QUANTIFICATION OF COCAINE TRACES ON BANKNOTES

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Abstract

Money suspected of being involved with drug related crime can be seized by law enforcement agencies and forensically tested for traces of drug contamination. Forensic experts compare these results with background levels found on ‘normal’ banknotes, and opine as to whether the contamination indicates involvement with illicit activities. Experiments were conducted to establish the efficiency of using thermal desorption tandem mass spectrometry (TD-MS/MS) for quantitative banknote analysis, and to compare with other techniques available. Certified standards of deuterated cocaine and cocaine hydrochloride were used for instrument calibration, optimisation, or as an internal standard.

Solvent extraction from a quarter of a ‘normal’ banknote (obtained from a bank) using methanol was conducted. The extraction method involved twice washing and then a final rinse. Extraction efficiencies were calculated to be 95%, with a standard deviation of +/- 4.

Extracts were spiked with 500 ng/mL deuterated standard, and injected into the TD-MS/MS in selected reaction monitoring mode. Isotope dilution using known amounts of cocaine hydrochloride and deuterated cocaine was used to establish a response factor. The peak areas of the deuterated internal standard were compared with the cocaine extracts, and the response factor was used to calculate the amount of cocaine. Average amounts of 4.6 µg and 7.7 µg were detected on individual banknotes, and batch tests of banknotes, respectively.

GC/MS gave initial results of approximately 20 µg cocaine per banknote, following drying and reconstitution of the extract. Further research of internal standards and response factor is needed. Simple filtration clean up, or drying under nitrogen and reconstituting in mobile phase was applied for HPLC, however, tests proved inconclusive with further work required into clean up steps. Fluorescence work identified self-quenching and potential interferents from banknotes.
Overall the TD-MS/MS work proved successful and found that amounts of cocaine on banknotes can be established using isotope dilution, with approximately 10% error. GC/MS could be a useful comparative tool.
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Abbreviations

APCI – Atmospheric pressure chemical ionisation
FSS – Forensic Science Service
GC – Gas chromatography
GC/MS – Gas chromatography/mass spectrometry
HMRC – Her Majesty’s Revenue and Customs
HO – Home Office
HPLC – High performance liquid chromatography
HSCIC – Health & Social Care Information Centre
LGC Ltd. – Forensic laboratory formerly known as the Laboratory of the Government Chemist
MDA – Misuse of drugs act
MDMA – Methyleneoxyamphetamine
MSA – Mass Spec Analytical Ltd.
MS/MS – Tandem mass spectrometry
ONS – Office for National Statistics
RF – Response Factor
RSD – Relative Standard Deviation
SIM – Selected ion monitoring
SPE – Solid phase extraction
SRM – Selected Reaction Monitoring
Std Dev – Standard Deviation
TD-MS/MS – Thermal Desorption-tandem Mass Spectrometry
THC – Tetrahydrocannabinol
UN – United Nations
UNODC – United Nations Office on Drugs and Crime
UK – United Kingdom
USA – United States of America
UV – Ultraviolet
1.0 Introduction

1.1 Forensic Analysis

The use of forensic analysis to provide scientific evidence in the investigation of crimes and in subsequent trials has increased over the years (Ludwig & Fraser, 2014; Mennell, 2006; White, 1998). Every year, hundreds of thousands of cases are forensically analysed by the variety of in-house and private companies across the United Kingdom (UK). In 2004-2005, the former Forensic Science Service (FSS) alone dealt with the forensic aspects of 140,000 cases, and gave expert evidence in court on 2,500 of them (FSS, 2005). Developments in instrument sensitivity and computer technology have enabled a much wider scope of analysis for forensic scientists, now allowing the detection of traces which were previously too small to identify. Further developments continue to assist courts in maintaining a fair justice system, yet new knowledge comes under scrutiny, thus requiring more research to further support the ever changing role of forensic evidence (National Research Council (NRC) 2009; Parliamentary Office of Science and Technology (POST) 2005).

1.2 Tackling Drug Related Crime

The UK has seen the introduction of the Serious Organised Crime Agency in 2006 (now the National Crime Agency ‘NCA’), as well as other groups, who undertake one of the government’s highest priorities i.e. tackling drug use, drug dealing and related crimes, such as, money laundering (HM Government, 2010; Home Office (HO) 2007b).

In 2013/14, over 13,000 seizures, totalling 3.4 tons of cocaine were seized by Police and Her Majesty’s Revenue and Customs (HMRC) officers and £33.3 million of drug related criminal assets were seized from those involved with drugs (HO, 2007b). Such asset seizures have been performed in the UK using the Drug Trafficking Offences Act (1986) and subsequent amendments, and the Proceeds of Crime Act (2002). Both of these Acts declare that the proceeds of illicit activities
may be confiscated i.e. depriving traffickers and drug dealers of the proceeds of their criminal activity (Rees and Fisher, 2005; Bean, 2004). UK government has invested heavily in the effective seizure of drugs and associated assets. However, it has been reported that a set-up cost of £60 million yielded seizures of £24 million, a figure which will take time to provide a decent return. Despite this, there is little evidence to show that the drugs trade in the UK is decreasing significantly.

Drug dealing and trafficking both involve the transfer of money for payment of goods. Therefore such monies can be used to form evidence in part of an investigation. Banknotes which have been in close contact with drugs or other items which are contaminated with such drugs (e.g. the ‘dirty’ hands of a user/dealer) may well become contaminated themselves (Frederick, Pertaub and Kam, 2007). This is based upon Locard’s principle that ‘every contact leaves a trace’, and could relate to many different steps of transfer, such as primary, secondary or tertiary etc., as described in Table 1 (White, 1998).

<table>
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<th><strong>Table 1. Principles of Trace Transfer</strong></th>
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<td><strong>Primary</strong></td>
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</tr>
<tr>
<td><strong>Secondary</strong></td>
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<tr>
<td>Transfer from the contaminated object to another object (e.g. onto banknotes through handling the notes using contaminated hands).</td>
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<tr>
<td><strong>Tertiary</strong></td>
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<td>Transfer from the secondary object to a third (into a wallet used to store the aforementioned banknotes).</td>
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In the chain of people involved in the supply of drugs, those who deal/sell drugs will probably handle large amounts of money (in terms of paper currency), when compared with that of the user. Therefore, investigations have often been made into money or associated possessions discovered on suspected drug dealers/traffickers or found in their premises, and whether this can be linked to drugs (Noonan et al., 2005). Forensic techniques can be used to test banknotes for the presence of drugs (section 1.4). Such traces may have occurred through being in close contact with
drugs or items contaminated with drugs and can help to form links between the owner of the banknotes and drug related activities (Sleeman et al., 2000). Oyler, Darwin and Cone (1996) refer to a study which found that US$1 bills can bind with cocaine particles in a specific, reversible manner which may be one reason for its retention on banknotes. There may be several other plausible explanations for cocaine retention, and research has shown that banknotes can become contaminated by a variety of other mediums, for example via counting machines (Carter, Sleeman and Parry, 2003; Sleeman et al., 2000).

1.3 Drug Abuse & Cocaine

1.3.1 History of Cocaine

The coca plants (of the genus *Erythroxylum*) from which the tropane alkaloid, cocaine, is extracted, are native to South America. Products from these plants have been used in indigenous communities for thousands of years, although not perhaps in quite the same way as it has been recently abused (Constable, 2002). Coca leaves were used as good luck charms in some civilisations or chewed by others to enhance meditative states, reduce the effects of altitude sickness and overcome fatigue. When chewed with lime, the alkaloid is produced and is absorbed into the body. This method of administration, however, yields much less cocaine alkaloid than any modern cocaine powder and would have had reduced effects (Gold, 1993).

In 1859, the primary alkaloid from coca leaves (cocaine) was extracted by Albert Niemann and was subsequently used by doctors and scientists to treat a wide range of illnesses. Soon after, new methods of over-the-counter remedies were introduced, such as cocaine cigarettes, toothache drops and a new drink containing cocaine called ‘Coca-Cola’ (Gold, 1993). In the early 20th Century, the side effects of cocaine, addiction, mucous membrane breakdown and body dysfunction, had been discovered and cocaine was removed from ‘Coca-Cola’ and other remedies: This was followed in America by new legislation (Pure Food and Drugs Act, 1906, and the Harrison Narcotics Act, 1914), which limited the drug to a ‘prescription only’ medicine and was thereafter abused much less (Lakoski, Galloway and White,
Such legislations and limitations, combined with the addictive ‘need’ and desire for the drug led to the illegal trafficking of cocaine across the globe. Similar reductions in cocaine use were observed in Europe, and were partly due to supply problems as a result of the World Wars. Following the Wars, cocaine trafficking soon began again and was assisted by the change in societal mood in the 1960’s, with many believing that drug use was more acceptable (Constable, 2002; Gold, 1993).

Today, cocaine abuse is still threatening lives and economies, despite changes in the opinions of many in society, who now regard the drug’s use as dangerous. The issue is worldwide and effects millions of people every year, as hundreds of tons of cocaine are smuggled illegally across the Globe (Figure 1).

Figure 1. Global Cocaine Consumption and Transport Routes
(Used with permission of the publisher, United Nations Office on Drugs and Crime (UNODC, 2010))

1.3.2 Cocaine as a controlled substance

There are two main types of cocaine abuse today:-

- The salt in powder form, which is most often snorted.
- The free-base in crystal form (crack), the fumes of which are inhaled.
The cocaine hydrochloride salt and a stereoisomer are displayed in Figure 2. This is the most common type of cocaine abused (Cabovska, Norman and Stalcup, 2003).

![Figure 2. Structure of Cocaine Hydrochloride (a) and a stereoisomer (b)](image)

All structures were produced using ACD/ChemSketch (2015).

The free base (Figure 3) can be found in powder or crystal form, which is created using slightly different methods of precipitation from the salt. An additional step involving alkali ammonia or bicarbonate precipitates the cocaine from a cocaine hydrochloride solution (UNODC, 2012).

![Figure 3. Cocaine freebase structure](image)

Cocaine preparation for ‘street use’ can be extracted from the leaves in a number of ways (Figure 4). Often the leaves are pressed along with sulphuric acid and crushed to form cocaine sulphate, hydrochloric acid is then added to produce cocaine hydrochloride (Gold, 1993), which is then prepared as paste, crystal or powder for transportation.
In 1961, cocaine became an internationally controlled substance under Schedule 1 of the Single Convention of Narcotics Drugs (UNODC, 2012). A controlled substance is a compound listed in the legislation of a particular country for the purposes of restricting its use, production and possession. The Misuse of Drugs Act (1971) (MDA, 1971) and Misuse of Drugs Regulations (1985) (MDR, 1985) have been introduced in the UK and the drugs concerned are split into three classes (MDA, 1971) and five schedules (MDR, 1985). The most strictly controlled substances are those in Class A and Schedule 1. The class in which a drug falls within is based upon the perceived danger of the drug, and the Schedule is upon how it is controlled in terms of possession/prescription (MDA, 1971).

