3</td>
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<td>Sample 4</td>
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<tr>
<td>Sample 5</td>
<td>-47</td>
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<tr>
<td>Fingerprint natural</td>
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<td>Participant 1</td>
<td>-89</td>
</tr>
<tr>
<td>Participant 2</td>
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<td>Participant 3</td>
<td>-76</td>
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<td>Participant 4</td>
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<td>Participant 5</td>
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<tr>
<td>Fingerprint soap</td>
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<td>Participant 1</td>
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<td>Participant 2</td>
<td>-57</td>
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<td>Participant 3</td>
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</tr>
<tr>
<td>Participant 4</td>
<td>-49</td>
</tr>
<tr>
<td>Participant 5</td>
<td>-62</td>
</tr>
</tbody>
</table>

There are different approaches to minimise or correct ionisation suppression effects, including modification of chromatography conditions, sample preparation or use of internal standards (Annesley, 2003). In this study, an internal standard (isoniazid-d₄) was used to correct for the observed degree of ionisation suppression (see Figure 5.7). In order for the internal standard to correct for the ionisation suppression, the internal standard must co-elute with the analyte of interest. The internal standard and analyte will undergo the same ionisation suppression effects and using the ratio A/IS will “correct” for the degree of ionisation suppression. The use of the ratio A/IS for isoniazid and acetylisoniazid shows that the use of isoniazid-d₄ corrects for the ionisation suppression effects observed in the presence of a blank paper substrate for both concentration levels (20 ng/ml and 80 ng/ml).

An F-test was performed to determine whether there was a significant difference in the sample variance between two sample sets, blank paper and fingerprints (natural and after handwashing) compared to the reference standard. The results suggested that the two standard deviations (between a sample and the reference standard) were significantly different, with the exception of blank paper (methanol) for acetylisoniazid at both 20 and 80 ng/ml, and blank paper (methanol) and fingerprints (soap) for isoniazid at 20 ng/ml. Therefore Eq. 2.8 was used to compare the two experimental means for data sets where the sample variances were significantly different and Eq. 2.6 for those that were not significantly different (at $p = 0.05$).
Figure 5.7: Average peak area ratio analyte (A) to internal (IS) standard (± standard deviation, n = 5 measurements) for isoniazid and acetylisoniazid for blank solvent (methanol) extracted and spiked with (C1) 20 ng/ml and (D1) 80 ng/ml of standard solution containing 50 ng/ml isoniazid-d₄ as internal standard.

Compared to the reference standard of the same concentration, the ratio A/IS for the extracted samples (fingerprints natural and after handwashing, and blank paper with methanol) were still significantly different (at $p = 0.05$) at both concentration levels (Appendix E. 19 and E. 20). The only exception to this was for isoniazid samples at 20 ng/ml and acetylisoniazid methanol samples at 20 and 80 ng/ml, no significant difference was observed at $p = 0.05$. The use of the ratio A/IS reduced the variability (%RSD) between n = 5 samples to 1% for isoniazid and acetylisoniazid at 20 ng/ml. At the higher concentration level (80 ng/ml), the use of the ratio A/IS reduced the variability for n = 5 samples to 2% for isoniazid and acetylisoniazid. This suggests a good level of precision between samples. A high level of variability in the degree of ionisation suppression was initially observed for isoniazid and acetylisoniazid in the presence of a fingerprint. However, using the ratio A/IS for isoniazid and acetylisoniazid corrected for the sample matrix effects observed at both concentration levels (Figure 5.8 and Figure 5.9). The variability in the level of isoniazid and acetylisoniazid observed (at 20 ng/ml) between fingerprint samples (natural) for n = 5 participants was reduced to 10 and 32%, respectively. After washing hands with soap and water the variability was reduced to 1 and 9% for isoniazid and acetylisoniazid, respectively. This shows that the sample matrix effects are corrected for using an internal standard, particularly after handwashing. However, based on a t-test, there is a still a significant difference observed between the levels of isoniazid in the presence of a paper substrate (at both concentration levels) and fingerprints (natural at 80 ng/ml and after handwashing at 20 and 80 ng/ml) compared to a standard of the same concentration (at $p = 0.05$, Appendix E.
For acetylisoniazid a significant difference is observed for all matrices (blank solvent on paper and fingerprint samples natural and after handwashing) at both concentration levels (at $p = 0.05$, Appendix E. 20). The results suggest that the level of acetylisoniazid in natural fingerprint samples are not comparable due to the large variability (in ratio A/IS) observed between participants in Figure 5.8 and Figure 5.9 for the same concentration due to sample matrix effects.

Figure 5.8: Average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, n = 5 measurements) for isoniazid and acetylisoniazid in natural fingerprint samples extracted and spiked with (C2) 20 ng/ml and (D2) 80 ng/ml of standard solution containing 50 ng/ml isoniazid-d$_4$ as internal standard.

Figure 5.9: Average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, n = 5 measurements) for isoniazid and acetylisoniazid in fingerprint samples collected after handwashing extracted and spiked with (C3) 20 ng/ml and (D3) 80 ng/ml of standard solution containing 50 ng/ml isoniazid-d$_4$ as internal standard.

The matrix effects observed for acetylisoniazid are not corrected to the same degree as isoniazid, as the chromatographic peak for the internal standard does not coincide with acetylisoniazid resulting in a difference in the observed degree of ionisation suppression.
However, the sample matrix effects for acetylisoniazid are corrected best using isoniazid-d₄ as the internal standard.

5.2.3 Method recovery

The recovery of isoniazid and acetylisoniazid using the developed extraction method was assessed at 1, 5 and 10 ng of analyte residue on surface in the presence of fingerprints (n = 5 participants). The percentage recovery for isoniazid and acetylisoniazid at each level was calculated based on the original spiked concentration (see Table 5.4). The results indicate that similar percentage recoveries (58 – 61%) were obtained for isoniazid at 1 and 5 ng in fingerprint samples (natural and after handwashing). In contrast, a slightly higher value (64 – 70%) is observed at 10 ng (natural and after handwashing). This can be a result of the sample matrix effects observed due to ionisation suppression which can be concentration dependent and therefore relates to the sample matrix-to-analyte ratio. For acetylisoniazid, the presence of a fingerprint (natural and after handwashing) resulted in very high recoveries (139 – 186%). This was a result of sample matrix effects (in the form of ionisation suppression) which were not corrected for acetylisoniazid using isoniazid-d₄ as the internal standard due to a difference in the degree of ionisation suppression.

There are a number of limitations, including the fingerprint variability (volume and size), matrix effects (ionisation suppression) and lower percentage recoveries, which will influence the fingerprint results obtained in this study. The difference in the levels of isoniazid and acetylisoniazid in the presence of a fingerprint sample compared to a standard of the same concentration highlights it is not possible to accurately determine the percentage recoveries of the analytes of interest in fingerprint samples or carry out quantitation using the current method.

Table 5.4: Percentage recovery range for isoniazid and acetylisoniazid for (n = 5 measurements) spiked fingerprints (natural and after washing hands with soap).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Percentage recovery (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fingertips (natural)</td>
<td>Fingertips (soap)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoniazid</td>
<td>Acetylisoniazid</td>
<td>Isoniazid</td>
<td>Acetylisoniazid</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>10 ng/ml</td>
<td>58</td>
<td>147</td>
<td>61</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>50 ng/ml</td>
<td>56</td>
<td>148</td>
<td>59</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>64</td>
<td>186</td>
<td>70</td>
<td>181</td>
<td></td>
</tr>
</tbody>
</table>
5.2.4 Method performance

A validation study was carried out for qualitative confirmation/identification measurements according to the Scientific Working Group for Forensic Toxicology guidelines to evaluate the analytical performance of the developed method. A set of experiments were performed to determine the reliability of the method for its intended use and evaluate the limitations of the method, including the working range, sensitivity (limit of detection) and sample stability. Definitions of terms used are detailed in Chapter 2, section 2.3.7.1.

5.2.4.1 Working range

A linear calibration curve for isoniazid and acetylisoniazid in standard solution was prepared with a concentration range of 50 – 1000 ng/ml in methanol. Each calibration standard was prepared from a stock solution (at 5000 ng/ml in methanol). The stock solution was prepared using isoniazid and acetylisoniazid. Calibration standards were prepared at 50, 100, 200, 400, 600, 800 and 1000 ng/ml in methanol by appropriate dilution of the stock solution. Ten microlitres of the calibration standard was added to the paper substrate (Whatman 1-Chr grade paper, 2 x 2 cm) to produce 0.5, 1, 2, 4, 6, 8, and 10 ng of analyte residue on surface. The samples were subsequently extracted using the method described in section 5.2.1. Each calibration standard was measured five times over five consecutive days. A blank mobile phase injection was performed after each calibration standard in triplicate to evaluate the potential for carryover at each concentration level. No carryover was observed at any calibration level for isoniazid, acetylisoniazid or the internal standard (isoniazid-d4). An overview of the calibration curve data for isoniazid and acetylisoniazid over five separate runs is shown in Appendix D.1. The results suggest that the analytes of interest are not stable in the standard solution (50:50 (v/v) ACN in H2O + 0.1% formic acid) as the linearity (R2) over the concentration range is decreased over five consecutive days, particularly for acetylisoniazid. This was confirmed by the analysis of freshly extracted calibration standards where linear calibration curves were obtained over n = 5 days (Appendix D.2).

Additionally, the precision of the method for the retention time and peak area ratio A/IS, the relative standard deviation (%RSD) was calculated for the repeated measurements for each standard of the calibration curve. The method provided good RSD values of 0 – 3% for the repeated measurements (peak area ratio A/IS) in a single run (n = 5), demonstrating that the method has a satisfactory level of repeatability. In contrast, poor peak area ratio A/IS reproducibility was observed due to the stability of isoniazid and acetylisoniazid in 50:50
(v/v) ACN in H₂O + 0.1% formic acid. The reproducibility of freshly prepared calibration curves over \( n = 5 \) days produced RSD values of 1 – 9% for isoniazid and acetylisoniazid, with an exception for isoniazid for 5 ng/ml at 19%. The correlation coefficients \( (R^2) \) were calculated with \( R^2 > 0.998 \). Standards were therefore prepared daily for each run.

5.2.4.2 Limit of detection

The sensitivity of isoniazid and acetylisoniazid was determined using the method outlined in section 2.3.7.3. Six standards were prepared to produce 10, 20, 40, 60, 80 and 100 pg of analyte residue on surface, including a blank sample substrate and blank solvent (methanol). The limit of detection was determined to be the concentration at which reproducible and reliable measurements were obtained for the lowest analyte concentration (Armbruster et al., 1994). The acceptance criteria were based on the retention time within ±2% (for \( n = 5 \) measurements) and repeatability of the measurement ≤20% (Armbruster et al., 1994). Figure 5.10 shows the trend of the standards extracted from paper used to evaluate the limit of detection. For isoniazid and acetylisoniazid, the minimal detectable limit was 10 pg of extracted analyte on paper (ratio A/IS 0.003 for both isoniazid and acetylisoniazid).

![Figure 5.10](image-url)

**Figure 5.10:** Evaluation of the limit of detection for isoniazid and acetylisoniazid by analysing progressively more dilute standards using extracted analyte standards in methanol. Data represented as the average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, \( n = 5 \) measurements) with error bars indicating the standard deviation.
5.2.4.3 Sample stability – processed sample

The stability of the analytes in standard solution was evaluated over \( n = 5 \) days. Analyte standards (containing isoniazid and acetylisoniazid) in methanol were prepared at 50, 100, 200, 400, 600, 800 and 1000 ng/ml. Ten microlitres of analyte standard (in methanol) was spiked onto paper (2 x 2 cm) to produce 0.5, 1, 2, 4, 6, 8, and 10 ng of analyte residue on surface. The samples were extracted using the method described in section 5.2.1. The sample stability of isoniazid and acetylisoniazid in standard solution (50% (v/v) ACN in H\(_2\)O with 0.1% formic acid) was evaluated. Each standard was measured \( n = 5 \) times to provide an average peak area ratio \( A/IS \). The samples at each concentration level were analysed after sample preparation to establish the time zero response (run 1). Each subsequent analysis of the standards was performed in 24 hours increments; run 1 = 0 h, run 2 = 24 h, run 3 = 48 h; run 4 = 72 h and run 5 = 96 h. Figure 5.11 shows the level of stability for isoniazid and acetylisoniazid at each concentration level. The results show an increase in the ratio \( A/IS \) over the five consecutive days. This is because the average peak area (\( n = 5 \) replicate measurements) for all analytes decreased, but more rapidly for isoniazid-d4, resulting in a higher ratio \( A/IS \) over time. Therefore, analytes were only considered to be stable during the first run (10 h). Samples were prepared daily to prevent degradation.

5.2.5 Patients on treatment

A total of 27 participants (10 female and 17 male) were recruited who were prescribed isoniazid as part of their treatment for tuberculosis. The dose prescribed is dependent on the weight of the patient (5 mg/kg). However, the maximum dose of isoniazid prescribed is 300
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mg. The medication is to be taken daily on an empty stomach before breakfast. All participants admitted having taken their tablets on the day of fingerprint sample collection. Information about each participant including age, gender and dose of medication are detailed in Table 5.5.

Table 5.5: Participant information, including gender (F = female, M = male), age (years) and dose of medication, for each patient on treatment.

<table>
<thead>
<tr>
<th>Participant number</th>
<th>Gender</th>
<th>Age</th>
<th>Dose of isoniazid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-2017-FP006</td>
<td>M</td>
<td>23</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP011</td>
<td>F</td>
<td>23</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP013</td>
<td>M</td>
<td>58</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP014</td>
<td>F</td>
<td>37</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP015</td>
<td>M</td>
<td>24</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP016</td>
<td>F</td>
<td>77</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP017</td>
<td>M</td>
<td>27</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP018</td>
<td>F</td>
<td>49</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP019</td>
<td>F</td>
<td>50</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP030</td>
<td>M</td>
<td>49</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP031</td>
<td>M</td>
<td>36</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP032</td>
<td>M</td>
<td>38</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP033</td>
<td>M</td>
<td>50</td>
<td>900</td>
</tr>
<tr>
<td>TB-2017-FP034</td>
<td>F</td>
<td>21</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP035</td>
<td>M</td>
<td>39</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP036</td>
<td>M</td>
<td>61</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP037</td>
<td>M</td>
<td>37</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP038</td>
<td>M</td>
<td>29</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP039</td>
<td>M</td>
<td>87</td>
<td>250</td>
</tr>
<tr>
<td>TB-2017-FP040</td>
<td>F</td>
<td>25</td>
<td>250</td>
</tr>
<tr>
<td>TB-2017-FP042</td>
<td>F</td>
<td>33</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP043</td>
<td>M</td>
<td>40</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP044</td>
<td>M</td>
<td>41</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP045</td>
<td>F</td>
<td>43</td>
<td>250</td>
</tr>
<tr>
<td>TB-2017-FP046</td>
<td>M</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td>TB-2017-FP047</td>
<td>M</td>
<td>56</td>
<td>300</td>
</tr>
<tr>
<td>TB-2017-FP048</td>
<td>F</td>
<td>45</td>
<td>300</td>
</tr>
</tbody>
</table>

Figure 5.12 shows the fingerprint results obtained for all participants on treatment. From 135 natural fingerprint samples collected, isoniazid and acetylisoniazid were detected in 102 (76%) and 125 (93%) samples, respectively. The majority of fingerprint samples analysed were positive for isoniazid and acetylisoniazid. However, in some cases only one of the analytes of interests was detected. Nineteen percent (26 samples) of fingerprint samples collected was positive for the metabolite acetylisoniazid only. In contrast, two samples (1%) were positive for isoniazid only. Additionally, 6% (eight samples) was negative for isoniazid and acetylisoniazid.
Figure 5.12: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for isoniazid and acetylisoniazid in natural fingerprints from (n = 27) participants on treatment for tuberculosis. Solid line represents limit of detection (LOD).

The lack of detection for either isoniazid or acetylisoniazid in fingerprint samples was only observed for three participants, namely TB-2017-FP030 and TB-2017-FP-037 and TB-2017-FP045. For participant TB-2017-FP030, none of the analytes of interest were detected in the right thumb, ring and little finger in samples collected as natural fingerprints. The remaining fingerprint samples (index and middle finger) were positive for acetylisoniazid only. Additionally, all fingerprint samples collected from participant TB-2017-FP037 were negative for isoniazid and acetylisoniazid, with the exception of the right index finger which was positive for isoniazid only. For participant TB-2017-FP045, isoniazid and acetylisoniazid were not detected in the right thumb. However, the right index and middle finger were positive for acetylisoniazid and the remaining fingerprint samples were positive for both analytes. The discrepancies observed between the fingerprint samples from the same participant highlights the difficulty associated with the use of fingerprints to monitor compliance. It is possible that the levels of isoniazid and acetylisoniazid were below the limit of detection and therefore a negative fingerprint result was obtained. This could be either due to a low concentration of analytes or a poor fingerprint deposition. However, to monitor whether a patient is compliant with their treatment, the presence of either the antibiotic (isoniazid) or the metabolite acetylisoniazid would be significant. Overall, based on this principle 94% (127 samples) of all fingerprints was positive (see Table 5.6). The findings show that fingerprint samples could potentially be used to monitor compliance with tuberculosis treatment. However, in order to understand the discrepancy in results obtained,
a corresponding blood sample would be required. Additionally, it is important to understand the discrepancy in fingerprint samples from the same participant.

Table 5.6: Detection rate (percentage) for isoniazid and acetylisoniazid in natural fingerprint samples from tuberculosis patients on treatment.

<table>
<thead>
<tr>
<th>Fingerprint sample</th>
<th>Isoniazid</th>
<th>Acetylisoniazid</th>
<th>Isoniazid or acetylisoniazid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right thumb (n = 27)</td>
<td>74</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Right index finger (n = 27)</td>
<td>74</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>Right middle finger (n = 27)</td>
<td>74</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Right ring finger (n = 27)</td>
<td>81</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Right little finger (n = 27)</td>
<td>74</td>
<td>89</td>
<td>93</td>
</tr>
<tr>
<td>Based on all fingerprints (n = 135)</td>
<td>76</td>
<td>93</td>
<td>94</td>
</tr>
</tbody>
</table>

5.2.6 Patients who completed treatment

A total of 6 participants (3 female and 3 male) were recruited who completed their tuberculosis treatment. The participants were recruited between 2 – 8 months after completion of their treatment. Information about each participant, including age, gender, dose of medication, treatment completion date and sample collection date are detailed in Table 5.7.

Table 5.7: Participant information, including gender (F = female, M = male), age (years) and dose of medication for each patient on tuberculosis treatment.