The Acts cover both the possession of a controlled substance and other activities such as importation, exportation, production, supply and cultivation of those drugs listed in the Act (Bennett & Holloway, 2005). Cocaine is one of the most prevalent
illicit substances and in the UK is categorised under Class A (the highest classification) within the MDA (1971) and Schedule 2 (a highly controlled substance only available as prescription), (Home Office Statistical Bulletin (HO), 2007).

1.3.3 Pharmacology

The effects that cocaine has on an individual can vary dramatically depending on the person, dosage and route of administration. There are various routes of administration (Lakoski, Galloway and White, 1991):

- Snorting or rubbing on the gums enabling the drug to pass into the blood system through the mucous membranes via diffusion, (a method most commonly used),
- inhaling fumes when cocaine’s base (crack) is heated to pass aerosol vapour into the lung tissue, wherein transfer into the blood vessels occurs (less common, with much more significant ‘highs’ and ‘lows’),
- cocaine hydrochloride can be dissolved and injected directly into the bloodstream (with similar dosage to snorting, injection is not the preferred method); or
- as an elixir in tea where it is ingested (lowest dosage, uptake and effects).

Cocaine is classed as a stimulant, which interacts with the dopamine pathways from the brain, leaving excess dopamine within the synapse. It also constricts blood vessels and can raise blood pressure and heart rates (Lakoski, Galloway and White, 1991). It is said to enhance sensations and make the user more confident and alert and has been shown to reduce hunger and altitude sickness. In addition to the physical effects and dangers, another problem relating to cocaine use is that of psychological dependence as the body builds a tolerance to ever increasing doses, and the after-effects of ‘coming down’ after the stimulant effect wears off make the user want more (Constable, 2002). Almeida, Cassella and Pacheco (2015) also refer to cocaine causing drug users to become violent with prolonged use, and UNODC (2012) reports malnutrition, hallucination and paranoid psychosis.
1.3.4 Cocaine Abuse and Related Crime

In 2008, the illegal cocaine market in the United States of America (USA) and Europe was estimated to be worth $60 billion; this figure is based upon the production of approximately 220,000 tons of dried cocaine annually (a decrease of approximately 15% over recent years), (UNODC, 2014).

Recent studies have shown that cocaine is the second most commonly used drug in the UK, and within the past year was used by 2.2 million people throughout Europe (Health & Social Care Information Centre (HSCIC), 2014). The number of people using cocaine has increased since the mid-nineties (Figure 5); however despite it becoming slightly less popular in recent years, there were 169 cocaine related deaths reported in the UK in 2013 (Office for National Statistics (ONS) 2014).

Worldwide, there are approximately 17 million cocaine users (UNODC, 2014). Although this may represent a rather small percentage (<0.5% globally), it still plays a role in damaging lives and economies; therefore government strategies to
tackle both the supply and use of illicit substances have grown in recent years (Constable, 2002; HO, 2007a; UNODC, 2014).

A crime survey in the UK found that approximately 50% of arrestees who were cocaine users were responsible for property crime, using it to fund their drug habit (Holloway et al., 2004), which indicates the large scale of additional crimes that relate to drug abuse. Furthermore, drug use and dealing can lead to gang/dealer related crimes, which consequently harm lives and communities (Bennett, 2005; Bean, 2004). In the regions where cocaine is cultivated, territorial disputes between dealers and violent armed groups cause serious problems for innocent locals and police authorities (Almeida, Cassella and Pacheco 2015).

Drug trafficking involves the transfer of illicit substances, across the globe, and can severely effect economic growth, development and even destabilise political states. In the USA in 2013, approximately 10% of all cases reported to the United States Sentencing Commission were related to cocaine trafficking (United States Sentencing Commission (USSC) 2013). Additionally, serious threats to societies have been introduced with reports of violent crime and murder, stemming from drugs trafficking and the need to establish strong supply routes (UNODC, 2010). As cocaine is transported from its native region abroad, many different individuals or groups will ‘cut’ the cocaine with various agents, bulking it out and diluting its overall purity. This is then sold on to make a larger profit. The route cocaine takes can vary, but in its simplest form can travel through multiple hands (Figure 6).

The compounds chosen can be diluents (substances purely chosen to bulk out the cocaine) or adulterants (compounds with similar surface properties of cocaine) which enhance its perceived value as being more ‘pure’. Common adulterants include benzocaine or lidocaine (which have similar anaesthetic properties) or caffeine (which has a stimulant effect) (Home Office Factsheet, 2015). Diluents can often include boric acid (insecticide) and tetramisole (animal worming ingredient).
The bulking stages significantly increase the value of the final volume of substance: The difference between the price of cocaine upon production in South America, versus its distribution price to users is large and helps to clarify why the illicit market still remains (Figure 7).

Figure 7 shows that the potential profits from drug dealing or trafficking are 100 times greater than that during production. The constant influx of new users who become addicted adds to the demand for the drug, fuelling drug related crime.
1.4 Techniques & Methodology

1.4.1 Detecting Cocaine on Banknotes and Other Items

A variety of methods exist for the detection and quantification of trace substances on forensic items. The method employed in each instance varies depending upon:

- The suitability to the particular substance matrix.
- The speed of analysis.
- Cost.
- Limit of detection.
- Accuracy.
- Overall objectives.

It is not common to find a method which encompasses all of the ideal abovementioned factors, and there will often be a trade-off between them. A quick analysis method with little sample preparation can assist with large scale throughput, helping with statistical evaluation of data, whereas if high accuracy is needed, a slower quantitative technique may be more suited. Within this section, a number of different methods of analysis are discussed, focusing on the detection and quantification of traces of cocaine on items.

1.4.2 Thermal Desorption-Tandem Mass Spectrometry

Sleeman et al. (2000 & 2005) reported a method for the detection of drugs on banknotes using thermal desorption-tandem mass spectrometry (TD-MS/MS), and background studies have shown that it is common to detect trace levels of cocaine on most banknotes taken from general circulation in the UK. When a banknote is inserted between two heated blocks for 1-2 seconds (Figure 8), drug contamination present on the banknote will vaporise and is drawn into the ionisation chamber in a stream of atmospheric air. Molecules enter a plenum chamber and pass through a corona discharge region where atmospheric pressure chemical ionisation (APCI) takes place (either positive [M+H]^+ or negative [M-H]^-, M). The ions then pass through an orifice into the first quadrupole region.
Figure 8. Specially adapted thermal desorption unit used to extract drugs from the surfaces of banknotes for analysis using tandem mass spectrometry (designed by Mass Spec Analytical Ltd. Bristol, UK)

The triple quadrupole tandem mass spectrometer, which is programmed to operate in selected reaction monitoring (SRM) mode, is highly specific as it can separate a number of different ions and subsequently their fragments. This allows two stages of differentiation from other molecules with similar mass (Figure 9) at the ‘Q1’ and ‘Q3’ positions as described in the diagram below.

Figure 9. The triple quadrupole arrangement of the API 2000 used by Sleeman, Carter and Ebejer (2005)

(Adapted and used with permission of the publisher (Applied Biosystems, 2005))
In the case of cocaine, positive ions are formed through the transfer of a proton from protonated water molecules \([H_3O]^+\) to the cocaine molecule. These ions travel through the orifice into the first quadrupole. The first quadrupole (Q1) selects the precursor ions and focuses it onto the next quadrupole via electrostatic fields. The second quadrupole (Q2) uses collision associated dissociation to fragment the molecular ion (Figure 10), which then passes into the third quadrupole. The final quadrupole (Q3) selects two specific fragments of the ion to pass onto the detector (an electron multiplier tube). The overall response (given in counts per second) is representative of relative amount of each product ion.

**Figure 10.** Selected ionisation pathway examples of a cocaine ion \([M+H]^+\) to produce product ions at \(m/z\) 182 and 105

This is a rapid process which allows a large number of banknotes (approximately ten per minute) to be analysed over a relatively short period of time (Ebejer *et al.* 2005). The responses are recorded in real-time, and require no sample preparation as the drugs are desorbed using the hot metal blocks. Absolute quantification of the exact amount is not possible due to losses observed in the open thermal desorption unit. The losses are greater than those observed in the use of other techniques, such
as, LC-MS/MS or GC/MS, which inject liquid samples directly into a closed system.

The method (Thermal Desorption Atmospheric Pressure Chemical Ionisation Tandem (APCI) Mass Spectrometry (TD-APCI-MS/MS)) in SRM mode is used by Mass Spec Analytical Ltd. (MSA). MSA (to which Sleeman et al. (2000) refers) conducts this form of analysis for the police and other authorities, and evidence of this nature is used in the courts of law frequently.

This method has been used to analyse over 125,000 individual banknotes taken from banks, shops and other sources across the UK to establish the background levels of contamination found on notes from ‘general circulation’ (Mass Spec Analytical (MSA), 2015). General circulation in these terms refers to banknotes obtained during legal everyday transactions, such as, withdrawals from banks or shop/business transactions. By comparing the contamination detected on the general circulation samples with the contamination detected on a sample of banknotes believed to have been involved with illicit activities, any differences in contamination can be observed.

There are several considerations when reviewing the contamination:

- Which drug(s) has been detected.
- The proportion of banknotes contaminated with a certain drug.
- The relative amounts of contamination on each note.
- Any pattern of contamination.

The general circulation database demonstrates that it is common to detect traces of cocaine on Bank of England banknotes at varying amounts, whereas other drugs (methylenedioxymethamphetamine (MDMA), amphetamine, diamorphine and Δ⁹-tetrahydrocannabinol (THC)) are much less prevalent (MSA, 2015). Therefore, the comparison of cocaine contamination is conducted by reviewing the relative amounts of contamination detected on a sample of banknotes from each exhibit. The comparisons are based upon reviewing the peak areas and height of the responses derived from each individual banknote. Since cocaine is more prevalent,
and the comparisons are more complex than mere identification alone, cocaine is where this research focuses its attention.

When reviewing the general circulation database, experts can determine what levels of cocaine are deemed within a normal range by considering the peak areas mentioned above. When analysed in an identical manner to the general circulation banknotes tested, if a proportion of banknotes from an exhibit are considered to be more highly contaminated than those found in general circulation (e.g. on banknotes withdrawn from banks), then it may be classed as unusual (Sleeman et al., 2000). The overall expert opinion may also be based upon the exhibit sample size and any additional information related to the exhibit (such as poor handling procedures and proximity to any bulk drugs).

Figure 11 demonstrates the differences seen in contamination between a sample of banknotes taken from general circulation and banknotes from an exhibit which were suspected of involvement with drug related crime. Each individual peak corresponds to a single contaminated banknote, and represents the sum of the two ions of interest; the larger the peak area and height, the greater amount of drug detected. In general, larger peak areas (representing larger amounts of contamination) are observed on the data set from Figure 11b (an exhibit suspected of being involved with drugs), when compared with the data set within Figure 11a (banknotes taken from a bank).