<table>
<thead>
<tr>
<th>Participant number</th>
<th>Gender</th>
<th>Age</th>
<th>Dose of isoniazid (mg)</th>
<th>Treatment completion date (2017)</th>
<th>Sample collection date (2017)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-2017-FP001</td>
<td>F</td>
<td>32</td>
<td>300</td>
<td>1 February</td>
<td>26 April</td>
</tr>
<tr>
<td>TB-2017-FP004</td>
<td>M</td>
<td>49</td>
<td>300</td>
<td>1 Jan</td>
<td>26 April</td>
</tr>
<tr>
<td>TB-2017-FP005</td>
<td>M</td>
<td>41</td>
<td>300</td>
<td>1 March</td>
<td>26 April</td>
</tr>
<tr>
<td>TB-2017-FP009</td>
<td>F</td>
<td>59</td>
<td>300</td>
<td>1 February</td>
<td>1 May</td>
</tr>
<tr>
<td>TB-2017-FP012</td>
<td>F</td>
<td>31</td>
<td>300</td>
<td>1 May</td>
<td>1 June</td>
</tr>
<tr>
<td>TB-2017-FP041</td>
<td>M</td>
<td>21</td>
<td>300</td>
<td>1 March</td>
<td>1 November</td>
</tr>
</tbody>
</table>

The fingerprint results obtained for these participants is shown in Figure 5.13. The results show that isoniazid is not detected in the fingerprint samples for participants who have completed treatment. Similarly, for acetylisoniazid, no fingerprint sample showed acetylisoniazid levels above the limit of detection, with the exception of participant TB-2017-FP009, which showed detectable levels of acetylisoniazid in all fingerprint samples. However, the levels of acetylisoniazid observed for this participant are lower compared to those observed for participants on treatment. Additionally, the levels observed for this
participant have a greater uncertainty (standard deviation) as the values are just above the limit of detection (ratio A/IS 0.003) for acetylisoniazid (see section 2.3.7.3).

Figure 5.13: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for isoniazid and acetylisoniazid in natural fingerprint samples collected from (n = 6) participants who have completed treatment for tuberculosis. Solid line represents limit of detection (LOD).

5.2.7 Negative control group

Ten participants (5 male and 5 female) were recruited as a negative control group to evaluate whether the analytes of interest or interfering compounds could be detected in natural fingerprint samples. Information about participants in the negative control group (including age and gender) are detailed in Table 5.8.

Table 5.8: Participant information, including gender (F = female, M = male) and age (years) for the negative control group.

<table>
<thead>
<tr>
<th>Participant number</th>
<th>Gender</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-2017-N001</td>
<td>F</td>
<td>25</td>
</tr>
<tr>
<td>TB-2017-N002</td>
<td>F</td>
<td>24</td>
</tr>
<tr>
<td>TB-2017-N003</td>
<td>M</td>
<td>23</td>
</tr>
<tr>
<td>TB-2017-N004</td>
<td>M</td>
<td>30</td>
</tr>
<tr>
<td>TB-2017-N005</td>
<td>M</td>
<td>24</td>
</tr>
<tr>
<td>TB-2017-N006</td>
<td>F</td>
<td>24</td>
</tr>
<tr>
<td>TB-2017-N007</td>
<td>M</td>
<td>21</td>
</tr>
<tr>
<td>TB-2017-N008</td>
<td>M</td>
<td>26</td>
</tr>
<tr>
<td>TB-2017-N009</td>
<td>F</td>
<td>23</td>
</tr>
<tr>
<td>TB-2017-N010</td>
<td>F</td>
<td>26</td>
</tr>
</tbody>
</table>

Isoniazid was detected in one fingerprint sample (TB-2017-N001, right thumb), resulting in a 2% detection rate (see Figure 5.14). In contrast, the metabolite acetylisoniazid was not detected in any fingerprint sample from the negative control group. The results demonstrate
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that (i) these analytes of interests are not detected in fingerprint samples from individuals who have not received any tuberculosis medication and (ii) “no interfering compounds” were detected at the same retention time with the same mass as the analytes of interest. Although a limited sample size was evaluated in this research, the results suggest that the detection of the isoniazid and acetylisoniazid in fingerprints is significant and arises from administration of the prescribed medication.

Figure 5.14: Fingerprint results average peak area ratio analyte (A) to internal standard (IS) (± standard deviation n = 5 measurements) for isoniazid and acetylisoniazid in natural fingerprint samples collected from (n = 10) participants from the negative control group. Solid line represents limit of detection (LOD).

5.2.8 Effect of washing hands

The sampling strategy for the analysis of isoniazid and acetylisoniazid in fingerprints was evaluated by washing hands with soap and water. This was to explore whether the analytes of interest could still be detected after an individual has washed their hands prior to sample collection.

5.2.8.1 Patients on treatment

Twenty-seven participants (10 female and 17 male) were recruited who were prescribed isoniazid as part of their treatment for tuberculosis. Two sets of fingerprint samples were collected for these participants (i) without preparation of the hands and (ii) after washing hands with soap and water. Information about each participant including age, gender and the dose of medication are detailed in Table 5.5.
Chapter 5: Monitoring Compliance with Tuberculosis Medication from a Fingerprint

Figure 5.15 shows the fingerprint results obtained for isoniazid and acetylisoniazid for each participant. After washing hands with soap and water, isoniazid was detected in 83 samples (61%). Additionally, the metabolite acetylisoniazid was detected in 117 samples (87%). Compared to the results obtained in section 5.2.5, the detection rate for both analytes has decreased (from 76 to 61% for isoniazid and from 93 to 87% for acetylisoniazid) by using a handwashing procedure prior to sample collection.

![Fingerprint results](image)

Figure 5.15: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for isoniazid and acetylisoniazid in samples collected after washing hands with soap and water for (n = 27) participants on treatment for tuberculosis.

A discrepancy was observed for the presence of the analytes of interest in fingerprint samples for 5 participants (TB-2017-FP019, TB-2017-FP035, TB-2017-FP037, TB-2017-FP042 and TB-2017-FP043). For participant TB-2017-FP019, all fingerprint samples were positive for acetylisoniazid only, with the exception of the right middle finger which was negative for both isoniazid and acetylisoniazid. Fingerprint samples for participant TB-2017-FP035 showed that acetylisoniazid was detected in the right middle, ring and little finger, whereas the right thumb and index finger were negative for both isoniazid and acetylisoniazid. For participant TB-2017-FP037, all fingerprint samples were negative for isoniazid and acetylisoniazid, which is surprising. However, natural fingerprint samples collected for this participant showed that only one fingerprint sample (right index finger) was only positive for isoniazid. The remaining fingerprint samples were negative for both analytes of interest. This questions whether this participant is compliant with their treatment, or if the method is not sensitive enough to detect these low levels of isoniazid and acetylisoniazid. Results obtained for participant TB-2017-FP042 showed that the right index
and little finger were positive for isoniazid only, whereas the right thumb and ring finger were positive for both isoniazid and acetylisoniazid. However, both isoniazid and acetylisoniazid were not detected in the right middle finger collected for this participant. Additionally, for participant TB-2017-FP043 all fingerprint samples were positive for acetylisoniazid only, except for the right middle finger.

The results show that although the majority of fingerprint samples are positive for tuberculosis medication, a discrepancy is observed between fingerprint samples for a number of participants. Collection of multiple fingerprint samples would be advantageous to confirm the presence of the analytes of interest. Additionally, based on the detection of either isoniazid or acetylisoniazid, 120 samples (89%) were positive for the presence of tuberculosis medication after handwashing (right thumb 89%; right index finger 89%; right middle finger 81%; right ring finger 93%; right little finger 93%).

5.2.8.2 Patients who completed treatment

Six participants were recruited within 2 – 8 months of completing their treatment for tuberculosis. Information about the participants, including age, gender, dose of medication, treatment completion date and sample collection date are outlined in Table 5.7. The fingerprint results obtained for these participants showed that isoniazid was not detected in any fingerprint sample for the 6 participants. In contrast, acetylisoniazid was present in 17% (5 samples) (see Figure 5.16).

![Figure 5.16: Fingerprint results (average peak area ratio analyte (A) to internal standard (IS), ± standard deviation n = 5 measurements) for isoniazid and acetylisoniazid in fingerprint samples collected after washing hands with soap and water for (n = 6) participants who have completed treatment for tuberculosis. Solid line represents limit of detection (LOD).](image)
This is a result of participant TB-2017-FP009, as all fingerprint samples were positive for acetylisoniazid, consistent with the results obtained for natural fingerprints. However, the lack of detection of isoniazid and acetylisoniazid in fingerprint samples for participants who have completed treatment at different time periods shows that the compounds are not present in the system long after completion of treatment. This is important as a long detection window would not be beneficial in monitoring compliance with tuberculosis treatment if an individual has not taken their medication for a couple of days or weeks.

5.2.8.3 Negative control group

A total of 10 participants (5 male and 5 female) were recruited as negative control subjects to evaluate whether the analytes of interest or interfering compounds could be detected in fingerprint samples collected after handwashing. Information about participants in the negative control group, including age and gender, are detailed in Table 5.8. The results show that both isoniazid and acetylisoniazid are not detected in fingerprint samples collected from participants who have never received tuberculosis medication. Additionally, no interfering compounds were present at the same mass and retention times of the analytes of interest. This shows that the detection of isoniazid and acetylisoniazid is significant as these analytes are not commonly observed in fingerprint samples. Although a 2% false positive rate was observed for natural fingerprints as 1 sample was positive for isoniazid (TB-2017-N001, right thumb), this signal was not observed after washing hands with soap and water.

5.2.9 Elimination profile

One participant was recruited who was completing their tuberculosis treatment to evaluate the drug elimination of isoniazid and acetylisoniazid in fingerprint samples. It is important to illustrate that if an individual is not compliant with their medication, the fingerprint sample is able to confirm this. However, in order to confirm non-compliance, the drug elimination of the analytes of interest in fingerprints must be short (e.g. 1 day). Otherwise the sample is not able to determine when an individual is not complying with their treatment based on a qualitative fingerprint test.

Fingerprint samples were collected from participant TB-2017-E001 on three occasions: (i) on the last day of their treatment (T = 0), (ii) two days after completing treatment (T = 2) and (iii) four days after completing treatment (T = 4). Fingerprint samples (n = 5) were collected natural and after washing hands with soap and water. The results showed that on the last day of treatment (T = 0) both isoniazid and acetylisoniazid were detected in all
natural fingerprint samples and after handwashing (Figure 5.17). However, isoniazid and acetylisoniazid were not present in any natural fingerprint samples and after handwashing on T = 2 and T = 4 days. The drug elimination profile for isoniazid and acetylisoniazid in fingerprint samples for TB-2017-E001 is shown in Figure 5.17.

As isoniazid and acetylisoniazid were not detected in fingerprint samples collected 2 and 4 days after completing treatment, the results indicate that the elimination of the antibiotic is short (<2 days). This demonstrates that when an individual is not complying with their treatment, the fingerprint results can confirm no analytes are present two days afterwards. However, a limitation of this experiment is that only participant was recruited. A larger group of individuals would have to be evaluated to determine the drug elimination profile of isoniazid and acetylisoniazid in fingerprint samples to confirm these preliminary findings, including various time periods to capture when the analytes of interest cannot be detected anymore.

Figure 5.17: Drug elimination profile of isoniazid and acetylisoniazid in natural fingerprints and after washing hands with soap and water for participant TB-2017-E001. Solid line represents the limit of detection (LOD).
5.3 Summary

The extraction of isoniazid and acetylisoniazid from paper was performed using 10% dichloromethane in methanol (section 5.1.5). Matrix effects were observed in the form of ionisation suppression for isoniazid and acetylisoniazid. However, the degree of variability in ionisation suppression was minimised with the use of an internal standard (isoniazid-d4) (section 5.2.2). The recovery values based on spiked fingerprints (natural and after washing hands) were 58 – 70% for isoniazid and 139 – 186% for acetylisoniazid (section 5.2.3). A linear response was obtained for isoniazid and acetylisoniazid (0.5 – 10 ng of analyte residue on surface) with correlation coefficients ($R^2 > 0.998$ (section 5.2.4.1). Peak area ratio $A/IS$ demonstrated good reproducibility 1 – 9% for isoniazid and acetylisoniazid, with an exception for isoniazid at 5 ng/ml (19%) (section 5.2.4.1). The limit of detection for isoniazid and acetylisoniazid was at 10 pg of analyte residue on surface (section 5.2.4.2). Additionally, the analytes of interest were stable in solution over 10 hours (section 5.2.4.3).

Isoniazid (76%) and acetylisoniazid (93%) can be detected in natural fingerprints from patients receiving tuberculosis treatment, even after a handwashing procedure (section 5.2.5). However, a lower detection rate was observed after washing hands (61% for isoniazid and 81% for acetylisoniazid) (section 5.2.8.1). These analytes are not detected in fingerprints from a negative control group, if samples are collected after washing hands (one sample was positive in natural fingerprints) (section 5.2.8.3). Additionally, isoniazid was not present in samples collected from patients who completed their treatment (2 – 8 months prior to recruitment). In contrast, acetylisoniazid was detected in 17% (five samples, all arising from the same participant) even after washing hands (section 5.2.8.2). However, the observed acetylisoniazid levels were lower compared to patients currently on treatment. This has shown that fingerprints could potentially be used to monitor compliance with tuberculosis treatment based on the presence of isoniazid and acetylisoniazid in fingerprint samples.

In addition, samples collected from a patient coming off treatment showed that isoniazid and acetylisoniazid were not detected in fingerprint samples collected two and four days after completing treatment. This suggests that there is a potentially short detection window for the analytes of interest, which is advantageous for monitoring compliance with tuberculosis treatment. However, further work is required to establish the detection window of the analytes of interest in fingerprints.
Chapter 6  Detection of Cocaine and Benzoylecgonine in Bodily Fluids using a Portable Mass Spectrometer

6.0  Introduction

One of the aims of this research was to evaluate the use of a portable screening method for the analysis of drugs of abuse in biological matrices, with an initial aim of developing a portable method for detecting cocaine in fingerprints. It was quickly established that a fingerprint sample proved too challenging for this novel detection set up. Additionally, the results in Chapter 3 (drug administration) and Chapter 4 (drug contact) highlighted the challenges associated with the use of fingerprints for drug testing. The unknown sample volume of fingerprints impacts the potential for quantification and the limited sample volume requires high sensitivity instrumentation. This pilot research study was therefore conducted to evaluate a portable screening system for drug testing using more conventional and established sample matrices, urine and oral fluid (saliva). The main advantage of a portable mass spectrometer system is the ability to analyse samples on-site (point-of-care). This in combination with a surface analysis technique that requires little to no sample preparation will allow rapid high throughput screening of samples. In addition, the rapid assessment of samples on-site will allow for subsequent samples to be collected and sent off to a laboratory for confirmation (e.g. workplace drug testing).

The potential use of portable mass spectrometers for on-site analysis of drugs has been investigated previously (O'Leary et al., 2015; Li et al., 2014). However, these methods rely on the use of ambient ionisation methods. The main disadvantage for ambient ionisation methods is selectivity. This is due to the need for a high resolution mass spectrometer to confidently assign peaks in a mass spectrum. Additionally, the lack of chromatographic separation of compounds prior to mass analysis makes it more vulnerable to ion suppression making quantitative profiling difficult. Ion suppression can be problematic in experiments where the ionisation of the analyte of interest is affected by the presence of other compounds, thereby limiting the sensitivity (Annesley, 2003).

This chapter explores the possibility of decoupling extraction from ionisation in a portable system, to reduce ion suppression effects and thereby improve sensitivity and selectivity. This enables the advantages of using a portable system for on-site analysis.
For the analysis of drugs of abuse in biological matrices (e.g. urine and oral fluid), a key application is the use of cut-off levels to determine a screening result as either positive or negative. There are two types of cut-off levels, screening and confirmation cut-off levels (Substance Abuse and Mental Health Services Administration, 2015; Substance Abuse and Mental Health Services Administration, 2008). A screening cut-off level is used to provide an initial presumptive result which qualitatively assesses the presence of a drug. A negative screening result does not always indicate that the sample does not contain any drugs, as it might contain drug levels below the cut-off used. Screening tests are usually based on immunoassay screening using antibodies as these are rapid and inexpensive to conduct. However, the main disadvantage of the use of antibodies is that they can bind to other substances and therefore cause false positive results. A screening result is therefore followed up with a confirmation test which is based on mass spectrometry. Confirmation tests are more selective and sensitive as they only monitor a few compounds compared to the initial screening of multiple drugs and drug metabolites using immunoassays.

For the purpose of this pilot research study, only a small sample size (n = 2 participants) was used to assess the potential suitability of the method for drug screening as the instrumentation was available for a limited time period. As a result, the possibility for method optimisation and development was therefore further restricted. Urine and oral fluid samples were collected from individuals attending an NHS drug and alcohol service for drug dependency. Informed consent was taken from participants prior to sample collection. Analysis of cocaine and benzoylecgonine in urine and oral fluid was carried out by a Plate Express™ system coupled to an expression Compact Mass Spectrometer (CMS) (see section 6.1.4). The following section will outline the sample collection and preparation methods for the study populations investigated (see section 6.1). All urine and oral fluid samples collected were stored (at 4 ºC) at the University of Surrey until required for analysis. All instrumentation and methodology used for the analysis of urine and oral fluid samples are detailed in section 6.1.5. The analysis of cocaine and benzoylecgonine in urine and oral fluid for both study populations (negative controls and drug dependent participants) are outlined in sections 6.2.1 and 6.2.2.

This chapter investigates the possibility of using a portable mass spectrometer set up (in combination with chromatography) for the purpose of drug testing from oral fluid and urine, in accordance with recognised workplace drug testing guidelines. For this study, the portable
system was evaluated in one location to demonstrate the potential to detect cocaine use. However, in order to demonstrate a point-of-care system, multiple locations would need to be tested. The system evaluated in this research can be placed in the back of the van for portability and on-site analysis.

6.1 Experimental

6.1.1 Chemicals and reagents
Quantisal™ collection devices were used for oral fluid samples (Alere Toxicology, UK). Sterilin™ polystyrene containers were used for the collection of urine samples (Scientific Laboratory Supplies, Nottingham, UK). Certified reference materials (CRM) of cocaine and benzoylecgonine were used to prepare drug standards (Sigma Aldrich, Dorset, UK). CRM of cocaine-d₃ was used as an internal standard (Sigma Aldrich, Dorset, UK). Optima grade LC-MS solvents of acetonitrile and water were used to prepare solutions and solvent mixtures (Fisher Scientific, Leicestershire, UK). Formic acid was added to the mobile phase solvents at 0.1% (v/v) (Fischer Scientific, Leicestershire, UK). Scintillation vials for sample preparation were purchased from Fisher Scientific, Loughborough, UK.

6.1.2 Study populations
Study participants from two populations (drug users and non-drug users as negative control group) volunteered to provide samples for the purpose of this pilot research project in 2015. Negative control samples were collected from non-drug users (n = 10) at the University of Surrey. Samples from cocaine users (n = 2 participants) were collected at an NHS drug and alcohol service, to demonstrate the application of the method. Informed consent was obtained from participants prior to sample collection. The samples collected for the purpose of this research project included oral fluid and urine.

6.1.3 Sample collection and sample preparation
6.1.3.1 Oral fluid
Oral fluid samples were collected from non-drug users (n = 5 females and n = 5 males) at the University of Surrey. Oral fluid samples (1 ml per participant) were collected using a syringe. The samples were used to prepare blank (negative control) and spiked standards for cocaine and benzoylecgonine. Oral fluid samples from drug users were collected using Quantisal™ oral fluid collection devices at an NHS drug and alcohol service (as described
the SOP in Appendix B. 1). The oral fluid samples were given a unique identifier (e.g. 04831605) to enable storage (at 4 °C) of samples in anonymised form at the University of Surrey. Both participants 04831605 and 04857599 admitted having taken cocaine in the past 24 h.

Oral fluid samples collected from the negative control group (n = 5 males and 5 females) were pooled and used to prepare blank and spiked drug standards of cocaine and benzoylecgonine to provide a calibration concentration range (see section 6.1.7). Oral fluid samples were prepared in two steps prior to analysis, which involved the preparation of spiked oral fluid drug standards and absorption of the spiked oral fluid standards using Quantisal™ oral fluid collection devices. A 1 µg/ml stock solution of cocaine and benzoylecgonine was prepared by dilution of the certified reference materials (CRM) of cocaine and benzoylecgonine (at 1 mg/ml in acetonitrile and methanol, respectively) in water. The pooled oral fluid sample was used to prepare spiked drug standards over the range 0 – 600 ng/ml by appropriate dilution of the stock solution. The spiked oral fluid samples were then placed in separate scintillation vials for absorption using Quantisal™ oral fluid collection devices. Once the volume indicator (1 ml ± 10%) on the Quantisal™ oral fluid collection device turned blue, the collection wand was placed in the transfer tube. The Quantisal™ oral fluid collection devices contain a stabilising buffer to stabilise drugs during transport and storage. This method was used to demonstrate the analytical performance of the detection of cocaine and benzoylecgonine in oral fluid using commercially available collection devices.

6.1.3.2 Urine

Urine samples were collected from non-drug users (n = 3 males) at the University of Surrey. Samples (60 ml) were collected in Sterilin™ polystyrene containers. Urine samples (60 ml) from participants (n = 2 males) at an NHS drug and alcohol service were also collected using Sterilin™ polystyrene containers. The urine samples were given the same unique identifier (e.g. 04831605) as the corresponding oral fluid sample to enable corroboration of samples. Samples were stored at 4 °C at the University of Surrey until required for analysis using the portable mass spectrometer set up.

Urine samples collected from non-drug users (n = 3 males) were pooled and used to prepare a blank negative control and spiked urine drug standards (see section 6.1.7). A 1 µg/ml stock solution of cocaine and benzoylecgonine was prepared in pooled urine by dilution of CRM
standards of cocaine (1 mg/ml) and benzoylecgonine (1 mg/ml). Spiked drug standards were prepared in urine by appropriate dilution of the stock solution over the range 0 – 600 ng/ml. For the analysis of oral fluid and urine samples collected from individuals at NHS drug and alcohol services, samples were analysed without sample preparation. Urine samples were collected in Sterilin™ polystyrene containers and no additive was added to stabilise the drugs up to the point of analysis to maintain the integrity of the sample.

6.1.4 Plate Express™ - Compact Mass Spectrometer (CMS)
The purpose of using chromatography in combination with a portable single quadrupole mass spectrometer is to enhance the selectivity of the portable system by separating the analytes prior to mass analysis in the detector. This is necessary as portable mass spectrometers lack the resolving power and sensitivity compared to laboratory-based instruments. Qualitative analysis was conducted by the use of a Plate Express™ system coupled to an expression compact mass spectrometer (CMS) by a chromatography column. The CMS is a portable single quadrupole mass analyser that was introduced in 2012, followed by the later release of the Plate Express™ system in 2015 (Advion Inc. Ithaca, USA). The instrument was designed to provide rapid information and increase the workflow of chemists by the identification of compounds and monitoring the progress of a reaction. However, for the purposes of this research, the system has been evaluated for the identification of drugs of abuse (cocaine and its metabolite benzoylecgonine) in bodily fluids, including oral fluid and urine.

The following sections outline the principal components present in the portable mass spectrometer system used, including the surface extraction using the Plate Express™ for sample introduction, chromatography method for the separation of analytes and mass analysis using a single quadrupole mass spectrometer.

6.1.4.1 Sample introduction
The Plate Express™ is designed for the extraction of analytes from a variety of different surfaces (such as glass, paper, aluminium foil and TLC plates). Sample introduction with the Plate Express™ system is facilitated by the use of an oval shaped elution head (4 x 2 mm, with a depth of 250 μm) under which the sample is placed. The Plate Express™ system is also equipped with a laser device to help target the correct sampling area for analysis. The system also features an automated head cleaning procedure with high pressure nitrogen gas, which is applied in between sample analysis to limit contamination and carryover. For
analysis the sample is loaded onto the sample substrate (e.g. Whatman 1-Chr paper) and placed under the elution head of the Plate Express™ system. Automated software is used to initiate sample extraction by driving the elution head down to make contact with the sample substrate. After contact with the sample substrate, a force is applied to seal the spot for extraction by compression of the spring inside the elution head. The elution head features an oval shaped knife edge which is used to seal against the sample substrate. The solvent from the binary pump is used to flush across the surface of the sample substrate to extract compounds. The knife edge is equipped with a frit to filter the extracted sample before introduction to the chromatography column for separation and subsequent mass analysis with the compact mass spectrometer.

6.1.4.2 Chromatography

An ultra-biphenyl column (50 x 2.1 mm, 5 µm) (Restek, Buckinghamshire, UK) was used for the separation of cocaine and benzoylecgonine in oral fluid and urine samples. A binary gradient method was applied to reduce run time and improve separation power. A binary pump was used to deliver gradient elution with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) (see section 6.1.5). After the extracted sample is introduced in the chromatography column, the analytes of interests are separated based on their affinity to the stationary phase. Ionisation and mass analysis of the separated compounds can then be accomplished allowing greater sensitivity using the compact mass spectrometer.

6.1.4.3 Plate Express™ - CMS Interface

An electrospray ionisation source was used as the interface between the chromatography column and the mass spectrometer. The mass spectrometer operates at high vacuum (10⁻⁶ mbar) to prevent collisions with air molecules, which can lead to fragmentation. Liquid chromatography is a high pressure technique, which operates at flow rates that would overwhelm the mass spectrometer (Harris, 2007). The main function of the interface is to remove most of the volatile solvent between the column and the mass spectrometer and to ionise the compounds of interest (McMaster, 2005). Further information on the principles of electrospray ionisation can be found in section 2.1.2.
6.1.4.4 Quadrupole mass spectrometer

A quadrupole mass spectrometer is a mass analyser consisting of four cylindrical or hyperbolic rods. The mass analyser is positioned between the ion optics and the detector and acts as a mass filter. A combination of a direct current (DC) and radio frequency (RF) voltage is applied to the rods to allow for the transmission of ions of a specific mass to the detector (Dunn, 2011). By varying the voltage applied with time, ions with a specific mass-to-charge ($m/z$) ratio will have a stable path to the detector. This makes it possible to scan across an entire mass range allowing multi-component analysis. Ion detection on the compact mass spectrometer is achieved by a dynode electron multiplier scanning every 2 $\mu$s. The stable ions that pass through the quadrupole mass analyser collide with the dynode and cause a cascade of secondary electrons to be released on contact. The amplified signal of the ion will finally reach the anode cup at the end of the detector where the current is measured (Harris, 2007).

6.1.5 Instrumentation and conditions

Analysis of all samples was carried out on a Plate Express™ system coupled to an expression compact mass spectrometer (Advion Inc., Ithaca, USA) at the University of Surrey (Guildford, Surrey, UK). Figure 6.1 shows a schematic diagram of the Plate Express™ system coupled to the compact mass spectrometer. Details of the specification of the compact mass spectrometer are shown in Table 6.1.

![Schematic diagram of Plate Express™ system coupled with compact mass spectrometer](schematic provided by Advion Inc.).

The Plate Express™ system was connected to a binary pump to allow gradient elution for the separation of cocaine and benzoylecgonine. Chromatographic separation was achieved by the adaptation of a previously developed method for the analysis of cocaine and benzoylecgonine on a Waters 2695 separation module and Micromass Quattro Ultima mass
spectrometer. The method was modified for the use on the Plate Express™ system as a lower flow rate was required to extract the sample beyond the Rheodyne valve (6 port injection valve), as this would otherwise lead to seal leakage.

Table 6.1: Specifications of compact mass spectrometer (CMS) used in this study.

<table>
<thead>
<tr>
<th>Type</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass range</td>
<td>10 – 2000 amu</td>
</tr>
<tr>
<td>Mass resolution</td>
<td>m/z 0.5 – 0.7 (at full width half maximum) at 1000 units sec⁻¹</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
</tr>
<tr>
<td>Expression CMS detector</td>
<td>32 kg</td>
</tr>
<tr>
<td>Rotary pump</td>
<td>30 kg</td>
</tr>
<tr>
<td>Dimensions</td>
<td></td>
</tr>
<tr>
<td>Expression CMS detector</td>
<td>66 x 28 x 56 cm</td>
</tr>
<tr>
<td>Rotary pump</td>
<td>26 x 23 x 46 cm</td>
</tr>
<tr>
<td>Power consumption</td>
<td></td>
</tr>
<tr>
<td>Expression CMS detector</td>
<td>300 VA maximum</td>
</tr>
<tr>
<td>Rotary pump</td>
<td>550 VA maximum</td>
</tr>
</tbody>
</table>

Table 6.2 shows the gradient used for the analysis of cocaine and benzoylecgonine on the Plate Express™ system using 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The sample extraction is initiated by the initial mobile phase composition used by passing the solution over the sample surface. After the sample extraction (120 s), the head disengages and the gradient elution is applied. Instrument optimisation was achieved by using a 1 µg/ml solution of cocaine and benzoylecgonine in 5% (v/v) acetonitrile in water.

Table 6.2: Binary gradient used for the separation of cocaine and benzoylecgonine on an Ultra biphenyl column (50 x 2.1 mm, 5 µm) on the Plate Express – CMS system.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (ml/min)</th>
<th>Solvent A (%)</th>
<th>Solvent B (%)</th>
<th>Gradient type</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>0.100</td>
<td>95.0</td>
<td>5.0</td>
<td>Step</td>
</tr>
<tr>
<td>2.00</td>
<td>0.100</td>
<td>95.0</td>
<td>5.0</td>
<td>Step</td>
</tr>
<tr>
<td>1.00</td>
<td>0.500</td>
<td>95.0</td>
<td>5.0</td>
<td>Linear</td>
</tr>
<tr>
<td>2.00</td>
<td>0.500</td>
<td>80.0</td>
<td>20.0</td>
<td>Linear</td>
</tr>
<tr>
<td>1.00</td>
<td>0.500</td>
<td>0.0</td>
<td>100.0</td>
<td>Step</td>
</tr>
<tr>
<td>1.00</td>
<td>0.500</td>
<td>95.0</td>
<td>5.0</td>
<td>Step</td>
</tr>
</tbody>
</table>

Table 6.3 details the optimised operating conditions used for mass spectral analysis of cocaine and benzoylecgonine on the Plate Express™ – CMS system. The source voltage and
source voltage dynamic were optimised to add selectivity and confirmation of the identification of compounds by producing mild in-source fragmentation of cocaine, benzoylecgonine and cocaine-d₃. Ten µl of standard solution (1 µg/ml in 5% (v/v) acetonitrile in water + 0.1% formic acid) was loaded onto the sample substrate (2 x 2 cm, Whatman 1-Chr paper) to check instrument response and mass fragmentation before each analysis run. Mass analysis of samples on the compact mass spectrometer was carried out in selected ion monitoring (SIM) mode. Using SIM mode increases the sensitivity of the mass spectrometer as more time is spent scanning for the compounds of interest. The mass spectrometer was set to only monitor a few \( m/z \) values for cocaine (\( m/z \) 304 and 182), benzoylecgonine (\( m/z \) 290 and \( m/z \) 168) and cocaine-d₃ (\( m/z \) 307 and \( m/z \) 171). Both the molecular ion and fragment ion were used to qualitatively confirm the presence of cocaine and benzoylecgonine.

Table 6.3: Operating conditions of Plate Express™ – CMS for the analysis of cocaine and benzoylecgonine in urine and oral fluid.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Operating condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample introduction</strong></td>
<td></td>
</tr>
<tr>
<td>Extraction time</td>
<td>120 s</td>
</tr>
<tr>
<td>Force</td>
<td>250 N</td>
</tr>
<tr>
<td><strong>Source parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>250 ºC</td>
</tr>
<tr>
<td>Capillary voltage</td>
<td>150 V</td>
</tr>
<tr>
<td>Source voltage</td>
<td>25 V</td>
</tr>
<tr>
<td>Source voltage</td>
<td>30 V</td>
</tr>
<tr>
<td>Gas temperature</td>
<td>200 ºC</td>
</tr>
<tr>
<td>Electrospray voltage</td>
<td>3.5 kV</td>
</tr>
<tr>
<td><strong>Detector settings</strong></td>
<td></td>
</tr>
<tr>
<td>Scan mode</td>
<td>Selected ion monitoring (SIM)</td>
</tr>
<tr>
<td>Dwell time</td>
<td>50 ms</td>
</tr>
<tr>
<td>Span</td>
<td>( m/z ) 0.3</td>
</tr>
</tbody>
</table>

Figure 6.2 shows an example of an extracted ion chromatogram of a spiked drug standard (at 200 ng/ml) in urine analysed with the Plate Express™ - CMS system. The respective retention times of cocaine and benzoylecgonine were 4.13 and 4.01 min. The chromatograms show the presence of the molecular ion as well as the fragment ion for cocaine (\( m/z \) 304 and 182) and benzoylecgonine (\( m/z \) 290 and 168) formed by the mild in-source fragmentation to confirm the presence of the analytes of interest.
Chapter 6: Detection of Cocaine and Benzoylecgonine in Bodily Fluids using a Portable Mass Spectrometer

6.1.6 Internal standard

A 1 µg/ml internal standard (IS) solution of cocaine-d₃ was used to plot the ratio of analyte (A) to internal standard (IS) against the concentration of spiked drug standard in urine and oral fluid. The IS solution was prepared by dilution of certified reference material of cocaine-d₃ (1 mg/ml in acetonitrile). For oral fluid standards, the IS solution was prepared in water (Fisher Scientific, LC-MS grade). The IS solution was spiked into the oral fluid drug standards to produce a final concentration of 150 ng/ml. The IS solution prepared in a pooled urine sample at 1 µg/ml was spiked into the urine drug standards to produce a final concentration of 100 ng/ml.

6.1.7 Method performance

The performance of the portable mass spectrometer was evaluated for qualitative confirmation/identification measurements according to the Scientific Working Group for Forensic Toxicology guidelines. A set of experiments were performed to determine the reliability of the method for its intended use and evaluate the limitations of the method, including the working range and sensitivity (limit of detection).

6.1.7.1 Linear working range

A concentration range of 0 – 600 ng/ml was selected to determine the linear working range of the method. All calibration curves were plotted as the concentration of the analyte, against the average ratio of the analyte (A) signal to the internal standard (IS) signal (n = 3). Calibration curves for both cocaine and benzoylecgonine were linear over the range 0 – 600 ng/ml.

Figure 6.2: Extracted ion chromatograms of (a) cocaine and (b) benzoylecgonine in a spiked urine standard (at 200 ng/ml containing 100 ng/ml internal standard cocaine-d₃) analysed using Plate Express™ - compact mass spectrometer (CMS) system.
ng/ml in both urine and oral fluid, and had $R^2$ values of at least 0.998 (see Figure 6.3 and Figure 6.4).

![Figure 6.3: Calibration curve of (a) cocaine ($m/z$ 182.1) and (b) benzoylecgonine ($m/z$ 168.1) in pooled urine containing 100 ng/ml internal standard (cocaine-d$_3$) analysed using Plate Express™ compact mass spectrometer (CMS) system.]

The precision of the method was evaluated by calculating the relative standard deviation (RSD) % of the repeated measurements ($n = 3$). The repeatability of the measurements was below 15%, except for benzoylecgonine in pooled urine at 50 ng/ml which provided an RSD value of 23%.

![Figure 6.4: Calibration curve of cocaine ($m/z$ 304.1) and benzoylecgonine ($m/z$ 290.1) in pooled oral fluid containing 150 ng/ml internal standard (cocaine-d$_3$) analysed using Plate Express™ compact mass spectrometer (CMS) system.]

### 6.1.7.2 Limit of detection and quantitation

The limit of detection is the concentration at which the instrument signal is significantly different from the blank or background signal (Miller & Miller, 2005). The limit of detection and quantitation of cocaine and benzoylecgonine were calculated based on Equations 6.1
Chapter 6: Detection of Cocaine and Benzoylecgonine in Bodily Fluids using a Portable Mass Spectrometer

and 6.2 for $n = 3$ replicate measurements of blank urine or oral fluid. Table 6.4 details the calculated LODs and LOQs for both analytes in urine and oral fluid.

$$\text{LOD} = y_B + 3s_B$$ \hspace{1cm} Eq. 6.1

$$\text{LOQ} = y_B + 10s_B$$ \hspace{1cm} Eq. 6.2

Table 6.4 shows the United States Substance Abuse and Mental Health Services Administration (SAMHSA) and European Union’s research project on Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) proposed cut-off levels for screening and confirmation tests for cocaine in urine and oral fluid (Substance Abuse and Mental Health Services Administration, 2015; Verstraete et al., 2011; Substance Abuse and Mental Health Services Administration, 2008). Compared to the SAMHSA guidelines for urine, the sensitivity of the developed method was below the recommended confirmatory cut-off concentration for cocaine, demonstrating its potential applicability. However, the SAMHSA and DRUID recommended cut-off levels for cocaine/benzoylecgonine in oral fluid are below the sensitivity of the developed method.

Table 6.5: United States Substance Abuse and Mental Health Services Administration (SAMHSA) and European Union’s research project on Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) recommended confirmation cut-off levels in urine and oral fluid.

<table>
<thead>
<tr>
<th>Sample matrix</th>
<th>Analyte</th>
<th>Concentration (ng/ml)</th>
<th>SAMHSA screening</th>
<th>SAMHSA confirmation</th>
<th>DRUID confirmation</th>
<th>LGC screening</th>
<th>LGC confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral fluid</td>
<td>Cocaine/benzoylecgonine</td>
<td>15</td>
<td>8</td>
<td>10</td>
<td>30</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>Benzoylecgonine</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>150</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

In comparison to commercial cut-off levels, the limit of quantitation calculated for urine using the portable mass spectrometer demonstrates that the level is below the screening cut-off level used for laboratory-based instruments. This shows the potential applicability of the portable system for drug detection in accordance with commercial cut-off levels, but not against recognised SAMHSA and DRUID guidelines. Additionally, quantitation limits
observed for oral fluid are slightly higher than those currently used by UK forensic providers
Private Communication with LGC (20 January 2014).

A limitation of the oral fluid screening method used with the portable system is that there
was no sample clean up or preparation step applied. As a result, the oral fluid is diluted by
the 3 ml of buffer used in the transfer tube and 0.5 ml of spiked IS solution. For analysis
using the Plate Express™ system only 10 µl of the resulting buffer solution was analysed
for screening purposes. It can be anticipated therefore that detection limits would be
improved using a modified collection device containing a small quantity of buffer as a
sample clean up method would not be feasible on-site.