The absolute quantities cannot be determined using this method and thus limit the available comparisons to ‘like with like’ analyses. As there is only one company currently using the individual banknote analyser in the UK and limited inter-lab comparison available, a technique allowing quantification would be of great value, for confirmation and scientific evaluation purposes. Further research is also needed to allow better comparison and interpretation of the data with other techniques, such as LC-MS/MS or GC/MS.
Figure 11. Cocaine Ion Count Intensity vs. Time, detected on (a) 125 sequentially tested banknotes taken from general circulation and classed as ‘normal’, and (b) 141 sequentially tested banknotes taken from an exhibit seized by Police and classed as unusual.
1.4.3 *Gas Chromatography Mass Spectrometry (GC/MS)*

In many cases of banknote contamination analysis, the banknotes are washed with a choice of solvent (sometimes acetonitrile, chloroform or methanol) to remove drug contamination. The solute is then tested by GC/MS or GC/MS/MS following a clean-up step (Esteve-Turrillas *et al.*, 2005; Jenkins, 2001) (Figure 12). The use of GC/MS to analyse the solute not only allows the detection of various contaminants, but also under certain circumstances, to quantify the amounts.

GC/MS analysis is carried out by injecting a known amount of the solute into the inlet, where it is vaporised, and transported onto the column using a carrier gas. In most circumstances of drug analysis a fused silica column is used, with longer lengths and varying parameter settings or coatings enabling higher resolution (Gough, 1991; UNODC, 2012). The stationary phase of the column is also of importance, as certain phases may not elute all of the drugs of interest.

Drugs are detected by comparing the retention times and spectra of different molecules with that of a standard. Although many drugs can be analysed in this manner, certain substances do not elute from the column or they may decompose in

---

Figure 12. Schematic of a GC/MS
the oven and need to be derivatised before analysis. Such sample preparation can be complex and adds to the time taken to analyse a sample.

GC can be used to quantify substances, and when analysing drugs an internal standard is most commonly used (Liu, Canfield and Wang, 2009; Gough, 1991). The internal standard chosen should not react with any of the species in the sample and is often added to the sample before analysis. The difference in ratios between the peak areas of known amounts of both substances can be used to calculate the concentration of the unknown sample.

A standard which behaves in a similar manner to the compound of interest is ideally chosen; this will have a similar chemical structure and the same functional groups, which will affect the compound’s behaviour e.g. volatility. If an isotopically labelled sample of the target compound is used, it should also behave in the same manner as the original target compound, however, they may have slightly different retention times to one another (see section 1.5.1).

The quantitative analysis of banknote washings using GC/MS involves the use of a closed system, and an internal standard. The application of this closed system has a greater accuracy than the open TD-MS/MS, although the GC/MS may struggle with matrix effects from other contaminants; e.g. finger grease and cosmetics.

Song, Zhang and Kohlhof (1996) report using GC/MS with positive ion chemical ionisation (CI) in selected ion monitoring mode, to help reduce any interference from other contaminants on banknotes. This allowed the [M+H]^+ ion (m/z 304) to be targeted and eliminate any other contaminants in the sample (Figure 13). They report that using GC/MS with electron ionisation creates too many interferents to be able to fully distinguish the cocaine peak from others in the sample, however the background seen in Figure 13 (obtained using chemical ionisation) is also still rather noisy with other peaks occurring.
Figure 13. Chromatograms obtained by analysing the extraction from a banknote (a) and the reference cocaine and internal standard halazepam (b). Both were obtained by using a GC/MS in positive chemical ionisation, and selected ion monitoring (m/z 304).

(Reprinted/used with permission from the publishers, Elsevier (Song, Zhang and Kohlhof, 1996)

Interference has not been reported in the research conducted by others using GC/MS for identification purposes, such as, Oyler et al. (1996). Oyler et al. (1996) also applied selected ion monitoring for quantification purposes using an internal standard but employed electron ionisation. Their results indicated good accuracy, with correlation coefficients of greater than 0.99, and a linear calibration curve between 0 and 500ng. Negrusz, Perry and Moore (1998) employed solid phase extraction (SPE) to isolate the analyte from complex biological matrices, prior to using GC/MS in selected ion monitoring mode. They used full scan mode to identify cocaine, followed by quantitation using SIM, and report acceptable accuracy and precision, and a correlation coefficient greater than 0.99.

It is also important to consider which solvent to use when dealing with extraction, as there are commonly two forms of cocaine which may be present on samples of banknotes; cocaine hydrochloride and its free-base (crack) (Gough, 1991). The solvent used must readily dissolve both forms of cocaine, or non-reproducible results may arise which do not fully reflect the contamination of banknotes.
1.4.4 Fluorescence Spectroscopy

Fluorescence is a highly sensitive type of photoluminescence. It is characterised by an excited electron being promoted to a higher energy level (excited state) through the introduction of light radiation, and returning to its ground state, following internal conversion, thereby emitting energy at a lower wavelength, causing it to fluoresce (Kealey & Haines, 2002).

The sample is usually a dilute liquid sample, which should not contain any interferents, such as contaminants, or other molecules which fluoresce in the same region when the same energy is applied. In Figure 14, the monochromator on the left represents the excitation beam entering the sample and the monochromator on the right represents the emission as the beam passes through the sample and leaves, entering the detector. Most spectrofluorometers allow you to analyse both the excitation and emission spectra of a species; the excitation when at a set emission wavelength, and the emission when at a set excitation wavelength.

![Figure 14. Fluorescence spectrophotometer used in fluorescence analysis](image-url)
The absorbance of energy is directly proportional to the concentration of the species absorbing it, allowing quantification, in accordance with the Beer-Lambert Law (equation 1.0).

\[ \text{Abs} = Ebc \]  

<table>
<thead>
<tr>
<th>Abs =</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>E =</td>
<td>Absorptivity</td>
</tr>
<tr>
<td>b =</td>
<td>Path length</td>
</tr>
<tr>
<td>c =</td>
<td>Concentration</td>
</tr>
</tbody>
</table>

For these samples, quantitation is performed by using a calibration curve of the sample species and comparing the relative peaks with that of a standard. More concentrated samples can show a non-linear behaviour, caused by interactions and molecular collisions, known as self-quenching. This involves collisions in the excited state (increasingly as the concentration is increased), causing a transfer of energy and returning to the ground state without fluorescence. Other forms of quenching can also occur, such as collisions with other species, the formation of complexes and other absorbing species altering the incident light. As direct comparisons are made, it is important to eliminate any interfering factors in the sample so as to gain the same response as the calibration sample (Kealy & Haines, 2002).

The electrons of different molecules absorb energy differently based upon the types of bond present in a molecule and the associated energy. The structure of cocaine lends itself to fluorescence in the Ultraviolet (UV) region due to its aromatic structure containing delocalised electrons (Figure 3), (Sharma & Schulman, 1999). Fluorescence has not often been reported in the detection of cocaine, however, Campanella et al. (1996) used it to quantify pure cocaine hydrochloride as well as for the detection of cocaine in illicit powders. They also found that lidocaine (a common cutting agent of cocaine) had a very similar absorbance spectrum and interfered in the absorption region of 230 nm.

A separation or clean up step could be advantageous due to interferent species and the complexities of banknote matrices. One main feature of fluorescence, however,
is the speed and ease of analysis, which becomes longer with further steps. Al Najjar (2004) has applied fluorescence in the detection of traces of illicit substances by using a fluorescent derivatising agent and capillary electrophoresis.

Almeida, Cassella and Pacheco (2015) found that the solvent used can assist with the detection of the appropriate compound and found that a Britton Robinson pH 4.0 buffer yielded the best results, most probably due to the high pKₐ of cocaine (~8.6) (Lu, Chen and Zhan, 2007). It was also found that a lower pH gave a better signal to noise ratio. The type of solvent and pH are important factors in assisting with fluorescence detection. This has to be reviewed in hand with whether it may destroy the banknote samples or remove additional matrices such as dyes, which in turn could cause interference.

1.4.5 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a similar principle to GC, wherein compounds are separated using a column. However, HPLC employs a continuous stream of a mobile phase to carry the sample into the column. Retention on the column depends on a compound’s individual affinity for the mobile and stationary phase, which affects its resolution and retention time. Different detectors can be used to identify the compounds as they elute. The most common detector with cocaine is a UV absorbance detector at 230 nm, as recommended by UNODC (2012).

Sun, Hall and Lau (2000) have reported using fluorometric detection in HPLC analysis of cocaine and its metabolites, based upon the fluorescence of the ring structure. They suggest that the limit of detection (LOD) is five times less than when using UV detection with HPLC; 0.05 μg/mL using fluorometric detection compared with 0.2 μg/mL using UV detection. Almeida, Cassella and Pacheco (2015) compared the quantification capabilities of fluorescence with HPLC-UV detection. Whilst their results imply that the fluorescence results are comparable with HPLC, it is still accepted that HPLC is a more reliable and proven technique, with the ability to separate and distinguish many similar compounds.
It could be argued that the HPLC separation capabilities, combined with greater accuracy and wide sample range, lend itself as a preferred method of analysis.

1.4.6 Summary

All of the aforementioned techniques are highly selective and sensitive, which is ideal for the analysis of traces of cocaine on items such as banknotes. Other, less selective techniques may be subject to interference, and may not be suitable for forensic use. Each individual technique has its advantages over the other; one is fast and requires no sample preparation, whereas the others have a better ability to quantify.

When choosing the best method, one has to consider a number of variables:-

- Objectives and aims.
- Sample preparation requirements (and clean up required if complex mixture).
- Estimated concentration range.
- Specificity requirements.
- Time taken (analysis and preparation).

1.5 Quantification of Cocaine Traces on Banknotes

As there are only low levels of contamination on banknotes, the methods applied for the extraction and subsequent analysis require more preparation than other methods in ‘bulk’ analysis. To date, the works of Sleeman, Carter and Ebejer (2005) have not achieved absolute quantification. Relative comparisons of amounts detected can be made using the TD-MS/MS, but the lack of absolute quantitation renders any inter laboratory comparisons difficult. Such comparisons could be useful in corroborating the forensic expert’s opinion as to whether a particular sample is unusual or not.

Most studies of quantification of cocaine traces on banknotes have been conducted using GC/MS, and some analyses using LC/MS methods. This could be due to the different parameters and extraction techniques and
efficiencies adopted. There are several researchers who have attempted to quantify traces on banknotes; the various authors, techniques and results are summarised in Table 2.