6.2 Results and Discussion

6.2.1 Detection of cocaine and benzoylecgonine in oral fluid

A total of n = 2 oral fluid samples were analysed using the Plate Express™ from drug users
to evaluate the analytical method for the detection of cocaine and benzoylecgonine. To
demonstrate the significance of the detection of cocaine and benzoylecgonine in those
samples, a pooled oral fluid sample was also analysed from a negative control group (n = 5
males and n = 5 females). The collection of oral fluid samples from these participants is
described in section 6.1.3. The limit of detection for cocaine and benzoylecgonine in oral
fluid using the portable set-up is provided in Table 6.4. The qualitative analysis of cocaine
and benzoylecgonine in oral fluid samples from both groups; negative control and drug users
is outlined in the following section.

Analysis of cocaine and benzoylecgonine in spiked oral fluid (at 200 ng/ml) showed that
both analytes could be detected with the current set-up of the portable system. The spiked
oral fluid standard provided a reference chromatogram for both cocaine and
benzoylecgonine, which was used to identify the presence of the analytes in blank oral fluid
and oral fluid samples collected from participants admitted having taken cocaine. A problem
that could arise from the use of a portable mass spectrometer that has lower resolving power
than laboratory-based instruments is the presence of interferences. Interferences could be
present in the sample from compounds that have the same mass-to-charge ratio as the
analytes of interest. However, the addition of chromatographic separation should improve
the selectivity of the instrument as previously described. Figure 6.5 shows chromatograms
for cocaine and benzoylecgonine in blank and spiked oral fluid samples. Cocaine and
benzoylcegonine are present in the spiked oral fluid samples with retention times of 4.13 min and 4.01 min, respectively. In addition, the mild in-source fragmentation that is produced in the source confirms the presence of the corresponding fragment ions for cocaine ($m/z$ 182) and benzoylcegonine ($m/z$ 168). In contrast, both the molecular ion and fragment ion for cocaine and benzoylcegonine are not present in the pooled blank oral fluid sample. This suggests that no interferences were present for both analytes.

Figure 6.5: Extracted ion chromatograms for (a) cocaine and (b) benzoylcegonine in blank oral fluid and (c) cocaine and (d) benzoylcegonine in spiked oral fluid standard (at 200 ng/ml).

Oral fluid samples collected from $n = 2$ participants who admitted having taken cocaine, were positive for both the presence of cocaine and its major metabolite benzoylcegonine. Figure 6.6 shows the chromatograms for cocaine and benzoylcegonine for both participants. Comparison of the spiked oral fluid standard against the participant’s oral fluid samples shows the levels of benzoylcegonine detected are in the same range as to those of the spiked oral fluid standard.
6.2.2 Detection of cocaine and benzoylecgonine in urine

A urine samples was collected from \( n = 1 \) participant to assess the detection of cocaine and benzoylecgonine from an individual admitted having taken cocaine. The detection window for drugs of abuse in urine after a single dose is 1.5 to 4 days, dependent of the drug. In contrast, oral fluid is used to determine recent drug use with a detection time of 5 – 48 hours (Verstraete, 2004). For chronic drug users, the detection time of drugs in urine can be longer (up to a week). The different matrices can therefore provide different information about the administration of drugs.

Urine samples were collected from \( n = 3 \) non-drug users as a negative control group at the University of Surrey. The urine samples were pooled and used to determine the presence of any interference that could be present in the sample matrix. The limit of detection of cocaine and benzoylecgonine in urine are detailed in Table 6.4. Figure 6.7 shows chromatograms for cocaine and benzoylecgonine in blank urine and spiked urine drug standard. In contrast to the blank oral fluid samples, there is a small peak observed at the same retention time for cocaine (4.13 min) in the blank urine sample. It is possible that this peak is from interference in the sample matrix as the corresponding fragment ion of cocaine (\( m/z \ 182 \)) is not present in the blank urine sample or that the concentration was too low to provide the fragment peak. The results indicate the importance of mass spectrometry and the ability for induced fragmentation to confirm the presence of compounds. The chromatogram for benzoylecgonine showed that no peaks were present for the retention time of both its molecular ion and fragment ion, indicating there are no interferences detected in the sample matrix.
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The qualitative analysis of cocaine and benzoylecgonine in the urine sample from the participant admitted having taken cocaine showed the presence of benzoylecgonine, but not the parent drug cocaine. As urine is the last excretion path for the elimination of drugs from the human body, it is to be expected that drug metabolites levels are higher than those for the parent drug. However, instability of drugs in urine can influence the interpretation of drug testing results (Dixon et al., 2015; Cone et al., 2003). This work has been published in Analytical Methods (Ismail et al., 2017).

Figure 6.7: Extracted ion chromatograms for (a) cocaine and (b) benzoylecgonine in blank urine and (c) cocaine and (d) benzoylecgonine in a spiked urine standard (at 200 ng/ml).

6.3 Summary

The use of a portable mass spectrometer for the analysis of cocaine and benzoylecgonine in urine and oral fluid was evaluated. The portable set up was equipped with a chromatography column to improve selectivity of the single quadrupole mass spectrometer. Analysis was performed on urine and oral fluid samples collected from non-drug users as well as cocaine users. No interferences were detected in the blank urine (see Figure 6.5) and oral fluid (see Figure 6.5) analysed. A limit of detection was achieved at 31 ng/ml and 17 ng/ml in oral.
fluid for cocaine and benzoylecgonine, respectively. For urine, the limit of detection was 10 ng/ml for cocaine and 21 ng/ml for benzoylecgonine (Table 6.4). In addition, the presence of cocaine and benzoylecgonine was confirmed in the oral fluid samples (n = 2) by mild-in source fragmentation (Figure 6.6). The limit of quantitation obtained in urine and oral fluid for both cocaine and benzoylecgonine were higher than recognised SAMHSA and DRUID guidelines. As the focus of this research was the use of the Quantisal™ oral fluid collection devices (which contains a 3 ml buffer solution), there is a possibility that dissimilar results would have arisen if other collection devices had been investigated or a sample clean-up was performed. This pilot research study showed the proof of concept for using a small (single quadrupole) mass spectrometer for drug analysis is bodily fluids with minimal sample preparation.
Chapter 7  Conclusions and Future Work

7.0  Introduction

A fingerprint is a challenging sample matrix for drug detection as the amount of analyte present in the sample is small. Significant challenges are attributed to fingerprint analysis due to the nature of the sample matrix. Not only does the sample size vary within and between individuals, but the limited sample volume offers minimal sample preparation. This inevitably affected the analytical performance and results. A fingerprint sample collection procedure was implemented to help control these variabilities (section 3.1.2). A simple sample preparation method was developed, which included a pre-concentration step (reconstitution in a small volume) to allow for the detection of the analytes of interest in fingerprints (section 2.3.4).

A primary aim of this research was to investigate the significance of the detection of drugs from fingerprints and to evaluate the suitability of a fingerprint sample to determine cocaine and heroin use. This was evaluated by detecting cocaine and heroin in fingerprint samples from individuals receiving treatment for drug dependency at NHS Drug and Alcohol Services (Surrey and Borders Partnership NHS Foundation Trust). To evaluate the presence of cocaine and heroin in fingerprints from drug administration, the detection of both the parent drug (cocaine and heroin) and respective major metabolites benzoylecgonine and 6-acetylmorphine was investigated. The presence of the major metabolites is toxicologically important as it is indicative of the administration of the drug rather than contamination picked up from a surface or handling the parent drug (Jacob et al., 2008). The possibility to use fingerprints for drug testing has become the subject of recent studies (Ismail et al., 2018; Costa et al., 2017; Zhang et al., 2015; Goucher et al., 2009). However, the reliability of a fingerprint sample matrix for the detection of drugs has not yet been investigated. Fingerprint samples were also collected from non-drug users after contact with the parent drug, to evaluate the presence of cocaine, heroin and respective metabolites in various scenarios (e.g. hand cleaning procedures and secondary transfer) (Chapter 4, section 4.2). A summary of the suitability for the use of fingerprints to detect cocaine and heroin use is outlined in section 7.1.
Chapter 7: Conclusions and Future Work

A secondary aim of this research was to evaluate the use of fingerprints to detect the medication used to treat tuberculosis (in particular isoniazid and its respective metabolite acetylisoniazid) and the possibility to use fingerprints to monitor compliance. Fingerprint samples were collected from individuals receiving treatment for tuberculosis at King Edward VII Hospital in collaboration with Frimley Health NHS Foundation Trust (Chapter 5). The use of fingerprints for a clinical application to monitor compliance with tuberculosis medication is summarised in section 7.2.

Finally, a pilot research study was carried out to evaluate the use of a portable mass spectrometer for drug testing purposes using more conventional matrices, such as urine and oral fluid from individuals receiving treatment for drug dependency (Chapter 6). Section 7.3 summarises the findings of this study.

7.1 Detection of Cocaine and Heroin use in Fingerprints

A liquid chromatography mass spectrometry (LC-MS) method was developed for the analysis of cocaine, heroin and their respective metabolites benzoylecgonine and 6-acetylmorphine in fingerprint samples from two study populations, including (i) a background population of non-drug users and (ii) individuals seeking treatment for drug dependency at NHS Drug and Alcohol Services (Chapter 2, section 2.2.2). All fingerprint samples were analysed by LC-MS at the University of Surrey, whilst oral fluid samples were sent to a commercial provider for analysis (Claritest, Norwich, UK). Comparison with oral fluid samples showed that discrepancies were obtained between the two matrices for a number of participants (Chapter 3, section 3.2.2). However, as the detection window of cocaine in fingerprints is unknown, the influence of this is difficult to determine. Therefore, results were based on participant testimony, which has limitations as these may not necessarily be accurate. The significance of the detection of the analytes of interest in samples was evaluated by comparing natural fingerprints (without any preparation of the hands) from the two study populations (Chapter 3, section 3.2.1). The analysis of fingerprint samples from the background population demonstrated that a threshold level needs to be proposed for natural fingerprints due to traces of cocaine and benzoylecgonine detected as a result of environmental exposure (Chapter 3, section 3.2.4). In contrast, heroin and 6-acetylmorphine were not prevalent (≤1%) in natural fingerprints collected from the background population (Chapter 3, section 3.2.3.2). Compared to cocaine, heroin is not as
prevalent on banknotes and is more likely to degrade (Carter et al., 2003; Jenkins, 2001). Therefore, environmental exposure to heroin is less likely to occur. Based on the results obtained, there was no requirement to propose threshold levels as the presence of the analytes of interest was significant to differentiate drug users from non-drug users. This demonstrates that it is possible to differentiate drug users from non-drug users based on the detection of the analytes of interest in fingerprints.

This is the first study to evaluate the significance of the detection of drugs in fingerprints by comparison to a background population and evaluating different sampling strategies, including (i) washing hands with soap and water, and (ii) wiping hands with alcohol free wipes (Chapter 3, sections 3.2.5 and 3.2.6). In order for fingerprints to be suitable for drug testing, it is important to evaluate the influence of the sampling strategy on the detection rate and to demonstrate that the test cannot be easily falsified by simply using a hand cleaning procedure prior to the test. The prevalence of the analytes of interest in fingerprint samples from non-drug users was reduced using a handwashing procedure (Chapter 3, section 3.2.7). If a handwashing procedure is used prior to a fingerprint test, there is no requirement to propose a threshold level in order to differentiate drug users from non-drug users. The use of a handwashing procedure is advantageous as it will reduce the possibility of contamination present on the hands that could lead to false positive results. However, it has been demonstrated that both the parent drug and metabolite (as contamination in street drugs) can be present in fingerprint samples after handling 2 mg of the parent drug (see section 4.2.1) even after the use of hand cleaning procedures (see sections 4.2.3 and 4.2.4). In light of this, no differentiation was possible between the presence of the parent drug and metabolite in fingerprint samples due to administration or handling of the drug.

A possible concern for drug testing from fingerprints is the secondary transfer of drugs between individuals. Cocaine and heroin can easily be transferred by individuals who have admitted having taken the parent drug in the past 24 h (section 3.2.9) and those who have handled a large amount (2 mg) of the parent drug, by shaking hands (section 4.2.5). The removal of secondary transfer of cocaine present in fingerprint samples from participants after shaking hands with an individual who had handled 2 mg of cocaine was not possible. In contrast, the use of a handwashing procedure removed all traces of secondary transfer of heroin present on the hands.
Based on the results obtained in this study, the relationship between the presence of the parent drug and metabolite did not provide additional information between participants who had positive or negative oral fluid results, or those who had handled or taken the drug.

Overall, it is possible to discriminate individuals from the background population from those who have taken cocaine or heroin recently based on the presence of the parent drug and metabolite in fingerprint samples (Chapter 3, sections 3.2.3 and 3.2.7). However, the detection of cocaine and heroin in fingerprints does not necessarily indicate administration of the drug, but rather that the individual has either handled or administered the drug. Depending on the application of the test, both of these results could be incriminating.

### 7.2 Detection of Tuberculosis Medication in Fingerprints

Fingerprints can be used to determine the presence of drugs of abuse, however in this study the detection of prescribed medication to treat tuberculosis was the main focus (Chapter 5). Tuberculosis is one of the top 10 cause of deaths and the formation of drug resistant tuberculosis is a great concern (World Health Organisation, 2017). The World Health Organisation (WHO) recommends the use of directly observed treatment (DOTS) to monitor adherence to treatment. However, this is not often used by clinicians. Current methods rely on clinical improvement, tablet counts and engagement with tuberculosis nursing teams to help assess adherence (Nackers et al., 2012; Osterberg & Blaschke, 2005). An alternative method to monitor compliance with medication would be advantageous to ensure patients are adhering to their treatment and help prevent the formation of drug resistant tuberculosis.

The complex nature of fingerprint analysis effected the analytical methodology for the analysis of tuberculosis medication in fingerprints in terms of matrix effects. The matrix effects in the form of ionisation suppression were observed for isoniazid and acetylisoniazid in the presence of fingerprints (section 5.2.2). However, with the use of an internal standard (isoniazid-$d_4$) these effects were minimised for isoniazid but not to the same degree for acetylisoniazid. The ionisation suppression effects could be overcome by changing the chromatographic conditions to separate the compounds and with the use of an appropriate internal standard for acetylisoniazid.

Isoniazid (76%) and acetylisoniazid (93%) were detected in natural fingerprint samples ($n$ = 135) collected from individuals who all admitted having taken their medication on the day
of sample collection (section 5.2.5). In contrast, the analytes of interest were not prevalent in fingerprints collected from the negative control group or participants who had completed their treatment (2 – 8 months prior to recruitment) (sections 5.2.6 and 5.2.7). The use of a handwashing procedure reduced the detection rate for isoniazid (61%) and acetylisoniazid (81%). The lower detection rate could be possible due to a lack of sensitivity of the method or not enough sweat was secreted from the fingertips after handwashing (section 5.2.8). However, the analytes of interest were not prevalent in the negative control group or participants who had completed treatment after the handwashing procedure (sections 5.2.8.2 and 5.2.8.3). In order to maximise the detection rate for isoniazid and acetylisoniazid to monitor adherence, collection of natural fingerprint samples would be advantageous.

Evaluation of the elimination profile of isoniazid and acetylisoniazid in fingerprint samples demonstrated that when a participant stopped their medication, neither analytes could be detected two and four days after completing tuberculosis treatment (section 5.2.9). The present findings show that it is possible to detect prescribed medication in fingerprints and that it is not possible to detect the isoniazid or acetylisoniazid two days after completing treatment. In light of this, fingerprints could be a potential sampling matrix to monitor compliance with tuberculosis medication, but further work is required to establish the detection window of isoniazid and acetylisoniazid in fingerprints.

7.3 Detection of Cocaine use in Urine and Oral Fluid using a Portable Mass Spectrometer

Evaluation of the use of a portable mass spectrometer to allow for the detection of cocaine use in urine and oral fluid was explored (Chapter 6). In this pilot research study, a chromatography column was used to help increase the selectivity of a portable single quadrupole mass spectrometer. Urine and oral fluid samples were collected from non-drug users as well as drug users receiving treatment for drug dependency at NHS Drug and Alcohol Services (section 6.1.3). No interfering compounds were detected in urine and oral fluid samples collected from non-drug users. For the oral fluid samples collected using Quantisal™ oral fluid collection devices, the subsequent buffer solution was used for analysis. The method was able to detect both cocaine and benzoylecgonine in the biological matrices collected from the drug users, without any sample preparation, with adequate sensitivity (<30 ng/ml).
7.4 Future Work

This research has highlighted challenges associated with the use of fingerprints as a sampling matrix for drug testing. The variable sample matrix and the absence of a fingerprint standard made it impossible to produce matrix matched calibration curves and the possibility for quantitative analysis. In order to minimise the variability of a fingerprint sample, the sample size and volume should be controlled. The influence of this would be an interesting next step to help compare results between individuals.

Although cocaine, heroin and their respective metabolites (benzoylcegonine and 6-acetylmorphine) can be detected in fingerprints collected from individuals who admitted having taken the drug in the past 24 h (Chapter 3, section 3.2.2), the source of the detection of these analytes cannot solely be attributed to administration of the drug, as these analytes can also be present after handling the parent drug (Chapter 4, section 4.2.1). In order to use fingerprints to detect cocaine or heroin use, more research would be required to explore strategies to differentiate individuals who have administered cocaine or heroin from those who have handled the parent drug. A hand cleaning procedure that is possible to remove all contact residue after handing the parent drug would be advantageous to support this. Additionally, the detection of other markers in fingerprints that are indicative of administration of cocaine or heroin would also be a step forward to overcome the challenges associated with the use of fingerprints to detect cocaine and heroin use. Alternatively, the use of imaging mass spectrometry could be used to explore differences in analyte distribution in fingerprints to help differentiate between drug administration and contact.

The application of fingerprint testing for clinical purposes was evaluated by exploring the detection of tuberculosis medication in fingerprints. Although all participants admitted having taken their medication, this was not confirmed for all participants in all the fingerprint samples collected (Chapter 5, section 5.2.5). In order to understand the discrepancy between the fingerprint samples collected from the same participant as well as between participants, corresponding blood samples should be taken to help corroborate results. Additionally, the fingerprint analysis method developed required further work. The most important limitation on the developed method was the observed matrix effects (ionisation suppression) and the absence of a suitable internal standard for acetylisoniazid to correct for the effects observed (Chapter 5, section 5.2.2). Additional work is required to improve the matrix effects observed, including improvement of the chromatographic
Chapter 7: Conclusions and Future Work

separation between the analytes of interest and evaluation of the use of washing the paper substrate prior to fingerprint deposition. This could help enhance the sensitivity of the method, which could improve the detection of isoniazid and acetylsalicylic acid in fingerprint samples.

The use of a portable drug testing system was able to detect cocaine use in urine and oral fluid samples from individuals admitted having taken cocaine in the past 24 h, without sample preparation. However, as this was a preliminary study, only a few participants were included in the study and optimisation of the method was limited. To improve sensitivity further, in particular with the use of oral fluid collection devices further work is required. The Quantisal™ collection device used consists of a 3 ml buffer solution, which will dilute the oral fluid sample and therefore affect the sensitivity of the method. A sample clean-up method or use of a smaller buffer solution could be investigated to improve sensitivity. Additionally, a more robust method evaluation is required to validate the use of the portable system. The use of a mobile drug testing device could have a wide range of applications (e.g. workplace drug testing, GP practices and hospitals) and this would be an interesting area of research.
References


Forensic Science Regulator 2013. Fingerprint examination - terminology, definitions and acronyms (FSR-I-402). In: HOME OFFICE (ed.).


Appendices

to

Investigation into the Significance of Monitoring Drug Use from a Fingerprint

by

Mahado Ismail
Appendix A: Analytical Methodology (Chapter 2):

A. 1 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A1 (20 mM ammonium acetate in 5% (v/v) acetonitrile in water) and solvent B1 (acetonitrile + 0.1% formic acid) starting at 80% aqueous and 20% organic solvent using gradient elution.

A. 2 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A2 (water + 0.1% formic acid) and solvent B1 (acetonitrile + 0.1% formic acid) starting at 80% aqueous and 20% organic solvent using gradient elution, see Table 2.1 for the LC-MS operating conditions.

A. 3 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A2 (water + 0.1% formic acid) and solvent B2 (MeOH + 0.1% formic acid) starting at 80% aqueous and 20% organic solvent, see Table 2.1 for the LC-MS operating conditions.

A. 4 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A1 (20 mM ammonium acetate in 5% (v/v) acetonitrile in water) and solvent B1 (acetonitrile + 0.1% formic acid) starting at 95% aqueous and 5% organic solvent using gradient elution, see Table 2.1 for the LC-MS operating conditions.

A. 5 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A2 (water + 0.1% formic acid) and solvent B1 (acetonitrile + 0.1% formic acid) starting at 95% aqueous and 5% organic solvent using gradient elution, see Table 2.1 for the LC-MS operating conditions.

A. 6 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A2 (water + 0.1% formic acid) and solvent B2 (MeOH + 0.1% formic acid) starting at 95% aqueous and 5% organic solvent, see Table 2.1 for the LC-MS operating conditions.

A. 7 Calibration curve (run 1) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H2O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.
A. 8 Calibration curve (run 2) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H2O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.

A. 9 Calibration curve (run 3) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H2O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.

A. 10 Calibration curve (run 4) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H2O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.

A. 11 Calibration curve (run 5) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H2O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.

Appendix B: Standard operating procedures (Chapter 2 & 3):

B. 1 Standard operating procedure for oral fluid sample collection.

B. 2 Standard operating procedure for fingerprint sample collection.

Appendix C: Forensic Science Ireland (Chapter 4):

C. 1 Forensic Science Ireland: Gas chromatography – mass spectrometry (GC-MS) analysis of cocaine.

C. 2 Forensic Science Ireland: Gas chromatography – mass spectrometry (GC-MS) analysis of heroin.

Appendix D: Calibration curve data (Chapter 5):

D. 1 Calibration curve (run 1) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H2O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 2 Calibration curve (run 2) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H2O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions. Calibration curve (run 3) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H2O + 0.1% formic acid.

D. 3

D. 4 Calibration curve (run 4) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H2O + 0.1% formic acid.
D. 5 Calibration curve (run 4) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid

D. 6 Calibration curve (run 1 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid

D. 7 Calibration curve (run 2 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 8 Calibration curve (run 3 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 9 Calibration curve (run 4 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 10 Calibration curve (run 5 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid

Appendix E: Statistical data (Chapters 3 and 4)

E. 1 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in fingerprints collected from cocaine/heroin users and a background population of non-drug users using different sample collection procedures (natural, soap and wipe).

E. 2 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected cocaine/heroin users and after wiping hands with alcohol free wipes (same participants).

E. 3 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in natural fingerprints collected from cocaine/heroin users and wiping hands with alcohol free wipes (same participants).

E. 4 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and after washing hands with soap and water (same participants).
E. 5 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in natural fingerprints collected from cocaine/heroin users in and wiping hands with alcohol free wipes (same participants).

E. 6 Kruskal Wallis test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users before and after shaking hands (secondary transfer).

E. 7 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in fingerprints collected from non-drug users before and after shaking hands with drug users.

E. 8 Kruskal Wallis test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users before and after shaking hands (secondary transfer).

E. 9 Kruskal Wallis test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprints collected from non-drug users after dermal contact with the parent drug (scenario 1), after wiping hands with alcohol free wipes (scenario 2) and after washing hands with soap and water (scenario 3).

E. 10 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine and benzoylecgonine levels in fingerprints collected from non-drug users after shaking hands with a participant with dermal contact of cocaine and after washing hands (scenario 4).

E. 11 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users after dermal contact with 2 mg of the parent drug.

E. 12 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users after dermal contact with 2 mg of the parent drug.

E. 13 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in fingerprint samples collected after wiping hands with alcohol free wipes from cocaine/heroin users and non-drug users after dermal contact with 2 mg of the parent drug.
E. 14 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in fingerprint samples collected after wiping hands with alcohol free wipes from drug users and non-drug users after dermal contact with 2 mg of the parent drug.

E. 15 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in fingerprint samples collected after washing hands with soap and water from cocaine/heroin users and non-drug users after dermal contact with 2 mg of the parent drug.

E. 16 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in fingerprint samples collected after washing hands with soap and water from drug users and non-drug users after dermal contact with 2 mg of the parent drug.

E. 17 Kruskal Wallis test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users after shaking hands (secondary transfer) and after washing hands.

E. 18 Kruskal Wallis test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine and heroin-to-6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users after shaking hands (secondary transfer) and after washing hands.

E. 19 Average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, n = 5 measurements) and statistical data for isoniazid in the presence of fingerprint samples (natural and after handwashing) and blank solvent (methanol) extracted from paper and spiked with 20 ng/ml and 80 ng/ml compared to the reference standard at the same concentration.

E. 20 Average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, n = 5 measurements) and statistical data for acetylisoniazid in the presence of fingerprint samples (natural and after handwashing) and blank solvent (methanol) extracted from paper and spiked with 20 ng/ml and 80 ng/ml compared to the reference standard at the same concentration.

**Appendix F: Publications**

F. 1 Publication: Ismail et al. (2017)

F. 2 Publication: Ismail et al. (2018)
Appendix A

A. 1 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A1 (20 mM ammonium acetate in 5% (v/v) acetonitrile in water) and solvent B1 (acetonitrile + 0.1% formic acid) starting at 80% aqueous and 20% organic solvent using gradient elution, see Table 2.1 for the LC-MS operating conditions.

A. 2 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A2 (water + 0.1% formic acid) and solvent B1 (acetonitrile + 0.1% formic acid) starting at 80% aqueous and 20% organic solvent using gradient elution, see Table 2.1 for the LC-MS operating conditions.
A. 3 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A2 (water + 0.1% formic acid) and solvent B2 (MeOH + 0.1% formic acid) starting at 80% aqueous and 20% organic solvent, see Table 2.1 for the LC-MS operating conditions.

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A. 4 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A1 (20 mM ammonium acetate in 5% (v/v) acetonitrile in water) and solvent B1 (acetonitrile + 0.1% formic acid) starting at 95% aqueous and 5% organic solvent using gradient elution, see Table 2.1 for the LC-MS operating conditions.

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A. 5 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A2 (water + 0.1% formic acid) and solvent B1 (acetonitrile + 0.1% formic acid) starting at 95% aqueous and 5% organic solvent using gradient elution, see Table 2.1 for the LC-MS operating conditions.

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A. 6 Chromatographic separation on a Waters Acquity UHPLC combined with a Q-ToF Premier using solvent A2 (water + 0.1% formic acid) and solvent B2 (MeOH + 0.1% formic acid) starting at 95% aqueous and 5% organic solvent, see Table 2.1 for the LC-MS operating conditions.

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A. 7 Calibration curve (run 1) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H₂O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.

A. 8 Calibration curve (run 2) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H₂O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.
A. 9 Calibration curve (run 3) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H$_2$O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.

A. 10 Calibration curve (run 4) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H$_2$O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.
A. 11 Calibration curve (run 5) of cocaine, benzoylecgonine, heroin and 6-acetylmorphine ranging from 0 – 100 ng/ml in 5% ACN in H₂O + 0.1% formic acid, see Table 2.1 for the LC-MS operating conditions.
Appendix B

B. 1 Standard operating procedure for oral fluid sample collection.

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STANDARD OPERATING PROCEDURE

Protocol for Oral Fluid Collection
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1.0 PURPOSE
This Standard Operating Procedure (SOP) outlines the procedure to follow with regard to oral fluid (saliva) swab collections. It contains instructions on specimen collection, handling and labelling. It will ensure that all oral fluid samples collected during the course of the study conform to specified requirements and that a normalised collection procedure is utilised.

2.0 POLICIES
Oral fluid specimens collected must be labelled with a barcode that corresponds to the fingerprints samples to be collected and will not contain the donor’s first name, last name or date of birth.

3.0 EQUIPMENT AND MATERIALS
3.1 Oral fluid swab kit
3.2 Disposable gloves

4.0 PROCEDURE
4.1 Research personnel will greet the research subject, identify themselves, and then indicate the collection procedure to the research subject.
4.2 The research subject will be approached in a friendly calm manner and their cooperation will be gained prior to oral fluid collection.
4.3 Following eating or drinking, a 10 min. waiting period is recommended before collection.
4.4 Participant’s name and gender will be recorded on the recruitment data spreadsheet.
4.5 Two lab submission forms will be provided for each participant, one for oral fluid and fingerprint collection. Another lab submission form will be provided for urine collection.
4.6 All sample containers and equipment needed to competently and efficiently carry out the oral fluid collection will be assembled prior to the procedure.
4.7 The research personnel will take a barcode from each of the submission forms and place them on the recruitment data spreadsheet under the participant’s name and corresponding sample.
4.8 The research personnel will put gloves on prior to oral fluid collection.
4.9 Collect two oral fluid swabs, as the amount collected in one swab is small. This will allow for multiple analyses to be carried out.
4.10 The research personnel will take the swab out of the sealed bag and ask the participant to churn saliva.
4.11 The swab will then be placed between the teeth and the cheek of the participant until the indicator on the swab turns blue. This indicates that enough saliva has been collected.
4.12 Once the indicator has turned blue, the swab is removed from the mouth and placed in the vial provided in the kit swab end first.
4.13 The lid of the vial should be pressed until sealed (a click should be heard).
4.14 Research personnel should remove the gloves before moving on to the next participant.

LABELING AND SPECIMEN TRANSFER
4.15 All oral fluid specimens must be properly labelled.
4.16 The research personnel will seal the container with self-adhesive barcodes that correspond to the fingerprint sample that will be collected from the participant immediately after oral fluid collection. The barcode strips are be available on the lab submission form.
4.17 Place the barcode strip over the vial with the indicated circle centered in the middle of the lid.
4.18 Another barcode from the lab submission form will be placed on the side of the vial.
4.19 Mark the sample with the date of collection.
4.20 Specimen must be placed into the chain of custody bag provided.
4.21 Sample must be accompanied with the lab submission form for LGC.
4.22 The Surrey research personnel will arrange for the samples to be shipped to LGC at the end of the collection day.

5.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE
5.1 Short term storage at 4 °C and long-term storage (over 2 weeks) at -20 °C.
B. 2 Standard operating procedure for fingerprint sample collection.

STANDARD OPERATING PROCEDURE
FINGERPRINT COLLECTION, LABELING AND HANDLING

STANDARD OPERATING PROCEDURE

Protocol for Fingerprint Collection

UNIVERSITY OF SURREY
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Appendix B

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1.0 PURPOSE
This Standard Operating Procedure (SOP) outlines the procedure to follow with regard to collecting fingerprints. It will ensure that all fingerprints collected during the course of the study conform to specified requirements and that a normalised collection procedure is utilised.

2.0 POLICIES
Fingerprint samples collected must be labelled with a barcode that corresponds to previous collected specimen (oral fluid) and may not contain the donor’s first name, last name or date of birth.

3.0 EQUIPMENT AND MATERIALS
3.1 Weighing balance
3.2 Fingerprint collection kit (10 finger capacity)
3.3 Self-adhesive barcode label
3.4 Disposable gloves

4.0 PROCEDURE
4.1 Research personnel will greet the research subject, identify themselves, and then indicate the collection procedure to the research subject.
4.2 The research subject will be approached in a friendly calm manner and their cooperation will be gained prior to fingerprint collection.
4.3 Participant’s name and gender will be recorded on the recruitment data spread sheet.
4.4 Two lab submission forms will be provided for each participant, one for oral fluid and fingerprint collection. Another lab submission form will be provided for urine collection.
4.5 All sample containers and equipment needed to competently and efficiently carry out the urine collection will be assembled prior to the procedure.
4.6 The research personnel will take a barcode from the oral fluid and fingerprint submission form and place it on the recruitment data spread sheet under the participant’s name and corresponding sample.
4.7 Prior to fingerprint collection, put on a pair of disposable gloves.
4.8 Press the ‘ON’ button on the weighing balance to turn it on.
4.9 Press the ‘TARE’ button to set the balance to zero.
4.10 Remove the first collection slide from the fingerprint collection kit. Place the collection slide on the weighing balance with the label side up.
4.11 Press the ‘TARE’ button again to set the balance back to zero.
4.12 Volunteer places the finger displayed on the label onto the collection slide (for example ‘left little finger’). The fingerprint must be placed within the rectangular guidelines.
   NOTE: When collecting the fingerprint make sure a full fingerprint is collected, not just their fingerprint. Volunteers do not need to ‘roll’ the finger when placed on the collection slide.
4.13 Volunteer must increase the pressure until the balance reads between 800 - 1200 g. If the volunteer is not able to apply the amount of...
Appendix B

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pressure required, the actual pressure (in grams) applied must be recorded and submitted with the collected fingerprint samples.

NOTE: Volunteer must not move the finger once placed on the collection slide to prevent smudging.

4.14 Volunteer must carefully lift the finger upwards, after providing the required pressure for collection.

4.15 The research personnel will remove the collection slide from the weighing balance and place it back into the storage box.

4.16 Repeat steps 4.6 to 4.9 until all ten fingerprints are collected.

5.0 LABELING AND TRANSFER

5.1 Prior to the collection of fingerprint samples, individual microscope glass slides will be labelled with a barcode corresponding to the oral fluid testing kit and identification of the finger ("little left finger").

5.2 All fingerprint storage boxes must be properly marked.

5.3 The research personnel will place the individual microscope slides in a storage box immediately after fingerprint collection. The storage box can contain up to 100 slides and therefore ten fingerprints from ten different participants.

5.4 Each of the storage boxes will be tagged with an identification barcode corresponding to each of the 10 participants. Each box will contain a representative of 10 samples per participant.

5.5 Mark the storage box using the date of collection.

5.6 Fingerprint storage boxes can be kept at room temperature, but must be away from any bright light sources.

5.7 Samples must be accompanied with the lab submission form.

5.8 The research personnel will arrange for the samples to be transported to the University of Surrey at the end of collections.

6.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1 Fingerprint samples will be stored at -20°C.
Appendix C

C. 1 Forensic Science Ireland: Gas chromatography – mass spectrometry (GC-MS) analysis of cocaine.
C. 2 Forensic Science Ireland: Gas chromatography – mass spectrometry (GC-MS) analysis of heroin.
C. 2 continued...
### Appendix C

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Fragmentor Voltage

Collision Energy

Ionization Mode: Unspecified

Counts vs. Acquisition Time (min)

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Library Spectrum

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</table>

*Note: Values are illustrative and not actual data.*
Appendix D

D. 1 Calibration curve (run 1) of isoniazid and acetylsalicylic acid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 2 Calibration curve (run 2) of isoniazid and acetylsalicylic acid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.
D. 3 Calibration curve (run 3) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H₂O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 4 Calibration curve (run 4) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H₂O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 5 Calibration curve (run 4) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H₂O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.
D. 6  Calibration curve (run 1 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H2O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 7  Calibration curve (run 2 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H2O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 8  Calibration curve (run 3 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H2O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.
D. 9 Calibration curve (run 4 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.

D. 10 Calibration curve (run 5 – prepared daily) of isoniazid and acetylisoniazid ranging from 0 – 100 ng/ml in 50% ACN in H$_2$O + 0.1% formic acid, see Table 5.1 for the LC-MS operating conditions.
Appendix E

E.1 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in fingerprints collected from cocaine/heroin users and a background population of non-drug users using different sample collection procedures (natural, soap and wipe).

| Analyte | Study population | Sample type | n₁ | n₂ | U calc | \(|z|_{calc}\) | Significant difference? (at \(p = 0.05\)) |
|---------|------------------|-------------|----|----|--------|----------------|-----------------------------------|
| Cocaine | Non-DU v DU      | N           | 13 | 65 | 0      | 5.67           | Non-DU < DU \(p < 0.001\)          |
|         | DU               | N v S       | 65 | 40 | 819    | 3.17           | S < N \(p = 0.002\)                |
|         | DU               | N v W       | 65 | 25 | 603    | 1.89           | No difference \(p = 0.059\)        |
| BZE     | Non-DU v DU      | N           | 5  | 61 | 27     | 3.04           | Non-DU < DU \(p = 0.001\)          |
|         | DU               | N v S       | 61 | 34 | 461    | 4.47           | S < N \(p < 0.001\)               |
|         | DU               | N v W       | 61 | 25 | 609    | 1.46           | No difference \(p = 0.144\)        |
| Heroin  | DU               | N v S       | 59 | 34 | 502    | 4.00           | S < N \(p < 0.001\)               |
|         | DU               | N v W       | 59 | 20 | 104    | 5.48           | W < N \(p < 0.001\)               |
| 6-AM    | DU               | N v S       | 60 | 35 | 807    | 1.88           | No difference \(p = 0.061\)        |
|         | DU               | N v W       | 60 | 20 | 557    | 0.48           | No difference \(p = 0.633\)        |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; Non-DU: non-drug users (background population; DU: drug users; N: natural fingerprints; S: fingerprints collected after washing hands with soap and water; W: fingerprints collected after wiping hands with alcohol free wipes. The significance level \((p)\) is 0.05.
E. 2 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected cocaine/heroin users and after wiping hands with alcohol free wipes (same participants).

| Analyte     | Study population | Sample type | n₁  | n₂  | Uₐₜₐₜ  | |z|calc | Significant difference? (at p = 0.05) |
|-------------|------------------|-------------|-----|-----|---------|--------|-------------------------------|
| Cocaine     | DU               | N v W       | 25  | 25  | 231     | 1.58   | No difference  
|             |                  |             |     |     |         |        | p = 0.114                    |
| BZE         | DU               | N v W       | 25  | 25  | 258     | 1.06   | No difference  
|             |                  |             |     |     |         |        | p = 0.290                    |
| Heroin      | DU               | N v W       | 20  | 20  | 43      | 4.25   | W < N  
|             |                  |             |     |     |         |        | p < 0.001                    |
| 6-AM        | DU               | N v W       | 20  | 20  | 250     | 1.35   | No difference  
|             |                  |             |     |     |         |        | p = 0.183                    |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; N: natural fingerprints; W: fingerprints collected after wiping hands with alcohol free wipes. The significance level (p) is 0.05.