Negrusz, Perry and Moore (1998) have extracted contamination from individual banknotes using hydrochloric acid and reconstitution in methanol following solid phase extraction (SPE) and drying. On this sample of 18 United States banknotes, between 0.14 and 10.02 μg of cocaine has been successfully extracted and quantified using GC/MS. These values are similar to those obtained by both Luzardo et al. (2011), and Wimmer and Schneider (2011), whose values range from 0 to 15 μg, both of whom tested Euros. The extraction method used by Wimmer and Schneider (2011) uses slightly different solvents, but similar principles and clean up steps. Luzardo et al. (2011) used the method employed by Jenkins (2001).

Jenkins (2001), used an acetonitrile wash whilst vortexing to remove the contamination from individual dollar bills, followed by SPE. The range of contamination detected (0-922 μg) was comparable with that reported by Oyler et al. (1996), who state that of the banknotes analysed, the range of contamination was between 0 and 1327 μg. The method employed by Oyler et al. (1996) involved using methanol as an extraction solvent for United States currency and deuterated cocaine as an internal standard. This was followed by SPE and GC/MS analysis.

It is curious that such different ranges have been found between Jenkins (2001) and Luzardo et al. (2011), since they use the same technique. However, it should be noted that the full details of the SPE are not given by Jenkins (2001), and also that the currency between the two authors differs. Given that the maximum cocaine levels on Euros are reported by different authors as 0.5 μg and 889 μg, and on US dollars as 10 μg to 1327 μg, there seems to be no particular variation between the currencies themselves. The main possibilities for the differences between authors are random variance and the technique or extraction method employed. Interestingly, the larger reported values appear to correlate with extraction efficiencies greater than 95%.
Esteve-Turrellas et al. (2005) state that, on Euro banknotes, between 1.25 μg and 889 μg was successfully extracted when using a similar method to that of Negrusz, Perry and Moore (1998). Again, the variation between values may be the result of several factors, for example different currencies having a different affinity for cocaine, the extraction methods used, or the variation of contamination of banknotes. The application of GC/MS and positive ion chemical ionisation has been applied by Song, Zhang and Kohlhof (1996), using an internal standard of halazepam for quantification. The extraction method used chloroform, followed by ethyl acetate to reconstitute after centrifugation and drying.

The technique employed by Jourdan et al. (2013), uses a method of extracting contamination from banknotes which has since become unpopular in favour of alternatives: Vacuuming the banknotes to remove surface debris and then testing them in batches has been abandoned by others for more reliable and efficient techniques. Sleeman, Carter and Ebejer (2005) discuss the use of vacuuming sets of banknotes to distinguish between ‘normal’ and ‘abnormal’ contamination levels, they also highlight that it can be more useful to test notes directly using a thermal desorption inlet for certain purposes. The low amounts of cocaine found by Jourdan et al. (2013) are unsurprising given that their technique leads to poor extraction efficiency. Arguably, only Bones, Macka and Paull, (2007) identify cocaine levels in such low amounts; maximum detection of less than 0.6 μg (twenty times smaller than the next highest detection value). The overall aim of Bones, Macka and Paull (2007) was to evaluate a new column, rather than solely consider the quantities of cocaine on notes and thus their focus may not have been on the specific extraction method or notes themselves.

The methods employed in many of the studies involve solvent extraction, leaving a liquid sample which will most likely degrade over a reasonably short time period (Johansen & Bhatia, 2007). Thus the liquid samples may not be suitable for re-analysis by other scientists/experts (such as defence experts), which can be of importance when dealing with the criminal justice system, especially considering the timescales often involved with complex cases.
The method employed by Sleeman et al. (2000) involves the analysis of only one end of a banknote, preserving the other end for any future analysis. The drawback, however, is that the exact same part of the banknote cannot be re-analysed to find identical results due to sample contaminant removal, whereas the solvent used to wash the banknote could be re-analysed within a reasonable time period. Such trace amounts on banknotes themselves could also degrade with time. The rate of any degradation is unknown with such low levels and it could be argued that the trace in solution is less stable than in its solid form. As the sample remains dry and unchanged, the degradation of the substances on the surface could well be less than that if it were a liquid sample.

The use of an internal standard, if spiked into the solute, could degrade at a similar rate, thus allowing prolonged analysis timescales (but could vary depending upon concentrations). Johansen & Bhatia (2007) employed a deuterated standard to quantify the cocaine contamination in body fluids and found that linear correlation of between 0.0001 and 4 mg/L could be achieved. The method differs from that employed by Sleeman, Carter and Ebejer (2005), yet uses the same type of tandem mass spectrometer, only with a different inlet port (LC/MS/MS).

Frederick, Pertaub and Kam (2007) suggest that the use of Raman microspectroscopy can identify single drug crystals on a banknote. This form of analysis, in comparison with all other techniques discussed is non-destructive as it leaves the sample unchanged. The usefulness of Raman microspectroscopy in the forensic context is, however, limited as it is unable to determine whether the banknote as a whole is ‘heavily’ contaminated with cocaine or whether it has normal levels of contamination. This may be counteracted with further studies using comparisons of ‘innocent’ sample banknotes and ‘contaminated’ banknotes, however would not remove the risk that a cluster of crystals may be found on a very small area of a ‘normal’ banknote, and are unable to put this into wider context. The method itself focuses on one cocaine particle only, and therefore it may need to be expanded to include a larger surface area of a banknote, which would be incredibly time consuming.
Table 2. Quantified Amounts of Cocaine Extracted from Banknotes Using Different Extraction and Detection Techniques

<table>
<thead>
<tr>
<th>Author</th>
<th>Currency</th>
<th>Technique</th>
<th>Extraction Method</th>
<th>Extraction efficiency</th>
<th>Sample Size (n)</th>
<th>Average Amount Per Note (µg)</th>
<th>Range From (µg)</th>
<th>Range To (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida et al. (2015)</td>
<td>Brazil R$</td>
<td>Fluorescence</td>
<td>Wash – MeOH</td>
<td></td>
<td>50</td>
<td>130</td>
<td>0</td>
<td>496</td>
</tr>
<tr>
<td>Bones et al. (2007)</td>
<td>Euro (Irish)</td>
<td>LC-MS/MS</td>
<td>Wash – MeOH</td>
<td>&quot;Quite high&quot;</td>
<td>45</td>
<td>n/a</td>
<td>$7^{10^{-6}}$</td>
<td>0.576</td>
</tr>
<tr>
<td>Di Donato et al. (2007)</td>
<td>Brazil R$</td>
<td>GC/MS</td>
<td>Wash - Water &amp; Eth Acetate</td>
<td></td>
<td>46</td>
<td>51</td>
<td>2.38</td>
<td>275.1</td>
</tr>
<tr>
<td>Esteve-Turrillas et al. (2005)</td>
<td>Euro (Spanish)</td>
<td>GC/MS/MS</td>
<td>Wash – MeOH</td>
<td>98%</td>
<td>16</td>
<td>155</td>
<td>1.25</td>
<td>889</td>
</tr>
<tr>
<td>Hudson (1989)</td>
<td>Canadian $</td>
<td>GC/Mfrag, GC/NPD</td>
<td>Wash - MeOH or Chloroform</td>
<td>35.6% - 72.9%</td>
<td>1 x 100 notes</td>
<td>&lt;0.01</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Jenkins (2001)</td>
<td>US $</td>
<td>GC/MS</td>
<td>Wash – Acetoneitrile</td>
<td>No info</td>
<td>50</td>
<td>28.75</td>
<td>0.01</td>
<td>922</td>
</tr>
<tr>
<td>Jourdan et al. (2013)</td>
<td>US $</td>
<td>GC/MS</td>
<td>Vacuum 1 side of note</td>
<td>No info</td>
<td>418 (groups of 10)</td>
<td>0.00234</td>
<td>0.00014</td>
<td>0.0947</td>
</tr>
<tr>
<td>Luzardo et al. (2011)</td>
<td>Euro (Canary)</td>
<td>LC-MS/MS</td>
<td>Wash – Acetoneitrile</td>
<td>No info</td>
<td>120</td>
<td>0.188</td>
<td>0</td>
<td>15.023</td>
</tr>
<tr>
<td>Negrusz et al. (1998)</td>
<td>US $</td>
<td>GC/MS</td>
<td>Wash – HCl</td>
<td></td>
<td>18</td>
<td>2.86</td>
<td>0.14</td>
<td>10.02</td>
</tr>
<tr>
<td>Oyler et al. (1996)</td>
<td>US $</td>
<td>GC/MS</td>
<td>Wash – MeOH</td>
<td>&gt;95%</td>
<td>136</td>
<td>70</td>
<td>0</td>
<td>1327</td>
</tr>
<tr>
<td>Rodrigues et al. (2013)</td>
<td>Brazil R$</td>
<td>HPLC</td>
<td>Wash – MeOH</td>
<td>98%</td>
<td>50</td>
<td>148</td>
<td>10</td>
<td>1110</td>
</tr>
<tr>
<td>Song et al. (1996)</td>
<td>GC/MS</td>
<td>Wash – Chloroform</td>
<td>n/a</td>
<td></td>
<td>1</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Wimmer &amp; Schneider (2011)</td>
<td>Euro</td>
<td>LC-MS/MS</td>
<td>Wash – MeOH</td>
<td>60-80%</td>
<td>64</td>
<td>0.106</td>
<td>0</td>
<td>12.4</td>
</tr>
</tbody>
</table>
Noonan & Beshire, (2005), highlight that the technique is not as sensitive as other methods, and can only usually detect contamination on banknotes which are more heavily contaminated with cocaine particles. Both authors indicate that interference occurs from the various dyes in the paper currency, in particular the lighter areas of the banknotes. This indicates that this form of analysis is not best for the application on banknotes. Due to the inherent interferences which can occur with fluorescence analysis, Almeida, Cassella and Pacheco (2015) compared the quantification capabilities with HPLC-UV detection, the results of which showed good correlation. They then only discuss the results of the fluorescence throughout their work, despite initially indicating reservations about its reliability. The extraction efficiency is reviewed by washing a banknote once, then spiking it and re-extracting. Their results of greater than 100% extraction efficiencies imply that there may have been residual cocaine on the banknotes following the initial extraction, which should be considered, or an issue with the fluorescence detection itself. Whilst it is desirable to extract all of a sample, complete removal is questionable.

1.5.1 Quantification using Isotope Dilution

Isotope dilution uses an isotopomer as an internal standard to enable quantification of the molecule of interest. Ideally an internal standard should be chemically similar to the molecule of interest, so that it behaves in the same way (i.e. its solubility). Additionally, its chromatographic retention time should also be similar in order to ensure that both species are subject to the same differences in instrument performance (i.e. retention times are in a similar region of low noise), (Sargent, Harrington and Harte, 2002).