E. 3 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in natural fingerprints collected from cocaine/heroin users and wiping hands with alcohol free wipes (same participants).

| Analyte     | Study population | Sample type | n₁  | n₂  | Uₐₜₐₜ  | |z|calc | Significant difference? (at p = 0.05) |
|-------------|------------------|-------------|-----|-----|---------|--------|-------------------------------|
| Cocaine-    | DU               | N v W       | 25  | 25  | 295     | 0.34   | No difference  
| to-BZE      |                  |             |     |     |         |        | p = 0.734                    |
| Heroin-     | DU               | N v W       | 20  | 20  | 40      | 4.33   | W < N  
| to-6-AM     |                  |             |     |     |         |        | p < 0.001                    |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; N: natural fingerprints; W: fingerprints collected after wiping hands with alcohol free wipes. The significance level (p) is 0.05.

E. 4 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and after washing hands with soap and water (same participants).

| Analyte     | Study population | Sample type | n₁  | n₂  | Uₐₜₐₜ  | |z|calc | Significant difference? (at p = 0.05) |
|-------------|------------------|-------------|-----|-----|---------|--------|-------------------------------|
| Cocaine     | DU               | N v S       | 40  | 40  | 516     | 2.73   | S < N  
|             |                  |             |     |     |         |        | p = 0.006                    |
| BZE         | DU               | N v S       | 36  | 34  | 260     | 4.14   | S < N  
|             |                  |             |     |     |         |        | p < 0.001                    |
| Heroin      | DU               | N v S       | 39  | 34  | 290     | 4.13   | S < N  
|             |                  |             |     |     |         |        | p < 0.001                    |
| 6-AM        | DU               | N v S       | 40  | 35  | 450     | 2.66   | S < N  
|             |                  |             |     |     |         |        | p = 0.008                    |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; N: natural fingerprints; S: fingerprints collected after washing hands with soap and water. The significance level (p) is 0.05.
E. 5 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in natural fingerprints collected from cocaine/heroin users in and wiping hands with alcohol free wipes (same participants).

| Analyte         | Study population | Sample type | \( n_1 \) | \( n_2 \) | \( U_{\text{calc}} \) | \(|z|_{\text{calc}} \) | Significant difference? (at \( p = 0.05 \)) |
|-----------------|-----------------|-------------|--------|--------|----------------|----------------|-------------------------------|
| Cocaine-to-BZE  | DU              | N v S       | 36     | 34     | 588           | 0.28           | No difference \( p = 0.778 \) |
| Heroin-to-6-AM  | DU              | N v S       | 39     | 33     | 353           | 3.28           | \( S < N \) \( p < 0.001 \) |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; N: natural fingerprints; S: fingerprints collected after washing hands with soap and water. The significance level \( (p) \) is 0.05.

E. 6 Kruskal Wallis test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users before and after shaking hands (secondary transfer).

<table>
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<tr>
<th>Analyte</th>
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<th>Sample type</th>
<th>( n_1 )</th>
<th>( n_2 )</th>
<th>( n_3 )</th>
<th>( \chi^2_{\text{calc}} )</th>
<th>d.f</th>
<th>Significant difference? (at ( p = 0.05 ))</th>
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<tbody>
<tr>
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<td>Non-DU v DU</td>
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<td>N v ST</td>
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<td>N v ST</td>
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<td>Non-DU v DU</td>
<td>N v ST</td>
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<td>6</td>
<td>67</td>
<td>11.12</td>
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</table>

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; ST: secondary transfer; N: natural fingerprints; d.f: degrees of freedom. The significance level \( (p) \) is 0.05.

E. 7 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in fingerprints collected from non-drug users before and after shaking hands with drug users.

| Analyte | Study population | Sample type | \( n_1 \) | \( n_2 \) | \( U_{\text{calc}} \) | \(|z|_{\text{calc}} \) | Significant difference? (at \( p = 0.05 \)) |
|---------|-----------------|-------------|--------|--------|----------------|----------------|-------------------------------|
| Cocaine| Non-DU          | N v ST      | 7      | 25     | 147           | 2.71           | ST > N \( p = 0.005 \)      |
| BZE     | Non-DU          | N v ST      | 2      | 5      | 6             | 0.39           | No difference \( p = 1 \)    |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; Non-DU: non-drug users; N: natural fingerprints; ST: secondary transfer. The significance level \( (p) \) is 0.05.
E. 8 Kruskal Wallis test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users before and after shaking hands (secondary transfer).

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<tr>
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<th>n₂</th>
<th>n₃</th>
<th>χ² calc</th>
<th>d.f</th>
<th>Significant difference? (at p = 0.05)</th>
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<td>N v ST</td>
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<td>N v ST</td>
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<td>59</td>
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BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; ST: secondary transfer; N: natural fingerprints; d.f: degrees of freedom. The significance level (p) is 0.05.

E. 9 Kruskal Wallis test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine in fingerprints collected from non-drug users after dermal contact with the parent drug (scenario 1), after wiping hands with alcohol free wipes (scenario 2) and after washing hands with soap and water (scenario 3).

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<th>n₃</th>
<th>χ² calc</th>
<th>d.f</th>
<th>Significant difference? (at p = 0.05)</th>
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<td>12</td>
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<tr>
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<td>1 v 2 v 3</td>
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<td>12</td>
<td>11</td>
<td>26.67</td>
<td>2</td>
<td>Yes, p &lt; 0.001</td>
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<tr>
<td>Heroin</td>
<td>1 v 2 v 3</td>
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<td>12</td>
<td>12</td>
<td>28.76</td>
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<tr>
<td>6-AM</td>
<td>1 v 2 v 3</td>
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<td>12</td>
<td>12</td>
<td>30.71</td>
<td>2</td>
<td>Yes, p &lt; 0.001</td>
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</tbody>
</table>

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; d.f: degrees of freedom. The significance level (p) is 0.05.

E. 10 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine and benzoylecgonine levels in fingerprints collected from non-drug users after shaking hands with a participant with dermal contact of cocaine and after washing hands (scenario 4).

<table>
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<th>n₂</th>
<th>U calc</th>
<th></th>
<th>z calc</th>
<th>Significant difference? (at p = 0.05)</th>
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<td>8</td>
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<tr>
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<td>ASH v AWH</td>
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<td>0</td>
<td>3.36</td>
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</table>

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; ASH: after shaking hands; AWH: after washing hands. The significance level (p) is 0.05.
E. 11 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users after dermal contact with 2 mg of the parent drug.

| Analyte       | Study population | Sample type | n₁  | n₂  | Uₘₐₖ | |z|ₘₐₖ | Significant difference? (at p = 0.05) |
|---------------|------------------|-------------|-----|-----|-------|-------|--------------------------------------|
| Cocaine       | DU v Non-DU      | N           | 65  | 12  | 777   | 5.44  | Non-DU > DU p < 0.001                |
| BZE           | DU v Non-DU      | N           | 61  | 12  | 732   | 5.45  | Non-DU > DU p < 0.001                |
| Heroin        | DU v Non-DU      | N           | 59  | 12  | 707   | 5.42  | Non-DU > DU p < 0.001                |
| 6-AM          | DU v Non-DU      | N           | 60  | 12  | 242   | 1.79  | No difference p = 0.073             |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; N: natural fingerprints. The significance level (p) is 0.05.

E. 12 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users after dermal contact with 2 mg of the parent drug.

| Analyte       | Study population | Sample type | n₁  | n₂  | Uₘₐₖ | |z|ₘₐₖ | Significant difference? (at p = 0.05) |
|---------------|------------------|-------------|-----|-----|-------|-------|--------------------------------------|
| Cocaine       | DU v Non-DU      | W           | 25  | 12  | 269   | 3.86  | Non-DU > DU p < 0.001                |
| BZE           | DU v Non-DU      | W           | 25  | 12  | 292   | 4.61  | Non-DU > DU p < 0.001                |
| Heroin        | DU v Non-DU      | W           | 20  | 12  | 88    | 1.25  | No difference p = 0.224             |
| 6-AM          | DU v Non-DU      | W           | 20  | 12  | 212   | 3.58  | Non-DU > DU p < 0.001                |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; W: fingerprints collected after wiping hands with alcohol free wipes. The significance level (p) is 0.05.
E. 14 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in fingerprint samples collected after wiping hands with alcohol free wipes from drug users and non-drug users after dermal contact with 2 mg of the parent drug.

| Analyte          | Study population | Sample type | n₁  | n₂  | Uₘₐₓ | |z|ₘₐₓ | Significant difference? (at p = 0.05) |
|------------------|------------------|-------------|-----|-----|------|-----|-------------------------------------|
| Cocaine-to-BZE   | DU v Non-DU      | W           | 25  | 12  | 99   | 1.66| No difference p = 0.102             |
| Heroin-to-6-AM   | DU v Non-DU      | W           | 25  | 12  | 222  | 3.97| Non-DU > DU p < 0.001               |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; W: fingerprints collected after wiping hands with alcohol free wipes. The significance level (p) is 0.05.

E. 15 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in fingerprint samples collected after washing hands with soap and water from cocaine/heroin users and non-drug users after dermal contact with 2 mg of the parent drug.

| Analyte          | Study population | Sample type | n₁  | n₂  | Uₘₐₓ | |z|ₘₐₓ | Significant difference? (at p = 0.05) |
|------------------|------------------|-------------|-----|-----|------|-----|-------------------------------------|
| Cocaine          | DU v Non-DU      | S           | 40  | 12  | 305  | 1.41| No difference p = 0.438             |
| BZE              | DU v Non-DU      | S           | 34  | 12  | 393  | 4.73| Non-DU > DU p < 0.001               |
| Heroin           | DU v Non-DU      | S           | 34  | 12  | 267  | 1.58| No difference p = 0.115             |
| 6-AM             | DU v Non-DU      | S           | 35  | 12  | 155  | 1.34| No difference p = 0.180             |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; S: fingerprints collected after washing hands with soap and water. The significance level (p) is 0.05.

E. 16 Mann-Whitney U-test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine ratio and heroin-to-6-acetylmorphine ratio in fingerprint samples collected after washing hands with soap and water from drug users and non-drug users after dermal contact with 2 mg of the parent drug.

| Analyte          | Study population | Sample type | n₁  | n₂  | Uₘₐₓ | |z|ₘₐₓ | Significant difference? (at p = 0.05) |
|------------------|------------------|-------------|-----|-----|------|-----|-------------------------------------|
| Cocaine-to-BZE   | DU v Non-DU      | S           | 34  | 12  | 172  | 0.80| No difference p = 0.438             |
| Heroin-to-6-AM   | DU v Non-DU      | S           | 33  | 12  | 329  | 3.36| Non-DU > DU p < 0.001               |

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; S: fingerprints collected after washing hands with soap and water. The significance level (p) is 0.05.
Appendix E

E. 17 Kruskal Wallis test (two-tailed) for the comparison of the cocaine, benzoylecgonine, heroin and 6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users after shaking hands (secondary transfer) and after washing hands.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Study population</th>
<th>Sample type</th>
<th>n_1</th>
<th>n_2</th>
<th>n_3</th>
<th>$\chi^2_{\text{calc}}$</th>
<th>d.f</th>
<th>Significant difference? (at $p = 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>Non-DU v DU</td>
<td>N v ST</td>
<td>8</td>
<td>8</td>
<td>65</td>
<td>21.24</td>
<td>2</td>
<td>Yes, $p &lt; 0.001$</td>
</tr>
<tr>
<td>BZE</td>
<td>Non-DU v DU</td>
<td>N v ST</td>
<td>8</td>
<td>8</td>
<td>61</td>
<td>18.87</td>
<td>2</td>
<td>Yes, $p &lt; 0.001$</td>
</tr>
<tr>
<td>Heroin</td>
<td>Non-DU v DU</td>
<td>N v ST</td>
<td>7</td>
<td>0</td>
<td>59</td>
<td>8.05</td>
<td>2</td>
<td>Yes, $p = 0.018$</td>
</tr>
<tr>
<td>6-AM</td>
<td>Non-DU v DU</td>
<td>N v ST</td>
<td>7</td>
<td>0</td>
<td>60</td>
<td>3.81</td>
<td>2</td>
<td>No, $p = 0.148$</td>
</tr>
</tbody>
</table>

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; ST: secondary transfer; N: natural fingerprints; d.f: degrees of freedom. The significance level ($p$) is 0.05.

E. 18 Kruskal Wallis test (two-tailed) for the comparison of the cocaine-to-benzoylecgonine and heroin-to-6-acetylmorphine levels in natural fingerprints collected from cocaine/heroin users and fingerprints collected from non-drug users after shaking hands (secondary transfer) and after washing hands.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Study population</th>
<th>Sample type</th>
<th>n_1</th>
<th>n_2</th>
<th>n_3</th>
<th>$\chi^2_{\text{calc}}$</th>
<th>d.f</th>
<th>Significant difference? (at $p = 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine-BZE</td>
<td>Non-DU v DU</td>
<td>N v ST</td>
<td>8</td>
<td>8</td>
<td>65</td>
<td>29.97</td>
<td>2</td>
<td>Yes, $p &lt; 0.001$</td>
</tr>
<tr>
<td>Heroin-6-AM</td>
<td>Non-DU v DU</td>
<td>N v ST</td>
<td>8</td>
<td>0</td>
<td>60</td>
<td>3.35</td>
<td>2</td>
<td>No, $p = 0.188$</td>
</tr>
</tbody>
</table>

BZE: benzoylecgonine; 6-AM: 6-acetylmorphine; DU: drug users; Non-DU: non-drug users; ST: secondary transfer; N: natural fingerprints; d.f: degrees of freedom. The significance level ($p$) is 0.05.
Appendix E

E. 19 Average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, n = 5 measurements) and statistical data for isoniazid in the presence of fingerprint samples (natural and after handwashing) and blank solvent (methanol) extracted from paper and spiked with 20 ng/ml and 80 ng/ml compared to the reference standard at the same concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration level 20 ng/ml</th>
<th>Concentration level 80 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s (n = 5) (ratio A/IS)</td>
<td>Ref. std (ratio A/IS)</td>
</tr>
<tr>
<td>Fingerprints natural</td>
<td>0.311 ± 0.031</td>
<td>0.314 ± 0.003</td>
</tr>
<tr>
<td>Fingerprints soap</td>
<td>0.324 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>Blank solvent (methanol)</td>
<td>0.337 ± 0.005</td>
<td></td>
</tr>
</tbody>
</table>

Note: s = standard deviation. For the two-tailed t-test: tcrit given in table (Miller & Miller, 2005).

E. 20 Average peak area ratio analyte (A) to internal standard (IS) (± standard deviation, n = 5 measurements) and statistical data for acetylisoniazid in the presence of fingerprint samples (natural and after handwashing) and blank solvent (methanol) extracted from paper and spiked with 20 ng/ml and 80 ng/ml compared to the reference standard at the same concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration level 20 ng/ml</th>
<th>Concentration level 80 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s (n = 5) (ratio A/IS)</td>
<td>Ref. std (ratio A/IS)</td>
</tr>
<tr>
<td>Fingerprints natural</td>
<td>0.554 ± 0.176</td>
<td>0.322 ± 0.004</td>
</tr>
<tr>
<td>Fingerprints soap</td>
<td>0.471 ± 0.042</td>
<td></td>
</tr>
<tr>
<td>Blank solvent (methanol)</td>
<td>0.387 ± 0.004</td>
<td></td>
</tr>
</tbody>
</table>

Note: s = standard deviation. For the two-tailed t-test: tcrit given in table (Miller & Miller, 2005).
Appendix F

F. 1  Publication: Ismail et al. (2017)
Appendix F

A diagnostic test for cocaine and benzoylecgonine in urine and oral fluid using portable mass spectrometry†

Mahado IsmaI,‡§ Mark Baumert,†‡ Derek Stevenson,§ John Watts,§ Roger Webb,§ Catia Costa*,‡ Fiona Robinson‡ and Melanie Bailey§

Surface mass spectrometry methods can be difficult to use effectively with low cost, portable mass spectrometers. This is because commercially available portable (single quadrupole) mass spectrometers lack the mass resolution to confidently differentiate between analyte and background signals. Additionally, current surface analysis methods provide no facility for chromatographic separation and therefore are vulnerable to ion suppression. Here we present a new analytical method where analytes are extracted from a sample using a solvent flush across the sample surface under high pressure separated using a chromatography column and then analysed using a portable mass spectrometer. The use of chromatography reduces ion suppression effects and this, used in combination with in-source fragmentation, increases selectivity, thereby allowing high sensitivity to be achieved with a portable and affordable quadrupole mass spectrometer. We demonstrate the efficacy of the method for the quantitative detection of cocaine and benzoylecgonine in urine and oral fluid. The method gives relative standard deviations below 15% (with one exception) and R² values above 0.990. The limits of detection for these analytes in oral fluid and urine are <30 ng ml⁻¹, which are comparable to the cut-offs currently used in drug testing, making the technique a possible candidate for roadside or clinic-based drug testing.

Introduction

Surface mass spectrometry is used in a wide array of disciplines to obtain chemical information from the surface of a sample. Since 2004, there has been an increase in techniques that can be used to liberate molecules from a surface under ambient pressure, followed by mass spectrometry detection. This has resulted in a step change in sample throughput, due to the fact that samples no longer need to be analysed under vacuum and require minimal sample preparation. Surface mass spectrometry techniques include desorption electrospray ionisation (DESI),## atmospheric pressure matrix-assisted laser desorption ionisation mass spectrometry (APMALDI),## plasma assisted desorption ionisation (PADI),## direct analysis in real time (DART),### MeVSecondary ion mass spectrometry (MeV-SIMS)### and desorption atmospheric pressure chemical ionisation (DAPCI).### These techniques have shown strength in many areas, but the absence of a chromatography step leaves the methods vulnerable to ion suppression effects.

In parallel to the development of ambient ionisation methods, a range of mass spectrometers have been miniaturised and tested for portable mass spectrometry.### Various combinations of these have been used in conjunction with methods such as DESI and paper spray to support portable analysis of drugs.### However, the limitation of miniaturised or portable mass spectrometers is that they are less powerful than lab based instruments in terms of resolving power and sensitivity.### This is problematic when encountering complex mixtures of samples. This combined with the issue of ion suppression effects limits the selectivity, sensitivity and quantification power of the portable mass spectrometry approaches to surface analysis.

In this work, we present a method that attempts to overcome the limitations of surface analysis by introducing a chromatography step. In the method, a solvent is flushed across the sample surface under pressure to extract analytes and the resulting solution is passed through a chromatography column before analysis using a portable mass spectrometer. The result is a low cost, sensitive and selective method of surface analysis.