There are various types of isotopes that can be used to label molecules, such as using a radioisotope or an isotope of a specific element, both of which can be used to substitute into the molecule, thus labelling it. Usually, an isotopically labelled molecule will have a mass of at least 3 higher than the molecule of interest to compensate for any occurrence of natural isotopes (e.g. $^{13}$C or multiples thereof). The chemical similarity of the isotopomer to the molecule of interest helps to remove variation in results in the case of change in instrument performance or
sample contamination, due to the ratio between the two samples remaining constant. It has, however, been reported by Wu et al. (2006) that cross contribution can occur at varying concentrations, giving slightly different responses under certain conditions. These can be overcome by using specific methods such as normalising the spike value to approximately the same as the sample or employing a polynomial calibration to the GC/MS results. One of the disadvantages of using isotopes is cost; they can often be expensive to buy. Furthermore, as they behave in the same manner, they may not elute separately using a GC alone and a mass spectrometer is typically required to identify the compounds by mass (Sargent, Harrington and Harte, 2002).

Webb & Carter (1997) discussed that using isotope dilution can improve the reproducibility using a tandem mass spectrometer by a factor of eight. Johansen & Bhatia (2007) have used deuterated cocaine standards in the quantification of cocaine in blood and urine, and obtained a linear correlation ($R^2 = >0.99$) between a concentration range of 0.0001 and 4 mg/ml. Additionally, Castiglioni et al. (2006) have applied the same technique and specifically looked at cocaine ion transitions $m/z$ 304/105 and 304/182 and the respective isotopic transitions $m/z$ 307/105 and 307/185. The $m/z$ 304 values are used by Sleeman et al. (2000) in the analysis of cocaine traces on banknotes using TD-MS/MS in SRM mode.

Through reviewing other research and literature into the quantitation of cocaine on banknotes, there appears to be a lack of continuity between the results. Furthermore, TD-MS/MS has not yet been used to quantify such traces, despite being the most commonly used technique for drug detection on banknotes. A comparison of some of the techniques would assist in providing further clarity of the reliability and reproducibility of results for each technique. Studies into using TD-MS/MS would help to establish if this is an additional comparative tool and further reinforce its use for both drug detection and quantitation. In the research of banknote contamination, a deuterated standard will be useful in allowing unknown amounts of contamination already present to be quantified using a variety of the techniques currently used, as already conducted on urban wastewater by Castiglioni et al. (2006).
1.6 Aims

The overall aim of this research is to establish whether trace levels of cocaine on banknotes can be quantified using TD-MS/MS as reported by Sleeman et al. (2000) and assign a particular numerical value to the contamination. The advantages of this form of analysis (speed of analysis, specificity and high throughput) lend itself to banknote analysis and could be enhanced by adding another method of evaluating contamination. Enhancement of this technique would enable further corroboration from other laboratories and allow further evaluation of the differences in levels between ‘normal’ banknotes and those involved in drugs related activities, thus further supporting an already important aspect of forensic evidence.

Establishing an appropriate method of quantification will allow further inter-lab comparisons to be conducted than are currently available. This will prove useful if the courts wish to scrutinise the precise amounts of contamination on banknotes and employing other independent companies/experts who can then apply different examination techniques. This is particularly pertinent with new developments and the implementation of forensic reviews (National Research Council (NRC) 2009, Parliamentary Office of Science and Technology (POST) 2005). Additionally, quantification will allow further assessment of findings where banknotes from an exhibit are reported as more heavily contaminated than banknotes taken from general circulation, giving experts further data with which to draw conclusions.

Almeida, Cassella and Pacheco (2015) have considered the differences between banknotes from general circulation sources and those directly involved with drug use or dealing, they also considered different areas from which banknotes were taken. They found that there was a significant difference (an average of approximately 30 times more) in the amounts of cocaine detected on seized banknotes compared with general banknotes, and found that the area from which banknotes are taken does not affect the extent of contamination. However it is important to consider that the regions of Rio de Janeiro where the samples were taken from may well have a much higher level of purity than those from Europe which may not display such a vast difference between ‘normal’ and ‘seized’ banknotes.
1.7 Objectives

1.7.1 Objective 1

Reviewing the work of others, a suitable liquid extraction method of cocaine on banknotes for a large range of contamination levels will be established. The sampling methods used will involve the solvent extraction of cocaine from banknotes using methods set out by other researchers and analysis of the solute using GC/MS and fluorescence.

1.7.2 Objective 2

Using extracts from real banknotes, and isotope dilution, quantities will be established. Comparisons with HPLC, fluorescence, GC/MS and direct injection into the TD-MS/MS will be employed.

1.7.3 Objective 3

The instrument responses using TD-MS/MS will be compared with the other methods used in order to establish the extraction efficiency of the thermal desorber. Losses from the instrument, differences in throughput speed of individual banknotes and other factors need to be considered.

2.0 Methodology

2.1 Preamble

Various researchers have speculated about the methods in which cocaine contaminates banknotes and how the traces are retained upon the banknote paper itself. Carter, Sleeman and Parry (2003) argue that banknotes can become contaminated using counting machines, which have a small residual amount of cocaine on them. Sleeman et al. (2000) also believe that inks on banknote paper or finger grease are a potential binding site for the drugs and retain a certain degree of
contamination. The lattice structure of the fibres of banknotes becomes larger once used, and an electron microscope shows that the cocaine particles can be positioned within these woven fibres (Figure 15), which may also be a reason for cocaine retention.

The images in Figure 15 were achieved by examining (a) a used £20 banknote taken from general circulation and (b) spiking a piece of cotton paper using pure cocaine hydrochloride powder (Johnson, 2009). The pure spike shows differences in the particle size and shape when compared with that on the ‘real banknote sample’. A real street sample of cocaine is not expected to show similar characteristics to that of a pure sample due to environmental factors, cutting agents and manufacturing differences. The size itself is largely related to the time taken to cool and crystallize; a process which is monitored and controlled with certified standards and not with ‘street drugs’.

![Figure 15. (a) cocaine within the lattice fibres of a used £20 banknote and (b) cotton paper spiked with pure cocaine hydrochloride standard (magnification (a)x1427 and (b)x1395) (Johnson, 2009)](image)
2.1 Methods Considered

There are several methods of analysis and extraction techniques that could be applied in the analysis of cocaine contamination on banknotes. If banknotes could not be used, modelling the contamination on blank pieces of paper would be required. Cotton paper has been established as having the most similar properties to banknote paper (Ebejer et al., 2007a) and may be used to carry out some of the experiments if it is not feasible to use real banknote samples.

It would be useful to use blank pieces of paper as it will enable the examination of how much is detected compared with a known ‘spiked’ value of cocaine. The drawback of using such spiking methods is recreating the matrices that are on banknotes, especially as there is no definitive explanation for its retention on banknotes. Additionally, there are methods available that overcome the need to spike using standard cocaine, such as, using an internal standard.

The methods considered are: -

a) A solution could be injected onto the surface of cotton paper, representing the banknote, however, the spread of the contamination may not be even due to wicking of the solution onto the porous surface. Also, as this uses a solution, the particles of cocaine may well be smaller in size than those found on ‘real’ banknotes and may act differently on the surface or within the fibres. The cotton paper will also not have the inks which could bind the cocaine to banknotes.

b) The piece of blank paper could be placed inside a box and shaken with dust particles that contain a quantity of cocaine (Ebejer et al. 2007a). This allows the dirt and finger grease to contaminate the paper, yet does not guarantee full cocaine contamination with the spike.

c) Microspheres have been used to evenly distribute explosive contamination onto surfaces using an inkjet system (MacCrehan & Bedner, 2004). Applying this method would be the most even, however would not incorporate the inks and dirt that most banknotes will have acquired.
d) A liquid sample of a known concentration of cocaine could be added to a smooth surface (e.g. a glass slide) and allowed to dry. The residue left behind can then be wiped off of the surface using a medium of choice (cotton paper). This method does not account for the dirt factor or have a good chance of even distribution.

e) Alternatively, real banknotes could be used and a deuterated solvent added to the extract to allow quantification. This removes the need for spiking as most banknotes are contaminated anyway and also have the dirt, inks and grease on them already. Used banknotes would be required, as new banknotes have not had the opportunity to gain significant amounts of background contamination or build-up of matrices.

It was decided that the most representative sample would be that of real banknotes taken from various sources (e). This removes the speculative arguments over how the contamination occurs and is retained, and also includes the finger grease, inks and other matrices on the paper. The use of a deuterated standard will enable the extract from the banknotes to be quantified, and comparisons with different extraction techniques will establish how efficient the methods are.

3.0 Preparation of Real Banknote Samples

3.1 Experimental

The work of Esteve-Turrillas et al. (2005) provided a basis upon which to extract the cocaine from the banknote efficiently. With permission from the Bank of England, approximately one quarter of each end a banknote sample (£20 Bank of England banknote) was removed for analysis. The purple coloured quarter was cut off and placed inside a test tube, 5 mL of methanol was added and the tube agitated ultrasonically for five minutes. The quarter banknote was removed and placed into a new test tube and the process of washing repeated. Both solvents were combined and a known volume ’spiked’ using an equal amount of deuterated cocaine standard. These were weighed gravimetrically to review error. Each sample was injected 15-
20 times into the TD-MS/MS. The opposite (white coloured) quarter end of the banknote was analysed directly by TD-MS/MS.

Figure 16. Diagram of the experiment process when analysing banknotes contaminated with cocaine

The experiment was then transposed onto the opposite ends of the banknote where possible i.e. the piece which had been washed was thermally desorbed, and vice versa, as represented in Figure 16. Batch testing of ten banknotes washed a quarter (purple) end of these banknotes (Figure 17) in 35 mL of methanol. The agitation was repeated as per a single banknote, but with 30 mL of methanol, and a final rinse with 5 mL methanol to remove any remaining residue.

Figure 17. Batches of ten banknotes with quarter ends cut off

To concentrate the samples for analysis and identification, the solute was dried under a stream of nitrogen at approximately 40°C. It was reconstituted in HPLC
grade water for testing using the spectrophotometer, and reconstituted in mobile phase for HPLC.

Other proportions of banknotes can be tested: A quarter was chosen for the £20 notes due to the positioning of the holographic strip. This shiny strip could retain cocaine differently to that of the paper and was thus avoided.

**4.0 Fluorescence**

**4.1 Instrumentation & Standards**

A Hitachi F2500 spectrophotometer with Xenon lamp source was used in emission, absorption and 3D mode. Limits were set for emission between specific wavelengths of 260 nm and 410 nm, with an excitation energy of 230 nm. Results were reviewed using ‘FL Solutions’ software. A standard 10 mm quartz cuvette was used to house each sample.

Certified cocaine hydrochloride powder solids were obtained from Sigma-Aldrich (Missouri, USA), with purity values of >98% (obtained using TLC). Certified deuterated cocaine liquid standards were obtained from Cerilliant (Texas, USA) in 1mg/mL ampoules, with purity values of 99%.

**4.2 Preliminary Experiments**

Different types of paper were tested to establish whether any interference was caused by dyes. A strip of paper (cotton paper, partially printed paper or a real banknote) was dipped into water in the cuvette for approximately ten seconds. The water was then agitated and analysed. Various areas of the paper containing different dyes and colours were also analysed. Further studies considered handling activities to recreate normal contamination with finger grease and dust (Appendix E. Initial Experiments (Fluorescence and TD-MS/MS)).
Methanol, de-gassed methanol and de-ionised water were all used as potential solvents for the washing or reconstitution of banknote washings and any interfering factors analysed. Acetonitrile was another solvent that was tested due to its use in deuterated samples.

A 3D model was generated to investigate any fluorescence between 220 nm and 450 nm and to establish in what region cocaine fluoresced. 3D experiments were conducted on concentrations between 10 mg/mL and 0.1 mg/mL, followed by concentrations in the region of 0.001 mg/mL to establish a basic range. A 2D scan with a fixed emission wavelength was conducted on samples of cocaine hydrochloride concentrations ranging from 50 ng/mL to 1500 ng/mL in HPLC grade water to establish optimum parameters.

Repeat analyses using the same concentration were carried out, washing the cuvette between each sample to ensure that it was clean. Differential scans were taken of the various concentrations as well as the different solvents and papers, to remove background responses. Emission was set at 310 nm, and a range of 220-260 nm for the excitation.

**4.3 Preliminary Experiments Results**

The emission results shown in Table 3 show the responses obtained from testing different pieces of paper, including some paper obtained from banknote manufacturers with only partial print on it. It was found that cotton paper produced a small amount of fluorescence, which may indicate that concentrations below 250 ng/mL cannot be analysed. It was also interesting to note that, of all the paper analysed, the new issue £20 banknotes showed the lowest fluorescence response, in particular on the white section. It may also be noted that the paper upon which banknotes are printed do not commonly fluoresce and ultra violet security features are added to them, which glow under a lamp emitting at around 365 nm (Bank of England, 2008).
Table 3. Fluorescence emission results when analysing different pieces of paper rinsed in water (excitation 230 nm, emission 260-410 nm)

<table>
<thead>
<tr>
<th>Type of paper</th>
<th>Description of part analysed</th>
<th>Fluorescence Emission Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton paper</td>
<td>All</td>
<td>6400</td>
</tr>
<tr>
<td>Partially printed banknote paper</td>
<td>White (non-printed part)</td>
<td>7500</td>
</tr>
<tr>
<td>Partially printed banknote paper</td>
<td>Blue printed section</td>
<td>4850</td>
</tr>
<tr>
<td>Partially printed banknote paper</td>
<td>Purple printed section</td>
<td>20250</td>
</tr>
<tr>
<td>New (unused) (2007) issue GBP £20</td>
<td>White part of banknote (taken from general circulation)</td>
<td>2000</td>
</tr>
<tr>
<td>New (unused) (2007) issue GBP £20</td>
<td>Purple part of banknote (taken from general circulation)</td>
<td>6500</td>
</tr>
<tr>
<td>Old issue GBP £20</td>
<td>Part of banknote (taken from general circulation and expected to have residual contamination)</td>
<td>10000</td>
</tr>
</tbody>
</table>

Methanol produced a relatively high baseline, which may have been caused by other ring structures or oxygen molecules that absorb in the same region and cause interference. As the interference could have been caused by oxygen dissolved in the methanol, de-gassing was attempted using an ultra-sonic bath for several minutes. No improvement in results was observed after de-gassing. De-ionised water, in contrast, showed a very low baseline and was used in further experiments.

The cocaine excitation and emission wavelengths were established using a 3D scan (Figure 18) and instrument parameters were selected for optimum performance (Table 4). No response was observed at concentrations between 0.1 mg/mL and 10 mg/mL. Fluorescence at 230 nm (excitation) and 260-410 nm (emission) resulted with lower concentrations (around 0.001 mg/mL). These values are consistent with that used by Sun et al. (2000), who report using an excitation wavelength of 230 nm and emission of 315 nm.
Figure 18. 3D Plot of Cocaine Hydrochloride Standard 10 ng/mL (Excitation identified at 230 nm and emission at 350 nm)

Table 4. Parameter settings used during analysis of cocaine solutions in the fluorimeter

<table>
<thead>
<tr>
<th>Fluorimeter Setting</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement type:</td>
<td>Wavelength scan</td>
</tr>
<tr>
<td>Scan mode:</td>
<td>Emission</td>
</tr>
<tr>
<td>Data mode:</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>EX WL:</td>
<td>230.0 nm</td>
</tr>
<tr>
<td>EM Start WL:</td>
<td>260.0 nm</td>
</tr>
<tr>
<td>EM End WL:</td>
<td>410.0 nm</td>
</tr>
<tr>
<td>Scan speed:</td>
<td>1500 nm/min</td>
</tr>
<tr>
<td>Delay:</td>
<td>0 s</td>
</tr>
<tr>
<td>EX Slit:</td>
<td>5.0 nm</td>
</tr>
<tr>
<td>EM Slit:</td>
<td>5.0 nm</td>
</tr>
<tr>
<td>PMT Voltage:</td>
<td>700 V</td>
</tr>
<tr>
<td>Response:</td>
<td>0.08 s</td>
</tr>
</tbody>
</table>

When comparing a sample of 1000 ng/mL deuterated cocaine, with that of 1000 ng/mL cocaine hydrochloride, both showed absorption in the 320 nm region (Figure 19). Further experiments may be possible using deuterated standards, enabling compensation for the deuterated internal standard addition (if used).
Figure 19. The fluorescence response obtained from 1000ng/mL (a) deuterated cocaine solution in acetonitrile and HPLC grade water and (b) cocaine hydrochloride solution in HPLC grade water (Excitation 230nm)

Differential scan results were acquired with a background subtraction of the solvent. The highest responses were achieved at excitation of 230-240 nm. This is in line with the expected values and did not show any change or variation.

Repetitions of the 1250 ng/mL sample were analysed to review the instrumental variation (Table 5). The average response was 28649 +/- 1195 (n=6). Prior to each analysis, the cuvette was cleaned using HPLC grade water and ethyl acetate (to aid drying), and dried under a stream of air.

Table 5. Repeat responses from the analysis of 1250 ng/mL cocaine hydrochloride (excitation 230 nm)

<table>
<thead>
<tr>
<th>Sample Repeat</th>
<th>Fluorescence Emission Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30058</td>
</tr>
<tr>
<td>2</td>
<td>29825</td>
</tr>
<tr>
<td>3</td>
<td>29183</td>
</tr>
<tr>
<td>4</td>
<td>28008</td>
</tr>
<tr>
<td>5</td>
<td>27359</td>
</tr>
<tr>
<td>6</td>
<td>27460</td>
</tr>
<tr>
<td>Mean Value</td>
<td><strong>28649</strong></td>
</tr>
</tbody>
</table>
4.4 Calibration Curve

Cocaine hydrochloride standard was obtained Sigma-Aldrich. Nine different concentrations (100 ng/mL to 1500 ng/mL) of cocaine hydrochloride in water were prepared using serial dilution and analysed using the parameters in 2-D mode (Table 4).

The cuvette was thoroughly rinsed between each analysis and dried using a stream of air. At intervals between concentration changes, blanks were taken to ensure that the cuvette remained clean. A blank sample of water was analysed and the background (peak area) reading removed from each of the scans (Appendix A. Fluorescence Background Subtraction).

4.5 Calibration Results

4.5.1 Peak Area Responses

As the maximum peak height occurred at slightly different intervals and levels for repeat analysis, the peak area of the response was examined and plotted (Figure 20).

![Figure 20. The fluorescence response obtained from the analysis of 1000 ng/mL cocaine hydrochloride solution in HPLC grade water (Excitation 230nm)](image)

It was observed that the responses at increasing concentrations, when plotted, produced an R\(^2\) value of 0.95 with a straight line plot (Figure 21). The accuracy of the fit was investigated to establish whether a better line of fit could be found with a
higher $R^2$ value. Repeat experiments found similar results and investigations into possible reasons are detailed in the following sections.

![Graph](image.png)

**Figure 21. The fluorescence responses at increasing concentrations of cocaine showed variation**

### 4.5.2 Calibration Curve Errors

As the calibration curve did not fit a linear pattern, the relative errors were reviewed to establish what could have caused such variation in the results. It was also observed that the 1000ng/mL sample, although in mid-range, was an outlier and was removed to allow further investigation of the results (see section 4.5.3).

Error propagation was calculated using the serial dilution of the cocaine standards, reviewing the errors involved in each step: glassware, pipette, weighing and errors in fluorescence measurement (Appendix B. Dilution Error Analysis). Inverse squared linear regression calculations were used to establish the expected results, which were found to differ from the observed (Appendix C. Fluorescence Regression Analysis).

### 4.5.3 Fluorescence Quenching Theory

Self-quenching could explain the differences in the expected and observed results and was further investigated. During this analysis, the data point obtained for the 1000 ng/mL was shown to be an outlier (Appendix D. Calculating an outlier in the
Fluorescence calibration results). The outlier at this point was removed in order to plot a better line of fit.

A self-quenching factor can be assigned to the fluorescence values in order to account for the reduction in fluorescence with the increase in concentration (due to an increase in excited state collisions). Assuming that the cocaine analyte does not absorb, a derivation of the ‘Stern-Volmer’ equation (Equation 2.0) can be applied to the data in order to account for the quenching factor.

\[ \frac{I_0}{I} = 1 + K [Q] \]  \hspace{1cm} (2)

<table>
<thead>
<tr>
<th>I =</th>
<th>Fluorescence response with (I) and without (I₀) quencher</th>
</tr>
</thead>
<tbody>
<tr>
<td>K =</td>
<td>Stern-Volmer</td>
</tr>
<tr>
<td>Q =</td>
<td>Quencher concentration</td>
</tr>
</tbody>
</table>

With the addition of an offset baseline value (c), the Stern-Volmer equation is re-arranged to form:

\[ I = \left( \frac{I_0[Q]}{1+K[Q]} \right) + c \]  \hspace{1cm} (3)

The equation can be further re-arranged and letters transposed to create a calculation for the best curve fit for the data (2.2). The equation, which compensates for self-quenching, was:

\[ y = \frac{a[Q]}{1+b[Q]} + c \]  \hspace{1cm} \text{re-arranged to} \hspace{1cm} \[ y = \frac{c+a}{x^{-1}+b} \]  \hspace{1cm} (4)

<table>
<thead>
<tr>
<th>a =</th>
<th>Fluorescence constant</th>
<th>44.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>b =</td>
<td>Quenching constant</td>
<td>8x10^{-4}</td>
</tr>
<tr>
<td>c =</td>
<td>Baseline offset</td>
<td>426.8</td>
</tr>
<tr>
<td>x =</td>
<td>Concentration</td>
<td></td>
</tr>
<tr>
<td>y =</td>
<td>Observed fluorescence</td>
<td></td>
</tr>
</tbody>
</table>
The computer program ‘CurveExpert’ (Curve Expert 1.40, 2001) was employed to apply the specific equation and create a curved line of best fit. Upon the application of a suitable equation, a curve of results which fitted with the self-quenching theory was produced (Figure 22).