Recent developments in a related technique, liquid extraction surface analysis (LISA),## have demonstrated the capability of using a liquid microjunction to extract analytes from a sample surface. The estimated analytes are collected in
Appendix F

Analytical Methods

Sample preparation

Oral fluid. A stock solution (1 mg mL⁻¹) of cocaine and benzoylcegonine was prepared in water by dilution of the CRM on the day of analysis. The pooled oral fluid samples were used to prepare calibration standards over the range 0-600 ng mL⁻¹ in pooled oral fluid by dilution of the stock solution. Internal standards were prepared by dilution of the CRM in water and spiked into the calibration standards to produce a final concentration of 150 ng mL⁻¹. Commercially available oral fluid collection devices (Quantum collection device, Alere Toxicology, United Kingdom) were used to absorb the calibration standards prepared in pooled oral fluid. This sampling method was applied to simulate drug testing using oral fluid devices. When 1 mL (±10%) of oral fluid was collected in the collection pad, the indicator on the stick turned blue and was placed in the collection tube containing a buffer (3 mL). For the samples collected from individuals at the drug treatment center, internal standard solution was spiked into the buffer of the Quantum device to produce a final concentration of 150 ng mL⁻¹.

Urine. Urine was collected in calibrated pots and stored at 4°C until required for sample preparation. Samples were prepared on the day of analysis. Urine samples were pooled and subsequently used to prepare a stock solution of cocaine and benzoylcegonine in pooled urine at 1 mg mL⁻¹ by dilution of the CRM. Calibration standards over the range 0-600 ng mL⁻¹ were prepared in pooled urine by serial dilution of the stock solution. Internal standards were prepared at 1 mg mL⁻¹ in pooled (drug-free) urine and spiked into the calibration standards to produce a final concentration of 160 ng mL⁻¹.

Instrumentation

Plate Express™ with expression compact mass spectrometer (Advion Inc., Ithaca, USA). The Plate Express is designed for sample extraction from a variety of flat surfaces (such as glass, paper, aluminium foil and T&G plates). The system is equipped with an oral shaped clamping head of 4 x 2 mm, with a depth of 250 μm, a laser targeting device, and automated head cleaning with high pressure gas. The sample is placed under the clamping head and the motor drives the head down to touch the sampling plate, then drives further down to compress the spring and generate the force required to seal the spot for extraction. The head has an oral shaped knife edge that is used to seal against the surface material. Inside the knife edge there is a fin that filters the eluted sample before being introduced onto the column for separation and subsequent mass spectral analysis.

Fig. 1 shows the Plate Express™ connected to a binary pump used to generate a gradient (see Table 1) for chromatographic separation. Chromatographic separation was carried out using an Ultra Rapid columns (60 mm x 2.1 mm, 5 μm) purchased from Restek (Pennsylvania, USA) for the separation of cocaine and benzoylcegonine. Chromatographic separation was previously optimised for cocaine and related metabolites (benzoylcegonine
Fig. 1 Schematic of the Plate Express combined with expression compact mass spectrometer. The compact expression mass spectrometer is single quadrupole mass analyzer with a mass range 10 to 2000 amu. The detector is a dynode combined with an electron multiplier scanning every 2 s. The mass spectrometer weighs 22 kg, dimensions 65 x 26 x 36 cm (length x width x height) and a power consumption of 300 VA maximum. The instrument is equipped with a rotary pump weighing 20 kg with dimensions 26 x 28 x 46 cm. The mass resolution is comparable to other portable mass spectrometers, at 5 - 10 m/z full width half maximum at m/z 1000 units sec⁻¹. The sensitivity is 10 pg methamphetamine in SIM mode.

Table 1 Binary gradient method for separation of cocaine and benzoylcgonine using an Ultra 300 column (50 mm x 2.1 mm, 5 μm) with solvent A: 0.1% formic acid in water and solvent B: 0.1% formic acid in acetonitrile.

<table>
<thead>
<tr>
<th>Flow (μl min⁻¹)</th>
<th>A (%)</th>
<th>B (%)</th>
<th>Time (min)</th>
<th>Gradient type</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>95</td>
<td>5</td>
<td>0.1</td>
<td>Step</td>
</tr>
<tr>
<td>0.5</td>
<td>95</td>
<td>5</td>
<td>2.0</td>
<td>Linear</td>
</tr>
<tr>
<td>0.5</td>
<td>95</td>
<td>5</td>
<td>1.0</td>
<td>Linear</td>
</tr>
<tr>
<td>0.5</td>
<td>80</td>
<td>20</td>
<td>2.0</td>
<td>Linear</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>100</td>
<td>1.0</td>
<td>Step</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>95</td>
<td>1.0</td>
<td>Step</td>
</tr>
</tbody>
</table>

and eugonine methyl esters (M) on a Waters 2695 separation module and Micromass Quattro Ultima mass spectrometer (see Supplemental Material for an example chromatogram). This method was adapted for the analysis of cocaine and benzoylecgonine on the Plate Express system using the same optimised gradient with lower flow rate. An electrospray ionisation (ESI) source was used in positive ion mode for ionisation. Ion source and mass spectrometer settings were as followed: capillary temperature, 250 °C; capillary voltage, 150 V; source voltage, 25 V; source voltage dynamic, 40 V; gas temperature, 300 °C; ESI voltage, 5.5 kV. The source voltage and source voltage dynamic were optimised to produce null in-source fragmentation of the analytes of interest. Analysis was performed in selected ion monitoring (SIM) mode for cocaine (m/z 344 > m/z 182), benzoylecgonine (m/z 340 > m/z 168), cocaine-d₄ (m/z 347 > m/z 185) and benzoylecgonine-d₄ (m/z 353 > m/z 171).

Analysis of cocaine and benzoylecgonine in urine and oral fluid. 10 μl of sample was added to the paper (Whatman chromatography paper, grade 1 CR) and analysed immediately. Repli cate measurements (n = 3) were carried out by adding 10 μl of the sample onto three separate sample substrates taped to a microscope glass slide. A continuous solvent flow was used to extract the analytes from the sample substrate. The analytes were extracted and separated using a binary gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, as shown in Table 1. The initial flow rate was set to 0.1 μl min⁻¹ to move the extracted sample past the valve without causing too much back-pressure, as this would cause the seal to leak. Extraction of the analytes from the sample surface is achieved by the initial composition of the binary gradient.

Results and Discussion

Analytical performance - urine samples

Chromatographic separation of cocaine and benzoylecgonine in spiked urine samples. Extracted ion chromatograms (XIC) of blank and spiked (100 ng μl⁻¹ cocaine and benzoylecgonine) pooled urine are shown in Figs 2A and B, respectively. The run time for each sample was 7 min and the respective retention times for cocaine and benzoylecgonine were 4.13 min and 4.91 min. Confirmation of the presence of cocaine and benzoylecgonine was achieved by in-source fragmentation. XICs for the fragment ions corresponding to cocaine (m/z 182) and benzoylecgonine (m/z 168) are also shown in Fig. 2 (with the same retention times as the respective molecular ions). The XIC of blank (drug-free) urine (see Fig. 2A) showed no interferences from endogenous analytes for the fragment ions of both analytes.
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Analytical Methods

Blank Urine

A

B

m/z 187
m/z 290

m/z 185
m/z 290

Retention Time (min)

Intensity (A.U.)

Retention Time (min)

Intensity (A.U.)

Fig. 2. Extracted ion chromatograms (XIC) for (A) cocaine and (B) benzoylecgonine in blank urine. XICs for (C) cocaine and (D) benzoylecgonine in spiked 200 ng mL⁻¹ cocaine and benzoylecgonine urine standard.

Spiked Urine

C

D

m/z 182
m/z 304

m/z 168
m/z 290

Retention Time (min)

Intensity (A.U.)

Retention Time (min)

Intensity (A.U.)

Fig. 3. Calibration curve for cocaine (m/z 152) and benzoylecgonine (m/z 168) in pooled urine analyzed using Thermo Trace™ coupled with an electrospray ionization mass spectrometer (ESI MS) with cocaine-d₄ (m/z 156) as internal standard.

R² 0.0000

R² 0.6987

Peak Area Ratio (Cocaine to IS)

Peak Area Ratio (Benzoylecgonine to IS)

Cocaine

Benzoylecgonine

Retrieved from the Journal's Digital Library.
Appendix F

Analytical performance - oral fluid samples

Extracted ion chromatograms (XIC) of blank and spiked (200 ng ml⁻¹ cocaine and benzoylecgonine) pooled oral fluid are shown in Fig. 4A and B, respectively. The respective retention times for cocaine and benzoylecgonine were 4.12 min and 4.01 min. XICs for the fragment ions corresponding to cocaine (m/z 182.1) and benzoylecgonine (m/z 166.1) are also shown in Fig. 4 (with the same retention times as the respective molecular ions). The chromatograms of blank (drug-free) oral fluid (see Fig. 4A) showed no interferences from endogenous analytes for both analytes.

Calibration curves. Calibration curves for pooled oral fluid spiked with cocaine and benzoylecgonine were linear over the range 0–400 ng ml⁻¹ with R² values of at least 0.999 (Fig. 5). The limit of detection (LOD) for cocaine and benzoylecgonine in oral fluid was below 14 ng ml⁻¹ as shown in Table 3.

Method sensitivity. The sensitivity of the method was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ) for cocaine and benzoylecgonine using LOD = 3SDblank / S, and LOQ = 10SDblank / S, where SDblank is the mean blank signal and S is the standard deviation of the blank signal.

Table 2 Analytical performance characteristics for cocaine and benzoylecgonine in oral fluid

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Slope (ng ml⁻¹)</th>
<th>R²</th>
<th>LOD (ng ml⁻¹)</th>
<th>LOQ (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>0.015</td>
<td>0.999</td>
<td>1.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>0.016</td>
<td>0.998</td>
<td>0.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Fig. 4 Extracted ion chromatograms (XIC) for (A) cocaine and (B) benzoylecgonine in blank oral fluid. XICs for (C) cocaine and (D) benzoylecgonine in spiked (200 ng ml⁻¹ cocaine and benzoylecgonine) oral fluid standard.
Appendix F

Analytical Methods

Cocaine

Benzoylcegonine

![Graphs showing calibration curves for cocaine and benzoylcegonine](image)

Fig. 5. Calibration curves for cocaine (m/z 104) and benzoylcegonine (m/z 258) in pooled oral fluid analysed using Plasma Expector™ with an expression compact mass spectrometer (CMS) with cocaine-d5 (m/z 119) internal standard.

The blank signals, calculated LODs are given in Table 4 for urine and oral fluid, respectively.

Cut-off levels currently employed for drug testing based on urinalysis are 150 ng mL⁻¹ for initial screening and 150 ng mL⁻¹ for confirmatory tests for cocaine. Similar work by Kirby et al. investigated the use of a portable mass spectrometer combined with digital microfluidics for the quantitation of cocaine in diluted urine and achieved LODs of 48 ng mL⁻¹ for cocaine. The LODs presented in Table 4 demonstrate the applicability of this new analytical method for cocaine detection in the context of these cut-off guidelines with better sensitivity.

Cut-off levels used by UK forensic providers for oral fluid screening and confirmatory tests are 30 ng mL⁻¹ and 8 ng mL⁻¹ for cocaine, respectively. The LODs for oral fluid for cocaine and benzoylcegonine shown in Table 4 are slightly above the cut-off levels currently employed. A limitation in this preliminary study was that only one oral fluid collection device (Quantitrap™ collection kit) was tested. Furthermore, the oral fluid samples were diluted using the oral fluid collection device as the collection pad is stored in 3 ml of extraction buffer and a further 0.6 ml of internal standard was added to the buffer. Only 10 μl of the resulting buffer solution was used for analysis, therefore, the sensitivity was limited by the sampling collection method and could be improved by collecting oral fluid samples in a smaller volume of buffer. Future work will also explore the extraction efficiency from the surface, use of different extraction solvents or alternative substrates to improve the sensitivity. It should be noted that in the configuration described in this paper, the initial mobile phase composition is used as the extraction solvent.

Cocaine and benzoylcegonine in samples from patients:

Oral fluid. Analysis of oral fluid samples collected from two patients attending an NHS drug & alcohol service showed detectable signals for cocaine and benzoylcegonine. Fig. 6 shows extracted ion chromatograms obtained from the analysis of pooled oral fluid samples using the new analytical method described previously. Fig. 6A and B show an extracted ion chromatogram of blank (pooled) oral fluid sample which indicates that no peak was obtained for cocaine and benzoylcegonine. Analysis of oral fluid samples from patients shows that peaks corresponding to cocaine (4.12 min) and benzoylcegonine (4.44 min) are detected with corresponding fragment ions (seen in Fig. 6C and D).

Urine. In addition to the oral fluid samples, corresponding urine samples were collected from these patients. Analysis of the urine samples showed that benzoylcegonine was present. No peaks were observed for the parent drug (cocaine). This is to be expected because cocaine is metabolised through the body before being excreted and so metabolite concentrations are higher in urine than in oral fluid. Another possibility for the absence of cocaine is the fact that the samples were not stabilised and therefore the cocaine may have been converted to benzoylcegonine.
Oral fluid is used by law enforcement agencies for roadside drug testing. Current devices based on antibody assays lack specificity and quantitative power. This work demonstrates the proof of concept, and that a portable mass spectrometry system for these campaigns should be investigated further. Additionally, urine analysis is used in a wide array of clinical applications and a low cost system for urine screening opens up possibilities of citing such an instrument in accident and emergency departments or GP practices.

The set up described here costs approximately £50k to install, with the mass spectrometer accounting for the majority of this cost. This can be compared with lab-based equipment used in toxicology that is typically an order of magnitude more expensive. This set up described here could potentially be interfaced with other portable mass spectrometers and indeed other configurations with increased portability could be investigated in the future. The system described here has the limitation of coming together with a 30 kg roughing pump, but has been used previously from the back of a van and therefore road side testing either with the mass spectrometer used here or a lighter alternative, is a real possibility for the future.

**Conclusions**

The method presented here offers a low cost, flexible and portable set up for analysis of analytes on flat surfaces. Whilst we have demonstrated the proof of concept for mobile drug testing of oral fluid and urine, there is a wide range of potential applications for which this methodology could be used. We have developed a new way to screen and quantify cocaine and related metabolite (benzoylecgonine) in biological fluids, using a combination of surface extraction, liquid chromatography and portable mass spectrometry. We have demonstrated the proof of concept for testing for cocaine in urine and oral fluid from patients. We have shown relevant levels of sensitivity (50 ng/mL) in these matrices, good linearity (R² 0.998) and relative standard deviations below 23% for replicate measurements. We therefore conclude that this configuration could be a candidate for roadside drug testing investigations in the future.

**Acknowledgements**

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Analytical Methods

Institute of Health Research (INHEA) is thanked for funding the Clinical Research Network Portfolio (ID 17487). In addition, the authors thank Julien Deraiche and Vladimir Filipov from the Ion Beam Centre for their help with the instrument set up and Ingo Zudovski for assistance in the analysis of samples. The authors also thank Clive Aldred from Advion Biosciences for his support with the instrument and Hazim F El-Ghali for his support in data handling. We would also like to thank the EPSRC Impact Acceleration Account for funding this work.

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Noninvasive Detection of Cocaine and Heroin Use with Single Fingerprints: Determination of an Environmental Cutoff

Mahadeo Ismail,1 Derek Stevenson,3 Catie Costa,1 Roger Webb,3 Marcel de Puit,4,6 and Melanie Bailey1

BACKGROUND: Recent publications have explored the possibility of using fingerprints to confirm drug use, but none has yet dealt with environmental contamination from fingerprints. Here we explore the possibility of establishing an environmental cutoff for drug testing from a single fingerprint.

METHODS: Fingerprint samples (n = 100) were collected from the hands of 50 non-drug users before and after handwashing to establish separate environmental cutoff values and testing protocols for cocaine, benzoylcegonine, heroin, and 6-monoacetylmorphine. The cutoff was challenged by testing the fingerprints of drug-free volunteers after shaking hands with drug users. Fingerprints from patients who tested positive for cocaine (n = 23) and heroin (n = 24) were also collected and analyzed.

RESULTS: A different cutoff value needed to be applied, depending on whether the fingerprints were collected as presented or after handwashing. Applying these cutoffs gave a 0% false-positive rate from the drug-free volunteers. After application of the cutoff, the detection rate (compared to patient testimony) for washed hands of patients was 87.5% for cocaine use and 100% for heroin use.

CONCLUSIONS: Fingerprints showed enhanced levels of cocaine, heroin, and their respective metabolites in patients who tested positive for the substances, compared with the population of non-drug users surveyed, and a cutoff (decision level) can be established. The cutoff is robust enough to account for small increases in analytic observed after secondary transfer.

The possibility of drug testing from a fingerprint has become the subject of many recent research articles, due to the ease and noninvasive nature of sample collection, as well as the fact that the donor’s identity is embedded within the ridge detail of the fingerprint itself (1, 2). This provides, in prospect, the possibility of rapidly and noninvasively carrying out drug testing in a way that is difficult to falsify. Several methods have been proposed for detection of drugs in fingerprints, mostly focused on contact residues or standards (3–9). A few reports have dealt with excreted drug metabolites (10–12) by use of direct or surface mass spectrometry approaches. While these approaches are attractive from the point of view of a fast turnaround in some cases (<2 min per sample), their quantitative capabilities are still limited. In contrast, LC-MS is the technique of choice among toxicologists for drug testing in other matrices owing to its superior selectivity and quantitative power, afforded by the chromatographic separation of analytes before mass spectrometric analysis (13–15). Analysis of fingerprint drug residues by LC-MS has been demonstrated previously (16–18). The limitation of LC-MS for fingerprint residue analysis is that the fingerprint must first be extracted from the deposition substrate, which reduces sample throughput compared with direct mass spectrometry methods. Nonetheless, attempts have been made to explore the detection window of both lorazepam and caffeine in fingerprints (16, 17) and to relate the fingerprint level of caffeine to a blood or oral fluid sample (18).

Despite the interest in testing for drugs from a fingerprint, to our knowledge, no studies have explored the robustness of fingerprint testing itself. Cocaine is an especially common environmental contaminant (20), and this deserves attention before fingerprints could be considered a credible testing matrix. In hair analysis, cutoff levels (21, 22) are used to ensure that environmental exposure can be eliminated as a possible source, but this has never been considered for a fingerprint test, probably because fingerprint testing is far less mature.

Here we report on a new LC-MS protocol that determines the relative mass of heroin, cocaine, and the
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respective metabolites, 6-monooacetylmorphine and benzoylacetone, in fingerprint samples. Benzoylacetone rather than cocaine methyl ester was monitored owing to its longer half-life in urine and for compatibility with current drug testing regimes (25, 24). The method has been applied to the fingerprints (n = 99) from 50 individuals who notified to be non-users to establish an environmental cutoff value. The fingerprint samples of 13 cocaine users and 12 heroin users were then measured against these cutoffs to determine drug use over the environmental level. The cutoff was challenged by testing non-users after shaking hands with drug users.

Materials and Methods

SAMPLE COLLECTION

A favorable critical opinion for collection and analysis of samples was received from the National Research Ethics Service (NRES-REC reference: 14/LO/0346).

Fingerprints were collected on 2 × 2 cm squares of Whatman 1-Chi-grade chromatography paper, with a single fingerprint collected per sample. Kitchen scales (Sartorius’s) were used to measure the pressure applied during collection (800–1200 g for 10 s). Fingerprint samples from the right thumb and right index finger were collected (a) by pressing and (b) after handwashing from 50 participants who notified not to be drug users.