![Plot of results (baseline removed) including the applied quenching equation showing a curve](image)

**4.6 Real Banknote Analysis**

Real samples of banknotes were washed using the process detailed in section 3.0. Following a blank run and a standard check to ensure instrument performance, samples of the real banknotes were analysed using the spectrophotometer. A variety of parameter settings were altered to produce optimum responses, the main setting altered was the voltage. This was reduced from 700 V to 400 V.

**4.7 Results**

When testing the real banknote samples, the responses exceeded the limit of detection. Once the voltage had been reduced to 400 V, the samples were run again to get a response within the working range of the instrument. The curve expected (as seen in the calibration) produced two peaks (Figure 23), neither of which occurred at a wavelength of 320 nm. The expected response would have fallen
within the calibration curve range, and the quenching factor applied to calculate the amount of cocaine in each sample.

![Graph showing fluorescence intensity vs. emission wavelength.](image)

**Figure 23.** Wavelength scan plot of the real banknote response using the optimum parameters with the voltage reduced to 400 V, showing responses within the instrument threshold (Excitation 230nm)

The interference which occurred for each of the banknote samples tested showed similar results. There are several explanations for this response, the most likely being matrix effects. Oils and fats on the surface of fingers and hands fluoresce in a similar region to cocaine and may well cause fluorescence or indeed bind with the drug. Sample clean-up was considered, but was excluded due to the number of potential interferents from dyes, paper and grease related contamination.

**4.8 Fluorescence Summary**

Using fluorescence, it has been possible to establish suitable emission and excitation settings for cocaine in certain concentrations (Emission 230 nm, Excitation 260-410 nm). At higher concentrations (3000 ng/mL and above), fluorescence was not apparent at the optimised settings, however certain parameters could be altered to enhance the dynamic range of the fluorimeter. It has been observed that a non-linear increase in instrument response is created at cocaine
concentrations between 50 and 1500 ng/mL due to self-quenching. This can be compensated for when applying the Stern-Volmer equation to form a curve that fits the data.

Interferents within the sample altered the expected response from the cocaine on banknotes and caused further fluorescence in the same region as the cocaine. It is unsurprising that there are limited reports of cocaine analysis using this method (when in complex matrices) due to the many possible interferents. Campanella et al., (1996) report that even a simple mixture containing a common cutting agent (lidocaine) may cause interference and thus its use with banknote analysis could well be limited.

5.0 Thermal Desorption-tandem Mass Spectrometry

5.1 Instrumentation & Standards

A triple-quadrupole mass spectrometer (MDS Sciex, Concord, ON., Canada) with a custom built thermal desorption inlet mechanism (Mass spec analytical Ltd. Bristol, UK) was used. The inlet mechanism consisted of two heated plates, heated to 285°C, which liberate vapour from the surface of items or liquids inserted or injected between them. The liberated vapour becomes entrained in an air sample flowing into the APCI source where ionisation takes place. The TD-MS/MS in SRM mode was programmed to identify precursor and product ions of cocaine (protonated precursor ion $m/z$ 304 with products of $m/z$ 182 and 105) and a cocaine isotope (protonated ion $m/z$ 307 with products of $m/z$ 185 and 105). The resultant chromatograms were analysed using ‘Analyst’ 1.4 (MDS Sciex, Concord, ON., Canada) and peak areas obtained from PeakEdit v2.0 (Adapted from MATLAB).

Certified cocaine hydrochloride powder solids were obtained from Sigma-Aldrich (Missouri, USA), with purity values of >98% (obtained using TLC). Certified deuterated cocaine liquid standards were obtained from Cerilliant (Texas, USA) in
1mg/mL ampoules, with purity values of 99%. The deuterium content is attached to the nitrogen as part of the CH$_3$/CD$_3$ function (Figure 24).

Figure 24. The location of the 3 x deuterium atoms is important when reviewing fragmentation patterns of the molecule

It is important to consider this positioning when analysing the fragmentation data and setting up the MS/MS parameters during optimisation, since the mass of one of the fragments will differ to those originally obtained for normal cocaine. The 182 ion fragment will become 185 as the deuterium is located within this fragment, whereas the 105 ion fragment will remain the same (for fragmentation pathways of interest, see Figure 10).

5.2 Instrument Performance

Daily instrument checks were carried out by injecting 2 µL of a solution containing 1 ng/µL of a mixture of drugs five times. These include cocaine, diamorphine, MDMA, amphetamine and THC. The resultant peak heights were recorded and the average input into a Shewhart chart. The chart kept track of changes in the mean values and is set to alert the user if the mean over a certain number of points changes significantly. It is also used to identify when a parameter falls beyond two or three standard deviations. The user can address any issues accordingly, either through maintenance, cleaning, re-optimising or replacing the standards. The charts were updated quarterly to account for the normal deviations in performance expected with any type of instrumentation.
5.3 Response Factor

Mixtures of known amounts of deuterated standard and cocaine hydrochloride were injected into the system in varying proportions (detailed in Table 6). A response factor between the two isotopomers could then be established.

Table 6. The Proportions of Equal Concentrations of Cocaine Hydrochloride mixed with the Deuterated Standard

<table>
<thead>
<tr>
<th>Cocaine Standard</th>
<th>Deuterated Standard</th>
<th>Ratio of mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>20%</td>
<td>4:1</td>
</tr>
<tr>
<td>66.6%</td>
<td>33.3%</td>
<td>2:1</td>
</tr>
<tr>
<td>50%</td>
<td>50%</td>
<td>1:1</td>
</tr>
<tr>
<td>33.3%</td>
<td>66.6%</td>
<td>1:2</td>
</tr>
<tr>
<td>20%</td>
<td>80%</td>
<td>1:4</td>
</tr>
</tbody>
</table>

5.4 Preliminary Experiments

The thermal desorption work conducted as part of this research entails various experiments for method development. Initial experiments conducted are discussed and further background studies into TD efficiency are summarised in ‘Appendix E. Initial Experiments’.

5.4.1 Standard Solutions

Standard solutions containing 1 ng/mL cocaine hydrochloride powder (99% pure, obtained from Sigma-Aldrich) and 1 ng/mL deuterated cocaine solution (1 mg/mL obtained from Cerilliant) were dissolved and diluted in HPLC grade methanol and acetonitrile. An auto-sampler (Leap CTC A200S) was used to inject solutions of the samples in the thermal desorption region of an (Applied Biosystems, MDS Sciex, API2000) MS/MS, creating reproducible results by using a known volume of solution, and a particular speed of injection. As each new experiment was conducted, a standard mix of cocaine hydrochloride and deuterated cocaine were injected into the system. The ratios of the m/z 182/185 and m/z 105/105 product ions were calculated for use as a response factor in calculating the concentration of the cocaine.
5.4.2 Instrument Optimisation

In order to identify the compounds of interest, the instrument parameters were optimised for the appropriate compounds. Injected concentrations of 2 ng for both cocaine and deuterated cocaine were used for optimisation purposes.

Settings routinely used for cocaine detection at MSA were reviewed, and several adjustments made to enhance the response. When introducing the deuterated standard, the settings were again checked and optimised. It was found that the standard and deuterated cocaine molecules required different instrument settings for optimum responses. Following manual optimisation, automatic optimisation was used with an electrospray source, according to the manufacturer’s recommendations. The electrospray settings were found to be much more appropriate and specific to the cocaine standards used.

5.4.3 Solvents

Mixtures of the cocaine (deuterated and non-deuterated) were made up in methanol only, and repeated in acetonitrile and water only. In the event that evaporation and reconstitution was required water was not considered a suitable solvent, since methanol is easier to dry and has also been used as a solvent in other studies of cocaine extraction from banknotes (Esteve-Turrillas et al., 2005).

5.4.4 Degradation

Standards dissolved in acetonitrile have been found to produce the same results after a period of one month (Johansen & Bhatia, 2007). However as cocaine hydrochloride did not dissolve in acetonitrile alone, alternative solvents were used. These were tested for their degradation after periods of two and four weeks. The peak areas as well as peak ratios of the methanol samples were assessed, comparing the results obtained from day one to four weeks following (Appendix F. Degradation of samples).
5.4.5 *Precursor Ion Scans and interference of results*

A precursor ion scan of the m/z 105 product ion, searching for the m/z 304 and m/z 307 ions respectively allowed comparison between the different solvents and to establish whether there was any interference between one targeted compound and its deuterated form. Each cocaine solution was tested separately to ascertain whether a response occurred for the target isotopomers of the same compound, despite it not being introduced into the system.

5.4.6 *Injection Speed and Temperature*

In order to reduce analysis error within the MS/MS, experiments varying the speed of injection and temperature of the heated blocks were conducted. Ten repeat injections of 8 μl (500 ng/ml) cocaine hydrochloride and deuterated cocaine solution (diluted in methanol) were injected into the heated blocks. The initial parameters included an injection speed of 50 μl sec\(^{-1}\) and lower temperatures than had been previously used. The full list of parameters can be found in Table 7.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Injection Speed (μl sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>30</td>
</tr>
<tr>
<td>225</td>
<td>50</td>
</tr>
<tr>
<td>250</td>
<td>80</td>
</tr>
<tr>
<td>285</td>
<td>50</td>
</tr>
</tbody>
</table>

5.4.7 *Analysis of Banknotes and Washings*

Experiments were carried out into the extraction efficiencies of the washing method. Tests were carried out to establish if a third wash step was required by washing for a third time and comparing the injected results between wash two and wash three. The first two washes were combined equally and weighed during the process of combining. The first wash extract always weighed more than the second wash extract, indicating that the majority of contaminants were removed during this first wash. The difference between wash two and three was so small that it was decided two washes with a rinse step was sufficient. If a third washing step was introduced,
this may require the sample to be dried and reconstituted more often, adding more
time and potential for errors to occur.

Multiple samples of banknotes were washed and the spiked washings injected and
analysed using the TD-MS/MS method (injected 20 times using specified
parameters). Twenty banknotes were used for method development purposes.
Seventy banknotes were washed individually and sixty banknotes were batch tested
for further studies. Details of the methods employed for washing and reconstituting
can be found in section 3.0 (Preparation of Real Banknote Samples).