Fingerprints were collected from individuals seeking treatment at drug rehabilitation clinics who notified to be taking either cocaine (n = 13) or heroin (n = 12) in the past 24 h. A fingerprint from each finger of the right hand (n = 5) was collected as described above. To investigate different sampling strategies, 8 of the participants were instructed to wash their hands thorougly with soap and water followed by wearing nitric gloves for 10 min to induce sweating, followed by removal of the gloves and finally depositing fingerprint samples.

Corresponding oral fluid samples were collected with a QuantisTM (Alere™) collection device. Oral fluid samples were analyzed by Claritec. Claritec screening uses immunoassay testing followed by LC-MS/MS quantification if screening is positive.

To test the possibility of secondary transfer of parent drug or metabolites, fingerprints (n = 5, right hand) were collected from drug-free volunteers. Hands were shaken with a drug user for approximately 2 s. Fingerprints (n = 5, right hand) were then collected from the drug-free volunteers after contact with the drug user.

MATERIALS

Drug standards (cocaïne, benzoylecgonine, heroin, 6-monooacetylmorphine, cocaine-d5, benzoylecgonine-d5, heroin-d5, and 6-monooacetylmorphine-d5) were prepared from certified reference materials (Ceraldian). Optima LC-MS grade solvents [methanol, dichloromethane, acetonitrile (ACN), and water] were used to prepare all solutions and solvent mixtures (Fischer Scientific). Formic acid (Fischer Scientific) was added to the mobile phase at 0.1% v/v. Artificial cocaine perspiration was purchased from Pickering Laboratories.

SAMPLE EXTRACTION AND PREPARATION

The paper samples were placed in a 2-mL Eppendorf microcentrifuge tube, following which the extraction solution (1.5 mL of 10% dichloromethane in methanol) was added. The tube was then centrifuged for 2 min (at 9500g centrifugal force). The paper was removed from the microcentrifuge tube and discarded. The solvent extract was evaporated to dryness under a stream of nitrogen at room temperature (20°C) and reconstituted in 100-μL mobile phase solution (50 ng/mL cocaine-d5, benzoylecgonine-d5, heroin-d5, and 6-monooacetylmorphine-d5 in 5% (v/v) ACN in water + 0.1% formic acid) before being water-mixed and transferred to a 300-μL glass microinsert vial, with 5 μL being injected onto the LC-MS/MS system.

Chromatographic separation was performed on a Thermo Scientific™ Ultimate3000 UHPLC system equipped with a binary solvent manager, column manager, and autosampler. Separation was performed on a Kinetex XB-C18 column (100 × 2.1 mm, 5 μm) operated at 30°C at a flow rate of 0.25 mL/min. Gradient analysis was performed with an initial mobile phase comprising 95% water (0.1% formic acid) and 5% ACN (0.1% formic acid) increased to 80% ACN (0.1% formic acid) and 20% water (0.1% formic acid) over 2 min and kept constant for 0.5 min before returning to the initial mobile phase composition (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.dinchem.org/content/10644/isure6). The samples were introduced to a Thermo Orbitrap Q-Exactive Plus mass spectrometer by the standard electrospray ionisation interface with a capillary temperature of 350°C and spray voltage of 3 kV (see Table 2 in the online Data Supplement). Positive mass spectra were acquired in full scan mode within a range of m/z 50–350 at a mass resolution of 70,000 at m/z 200.

METHOD VALIDATION

Extracted ion chromatograms for m/z 304.15 (assigned to cocaine), m/z 290.14 (assigned to benzoylecgonine), m/z 370.16 (assigned to heroin), and m/z 328.15 (assigned to 6-monooacetylmorphine) for supplemented (10 μL at 600 ng/mL) samples extracted from chromatography paper are shown in Fig. 1 in the online Data Supple-
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Peaks assignment was confirmed by verifying the exact mass peak within 5 ppm of the expected values by MS/MS fragmentation. The run time for each sample was 3 min, and the retention times for cocaine, benzoyllycgonine, heroin, and 6-monacetylmorphine were 2.12, 2.60, 1.35, and 2.06 min, respectively.

A linear calibration curve of added drugs in ACN was prepared with a working range of 500 pg/sample to 10 ng/sample. Each calibrator was prepared from a stock solution containing cocaine, benzoyllycgonine, heroin, and 6-monacetylmorphine in ACN at 5000 ng/mL. The stock solution was prepared from the certified reference material of the individual analyte at 1 g/L. Calibration standards were prepared at 50, 100, 200, 400, 600, 800, and 1000 ng/mL in ACN by dilution of the stock solution. Ten microliters of the calibrator were added to the sample substrate (Whatman 1-Chromatography paper, 2 × 2 cm) and allowed to dry overnight in the fume hood before being extracted and analyzed as detailed above. Each calibrator was re-injected 5 times. The mean peak area of the 5 repeated measurements was used to calculate the ratio analytic/internal standard (AIS) and is shown in Fig. 3 in the online Data Supplement. The R² value was >0.9995 for all analyses, and the precision was greater than ±1% (n = 25).

To determine limits of detection, 10 µL of solutions of the drug standard at 1, 2, 3, 4, and 5 ng/mL was pipetted onto paper substrates (2 × 2 cm) and allowed to dry in the fume hood. The subsequent sample was then extracted by using the developed extraction and analysis procedure. The limits of detection were determined as the mass of standard below which the analytic signal was no longer observed. The limits of detection (provided in Table 3 in the online Data Supplement) were 10, 30, 40, and 40 pg for cocaine, benzoyllycgonine, heroin, and 6-monacetylmorphine, respectively.

**Matrix Effects**

To test the matrix effects, samples were prepared as follows: 10 µL of supplemented standard solution (500 ng/mL) was deposited on a paper substrate in (a) ACN, (b) artificial sweat mix, (c) ACN after deposition of a fingerprint, and (d) ACN after deposition of a fingerprint from each of 4 participants after washing hands with soap, and (d) ACN after deposition of a fingerprint from each of 4 participants after wiping hands. In each case, extraction was performed as described earlier. Four replicate samples for each of the above sample were prepared and 5 injections of each were performed.

**Results and Discussion**

**Matrix Effects**

Fig. 3 in the online Data Supplement shows the ratio AIS obtained in each sample type, described in the previous section. For cocaine and benzoyllycgonine, no substantial difference was observed between the standards in the presence of ACN only and the standards in the presence of a fingerprint, and therefore no matrix effects were observed. For heroin and 6-monacetylmorphine, however, the presence of a fingerprint increased the ratio AIS by 29% and 17%, respectively, showing that the matrix effects needed to be considered for absolute measurements (i.e., mass per fingerprint). However, the close distribution of values (<5% variability) between the 4 participants studied here implied that there was no donor-dependent matrix effect. Additionally, given the small number of participants studied for matrix effects, a more thorough evaluation of participant dependence and fingerprint aging on matrix effects should be completed in the future. The presence of artificial cocaine sweat increased the ratio AIS by a factor of 3, showing that this standard was not representative of a fingerprint matrix. Therefore, in the absence of a validated standard, we took a previously used approach (16, 18, 19) and opted not to use a matrix-matched calibration curve. Subsequent results are therefore stated in terms of AIS rather than pg/fingerprint.

**Cocaine and Benzoylecgonine Detection in Fingerprints from the Background Population**

Fingerprint samples (right thumb and right index) were taken from 50 participants who testified not to be drug users. These were extracted and analyzed with the LC-MS method described earlier. Fig. 1 displays the ratio AIS peak area (5 replicate injections) corresponding to cocaine and benzoylecgonine. The limit of detection of the method (dashed line) shows detection of cocaine in 13 out of the 99 fingerprint samples and that of benzoylecgonine in 5 of the 99 fingerprint samples analyzed using this method. Note that for 1 sample, the LC-MS run failed—SUB016 (right index finger). There was clearly a requirement to impose an environmental cutoff on the data, particularly in the case of cocaine, as the method readily detected cocaine even in the fingerprints of nonusers. We propose an AIS cutoff of 0.026 for cocaine and 0.018 for benzoylecgonine on the basis of a 99% confidence limit calculated from the background samples, as indicated by the dotted line in Fig 1.

**Cocaine and Benzoylecgonine Detection in Fingerprints from Individuals Seeking Treatment for Drug Dependency**

Fingerprint samples (all fingers of the right hand) were taken from 13 participants who testified to taking cocaine in the past 24 h. These samples were extracted and analyzed with the same LC-MS method. Fig. 4 in the online Data Supplement shows the ratio AIS (5 replicate injections) corresponding to cocaine and benzoylecgonine for all 5 fingerprint samples collected. The data show the considerable variability between the fingerprint samples collected from the same participant, which could...
be expected owing to various factors that include the difference in surface area of the different fingers. Fig. 2 displays the same data, but for the same 2 fingers (right thumb and right index) as those presented for the background study. There was, in general, good agreement with patient testimony and the detection of cocaine and benzoylcegonine, with these compounds being detected above the cutoff in 100% and 85% of the fingerprint samples, respectively. There are, of course, obvious limitations of patient testimony—the patient may not have known what they were taking, may have forgotten when they had taken a substance, or deliberately falsified their information. This could be the case for participant 4, who tested negative in oral fluid and benzoylcegonine in the fingerprint samples. Benzoylcegonine was detected above the proposed cutoff only in 1 out of 2 fingerprints for participants 3 and 5, despite a positive oral fluid test result and cocaine detection in both fingerprints. This therefore shows an inevitable limitation of imposing an environmental cutoff, as benzoylcegonine was above the limit of detection in both fingerprints.

Table 4 in the online Data Supplement compares the oral fluid testing results to the fingerprint test results (based on the presence of a signal above the environmental cutoff). For participants 1 and 13, both cocaine and benzoylcegonine were detected at levels considerably greater than...
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Fig. 2. Ratio of analyte to internal standard (A/S) for peak areas corresponding to cocaine and benzoylegonine from the fingerprints (right thumb and right index) of 13 drug users, extracted from a filter paper and analyzed by LC MS.

Dotted line represents the limit of detection; dashed line represents the proposed cutoff.

than the environmental cutoff, despite the negative oral fluid test result. Sweat has a larger detection window than oral fluid (29), and therefore, we provide this as an explanation for the discrepancy in fingerprint and oral fluid results observed here. This is consistent with previous observations with paper spray mass spectrometry (42).

The model was challenged by the collection of fingerprints from 30 drug users working at the clinic before and then directly after shaking hands with 5 different drug users (Fig. 3). The levels of cocaine exceed the proposed cutoff for SUB002 (right thumb), SUB054 (right thumb), and SUB055 (right thumb and right in-

Fig. 3. Ratio of analyte to internal standard (A/S) for peak areas corresponding to cocaine and benzoylegonine from fingerprints (drug-free volunteers, with SUB as the subject number) deposited before and after shaking hands with drug users (SUB001 with participant 4, SUB002 with participant 5, SUB054 with participant 6, SUB055 participant 7, SUB056 with participant 8) testing positive for cocaine, extracted from a filter paper and analyzed by LC MS.

Dotted line represents the limit of detection; dashed line represents the proposed cutoff.
Table 1. Comparison of fingerprint and oral fluid screening for cocaine and benzylecgonine (BZE) in samples collected after handwashing from individuals seeking treatment for drug dependency.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Fingerprint screening results (with LOD as cutoff)</th>
<th>Oral fluid screening results</th>
<th>Patient testimony</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cocaine</td>
<td>BZE</td>
<td>Cocaine</td>
</tr>
<tr>
<td>1</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
<td>64 ng/mL</td>
</tr>
<tr>
<td>3</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;64 ng/mL</td>
</tr>
<tr>
<td>6</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;64 ng/mL</td>
</tr>
<tr>
<td>7</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;64 ng/mL</td>
</tr>
<tr>
<td>8</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;64 ng/mL</td>
</tr>
</tbody>
</table>

After shaking hands (Fig. 3), benzylecgonine was never observed to exceed the cutoff. This is important given that cocaine can chemically convert to benzoylecgonine (26, 27). This was true even after shaking hands with participant 7, who also had the highest levels of drug present in their fingerprints (Fig. 2). Therefore, if the testing regime requires benzylecgonine to be present in a fingerprint sample for a positive test result, the test is robust enough that secondary transfer presented here would return a negative result. This would result in a reduced detection rate of 85.4%, but no false positives from the data presented here.

Effects of Handwashing on the Detection of Cocaine and Benzylecgonine

The results showed good agreement with patient testimony for cocaine use but have so far only considered unwashed hands. Discrimination of contact residue from excreted drugs and metabolites would be essential for any quantitative test. Also, any test from a fingerprint must be robust enough to have a good detection rate even if an individual has washed their hands before being tested, otherwise the test would be easily falsified. Therefore, 8 patients who testified to not taking cocaine were asked to wash their hands with soap and water after initial deposition of fingerprints. The signals corresponding to cocaine and benzylecgonine are plotted in Fig. 5 in the online Data Supplement. Cocaine was present in all fingerprints even after handwashing (Table 1), and benzylecgonine was present in all but 1 of the fingerprints of participant 4, consistent with the data for unwashed hands for this participant. It is noteworthy that for participant 1, whose oral fluid tested negative, both benzylecgonine and cocaine were still detected in the fingerprints even after handwashing. Imposing the same environmental cutoff conditions as for the unwashed hands on these data is of course not appropriate here, because the background population had not washed their hands. Therefore, a cutoff based on the fingerprints of the background population after handwashing was imposed. The limit of detection was used here as the cutoff, because cocaine and benzylecgonine were detected in only 1 out of 100 and 0 out of 100 fingerprint samples, respectively. The detection rate was therefore 87.5% and the false-positive rate was 0.0%, based on the detection of benzylecgonine.

Heroin and 6-Monoacetylmorphine Detection from Fingerprints

Fingerprint samples (right thumb and right index) were taken from 50 participants who testified to not being drug users and extracted and analyzed by the same LC-MS method described in previous sections. No signals corresponding to heroin were observed in any sample, and a signal above the limit of detection (0.007 compared with 0.003) was observed for 6-monoacetylmorphine in only 1 of the 99 fingerprints tested.

As detailed in the previous section, fingerprint samples (all fingers of the right hand) were taken from 12 participants who testified to taking heroin in the past 24 h. These samples were extracted and analyzed by the LC-MS method described in the previous sections. Fig. 6 in the online Data Supplement shows the ratio A1S for peak areas (5 replicate injections per sample) corresponding to heroin and 6-monoacetylmorphine for all 5 fingerprint samples collected. The data again show the considerable variability between the fingerprint samples collected from the same participant. It is perhaps surprising to see the parent drug together with the metabolite in the fingerprint samples, as heroin is quickly metabolized by the body (28). It is possible that the detection of heroin and its metabolite in these fingerprint samples...
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![Graphs showing ratio of analyte to internal standard (A/S) for peak areas corresponding to heroin and 6-monoacetylmorphine from the fingerprints (right thumb and right index) of 12 drug users, extracted from a filter paper and analyzed by GC/MS. Dotted line represents limit of detection.]

Fig. 4. Ratio of analyte to internal standard (A/S) for peak areas corresponding to heroin and 6-monoacetylmorphine from the fingerprints (right thumb and right index) of 12 drug users, extracted from a filter paper and analyzed by GC/MS. Dotted line represents limit of detection.

Therefore, data on the background and extraction of metabolites, as the fingerprints were taken without handwashing before deposition. Fig. 4 shows the same data, but for the right thumb and right index finger corresponding to those used for the background study. Here, the data shows excellent agreement with patient testimony, with heroin and 6-monoacetylmorphine detected at levels above the limit of detection for all samples.

Table 5 in the online Data Supplement compares the oral fluid test results to the fingerprinting results. The fingerprints of participants 1, 2, 3, and 8 tested positive for both heroin and 6-monoacetylmorphine at levels considerably greater than the background population, despite negative oral fluid test results for these participants. This can be explained by either a longer detection window for heroin in sweat than in oral fluid or the prevalence of contact residues on the patients, as fingerprints were deposited without handwashing.

To investigate the potential for contact residue and secondary transfer, fingerprints from researchers working at a clinic session were taken before and directly after shaking hands with 3 different heroin users (participants 6–8). The level of heroin observed exceeded the limit of detection in only 1 case after working at the clinic and after contact with a drug user (see Fig. 7 in the online Data Supplement). It is therefore likely that

<table>
<thead>
<tr>
<th>Participant</th>
<th>Heroin</th>
<th>6-MAM</th>
<th>Morphine</th>
<th>6-MAM</th>
<th>Patient testimony</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
<td>Negative</td>
<td>Cocaine, morphine</td>
</tr>
<tr>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>3</td>
<td>1/2</td>
<td>2/2</td>
<td>Negative</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>4</td>
<td>1/2</td>
<td>2/2</td>
<td>Negative</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>5</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>6</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
<td>22.6 ng/mL</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>7</td>
<td>2/2</td>
<td>2/2</td>
<td>90 ng/mL</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>8</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>14</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;240 ng/mL</td>
<td>&gt;52 ng/mL</td>
<td>Heroin</td>
</tr>
<tr>
<td>15</td>
<td>2/2</td>
<td>2/2</td>
<td>138 ng/mL</td>
<td>32 ng/mL</td>
<td>Heroin</td>
</tr>
</tbody>
</table>

Table 2. Comparison of fingerprint and oral fluid screening for heroin and 6-monoacetylmorphine (6-MAM) in samples collected from individuals seeking treatment for drug dependency.
the heroin present in the fingerprints collected from the patient population would have come from a source other than contact with other users or surfaces within the clinic.

**Effects of Handwashing on the Detection of Heroin and 6-Monoacetylmorphine**

The eight patients who tested positive for heroin were asked to wash their hands with soap and water after initial deposition of fingerprints. The signals corresponding to heroin and 6-monoacetylmorphine are plotted in Fig. 8 of the online Data Supplement. 6-Monoacetylmorphine was present in all fingerprints, and heroin was present in some fingerprints but not those from participant 3, even after handwashing, as shown in Table 2. Therefore, a testing procedure that requires 6-monoacetylmorphine to be present in a fingerprint sample for a positive test would give a 100% detection rate with 0% false positives.

In summary, we have developed an LC-MS method for testing both cocaine and heroin use from a single fingerprint. Testing from a fingerprint is rapid and affords the opportunity for biometric identification directly from the sample, ensuring traceability. Although this is not explored here, the development of a fingerprint ridge detail before mass spectrometry analysis has been demonstrated (12) and could in theory be applied to fingerprint testing with the method presented here.

This is, we believe, the first study to explore the significance of reciting for drugs from a fingerprint, and therefore, the first effort dedicated to establishing an environmental cutoff. By testing the fingerprints from 50 blood donors, and fingerprints from nondrug users after testing with patients, we have constructed and tested an environmental cutoff for cocaine and heroin from a fingerprint. The cut-off level used here cannot be applied universally but it serves to illustrate the distinction between the fingerprints of drug users and nondrug users of cocaine and heroin.

**Author Contributions.** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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**Patents.** None declared.

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**References**


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