5.5 Response Factor Results (Section 5.3)

Once the peak areas of the two cocaine isotopes were identified, the ratio difference
between the two peaks was calculated. The relative response factor (RRF) was then
calculated for each of the mixtures, so that an average response could be identified
(5).

\[
R_{X/\text{is}} = \frac{\left( \frac{A_X}{M_X} \right)}{\left( \frac{A_{\text{is}}}{M_{\text{is}}} \right)} = \frac{A_X}{A_{\text{is}}} \times \frac{M_{\text{is}}}{M_X}
\]

<table>
<thead>
<tr>
<th>A</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Cocaine concentration</td>
</tr>
<tr>
<td>is</td>
<td>Deuterated internal standard</td>
</tr>
<tr>
<td>(x)</td>
<td>Cocaine hydrochloride</td>
</tr>
</tbody>
</table>

Table 8 details the results from the five repeat injections after they had been
averaged and the Response Factor calculation applied. The peak area responses
were not linear, however followed a logarithmic curve. Once the data
had been logged, a straight line graph was plotted achieving an \(R^2\) value of 0.997
(Figure 25).
Table 8. Ratio response of the various mixtures of cocaine and deuterated cocaine standards (n=5)

<table>
<thead>
<tr>
<th>Cocaine Standard % Content</th>
<th>Ratio</th>
<th>Peak Area Ratio 182/185 Ions</th>
<th>Log_{10} Peak area ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>1:1</td>
<td>1.249</td>
<td>0.097</td>
</tr>
<tr>
<td>33%</td>
<td>1:2</td>
<td>0.678</td>
<td>-0.169</td>
</tr>
<tr>
<td>20%</td>
<td>1:4</td>
<td>0.342</td>
<td>-0.466</td>
</tr>
<tr>
<td>67%</td>
<td>2:1</td>
<td>2.590</td>
<td>0.413</td>
</tr>
<tr>
<td>80%</td>
<td>4:1</td>
<td>4.950</td>
<td>0.695</td>
</tr>
</tbody>
</table>

Figure 25. The Log_{10} response of the mixtures at varying concentrations of cocaine and deuterated cocaine standards showed a straight line graph (obtained using the m/z 182:185 ion transitions).

The overall RF for the m/z 182:185 ions showed greater variation than the product ions m/z 105:105 ions (Table 9). Whilst this shows variation in the standards, when compared with the results of the real banknotes tested (see section 6.0) with an internal standard spike, a larger standard deviation occurred using the m/z 105:105 transitions, the opposite effect than with the standards. Of note, the RF of a 1:1 mix was obtained at the time of injection of every banknote solute. The response of the 1:1 mix varied daily and ranged from 0.96 to 1.32 throughout the analysis period. This variation has not been considered on an individual basis, since the error of the
overall process should outweigh any slight variation in response factor. This should, however be considered if further work is to be conducted as it could play an important role in error analysis.

Table 9. Response factors for the different ion transitions and their errors

<table>
<thead>
<tr>
<th>Ion Transition</th>
<th>Average Response Factor</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>182:185</td>
<td>1.2995</td>
<td>0.0588</td>
</tr>
<tr>
<td>105:105</td>
<td>1.342</td>
<td>0.0334</td>
</tr>
</tbody>
</table>

5.6 Preliminary Experiment Results

5.6.1 Standard Solutions (Section 5.4.1)

The amount of cocaine hydrochloride within each standard solution varied depending on the exact amount weighed out. Approximately 0.002 g of cocaine hydrochloride was weighed out for dilution into methanol. A final concentration of 1 μg/mL cocaine hydrochloride was obtained through two further sets of serial dilution. The peak area responses were re-calculated to account for the hydrochloride presence, and provide cocaine base values. The errors involved with serial dilution are considered in the making up of standards section in Appendix B. Dilution Error Analysis.

The response factor of the standards were analysed over a period of time. Whilst variations occurred, this could have been due to sample variation. The use of the deuterated spike in the sample means that any instrumental change should be accounted for since there is the same reference compound running through each injected sample. The instrumentation was also checked on a daily basis using Shewhart charts to determine if it was performing within set parameters.

5.6.2 Instrument Optimisation (Section 5.4.2)

Cocaine hydrochloride and deuterated cocaine standards were injected directly into the thermal desorption inlet of the TD-MS/MS and were used to optimise the
instrument settings. Known amounts of a mixture of cocaine hydrochloride and deuterated cocaine standards were also injected into the instrument. The peak areas produced with varying concentrations were recorded.

Table 10. The instrument parameters for the simultaneous analysis of cocaine hydrochloride and deuterated cocaine when detecting fragments m/z 304/105, m/z 304/182, and m/z 307/105 and m/z 307/185

<table>
<thead>
<tr>
<th>Q1 Mass (m/z)</th>
<th>Q3 Mass (m/z)</th>
<th>Dwell (msec)</th>
<th>Parameter Settings (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>304.15</td>
<td>105.03</td>
<td>20</td>
<td>FP 370 EP 10 CEP 24 CE 45 CXP 4</td>
</tr>
<tr>
<td>304.15</td>
<td>182.12</td>
<td>20</td>
<td>FP 360 EP 9 CEP 32 CE 27 CXP 6</td>
</tr>
<tr>
<td>307.17</td>
<td>105.03</td>
<td>20</td>
<td>FP 360 EP 10.5 CEP 28 CE 45 CXP 4</td>
</tr>
<tr>
<td>307.17</td>
<td>185.14</td>
<td>20</td>
<td>FP 360 EP 10.5 CEP 26 CE 27 CXP 8</td>
</tr>
</tbody>
</table>

FP = Focusing Potential  
EP = Entrance Potential  
CEP = Collision Cell Entrance Potential  
CE = Collision Energy  
CXP = Collision Cell Exit Potential

The responses without the electrospray mechanism were small and thus parameters needed to be altered slightly to obtain higher responses. This is to be expected
due to the differences between the direct injection into the thermal desorber and the electrospray source, which feeds a constant liquid spray into the ionisation chamber.

5.6.3 Solvents and degradation (Sections 5.4.3 and 5.4.4)

When reviewing the instrumental parameters, it was observed that there was a difference in responses when various solvents were used. It was also found that the cocaine hydrochloride became saturated at lower concentrations in acetonitrile than methanol.

The standards made in the acetonitrile/methanol mix were found to produce very similar results to when they were originally analysed four weeks previously. Split peaks produced variable heights, even when injected using an autosampler, with heights ranging by up to double the smallest peak height. Peak area was considered as a much more reliable form of reviewing peak size. The peak area results alone showed that there was a significant difference between responses after only two weeks, however, the ion ratios proved to be more consistent, even at four weeks after originally being made, as shown in Figure 26, and further discussed in Appendix E. Initial Experiments.

![Figure 26. Peak area ratio responses of deuterated cocaine:cocaine hydrochloride over 4 weeks](image-url)

Figure 26. Peak area ratio responses of deuterated cocaine:cocaine hydrochloride over 4 weeks

---

55
The chromatogram peak shapes in methanol showed little peak splitting compared with other solvents and thus each peak response was more consistent with one another (Figure 27). However, a decrease in peak areas over time could be attributed to degradation of the samples in this solvent, or alternatively due to changes in instrument performance. Additionally, it was observed that the more concentrated ‘stocks’ of standards degraded at a slower pace than weaker standard concentrations. When conducting experiments and spiking samples, it is therefore important to spike with standards which were produced not only at the same time, but also in the same concentrations. Methanol was used as the solvent of choice due to saturation levels, and stability.

![Figure 27. Ion count intensity responses of cocaine hydrochloride ion transitions (a) 307:105 & (b) 304:105 analysed on the same date. The ratios of the ion transitions were very similar to that observed in the following weeks.](image)

5.6.4 Precursor Ion Scans and Interference of Results (Section 5.4.5)

The results showed that no other response was detected for the ion transitions of choice, indicating the specificity of the instrument method used. It is not common using this instrumentation to find interferents. A precursor ion scan of the cocaine ions (m/z 304 and 307) found several isotopomers, all decreasing in value, in accordance with that expected based upon the abundance of each of the carbon isotopes.
5.6.5 Injection Speed and Temperature (Section 5.4.6)

The results were found to differ from those originally obtained in the degradation studies and had smaller variation and standard deviations. When the cocaine mixture was injected using various instrument settings, a range of responses were observed: Where lower temperatures were employed, the peak areas of each individual injection were reduced and at higher temperatures, the peaks became less smooth in shape with more peak splitting. Despite having a number of small peak splits, it was observed that the ratios of the results obtained using the parameters 285°C and 50 μl/sec produced the smallest standard deviations (0.046 and 0.039, ‘Appendix G. Testing varying parameters for TD-MS/MS optimisation’) and thus the least variation in results.

5.6.6 Analysis of Banknotes and Washings (Section 5.4.7)

The experiments conducted assisted with establishing the final extraction method. They were also used to generate preliminary findings upon which to clarify the objects set. The anticipated results appeared successful enough to maintain the objectives and begin analysis with updated methods.

5.7 Summary

The instrument can be used to simultaneously detect both cocaine hydrochloride and its deuterated standard. Depending upon the solvent used and concentration, some degradation of the sample occurs over periods of more than four weeks. Therefore, experiments using the same samples of low concentrations need to be conducted within one month (see section 5.4.4). However, if the samples are used for generating a response factor (with internal standard), as long as they both degrade at similar rates, then any impact of degradation over time is less significant.

The optimum parameters have been established to allow the best results for all four of the ions. The ion transitions used are those used as standard in MSA and those also applied by Castiglioni et al. (2006).
6.0 Thermal Desorption Banknote Analysis

6.1 Introduction

Thirty two individual banknotes (notes labelled 71 to 103) and one hundred notes tested in batches of ten (batches labelled A-J) were tested using the optimum parameters and extraction methods as set out in sections 3.0. The results of these experiments were recorded and the different aspects of the analytical data reviewed and reported accordingly (section 6.2-6.3). Samples of a mixture containing varying ratios of both cocaine hydrochloride and deuterated cocaine were injected into the TD-MS/MS for comparison with results from ‘real’ banknote samples (see 3.0).

6.2 Banknote Analyses Using Isotope Dilution

Samples of the banknote washings obtained using the method set out in section 3.0 were spiked with an equal quantity of 1000 µg/mL deuterated cocaine standard, effectively making the spike concentration 500 ng/mL. Known amounts of the solutions were injected into the system twenty times and the peak areas of each ion transition recorded.

6.3 Banknote Analyses Results

6.3.1 Extraction Efficiencies

Extraction efficiencies were calculated by comparing the TD peak area results from both ends of the banknote; one end prior to any washing, and the other end following the two wash and rinse approach (as detailed in section 3.0 and in Figure 28).

When comparing the results, several outliers were removed, as they showed vastly differing results to that expected and were accounted for by uneven distribution of contamination across the banknote. This is to be expected when using non-
controlled samples from unknown sources. The remaining results displayed an average extraction efficiency of 95% with a standard deviation of +/- 4.

![Diagram showing the process of washing extraction efficiencies](image)

**Figure 28.** Washing extraction efficiencies were established by comparing the Thermal Desorption results from each end of the note before and after washing

### 6.3.2 Injections of Banknote Washings

The multiple injection results were examined for reproducibility and found that within each data set, there was often an outlier. Once each data set from an injection of standards or banknote washings was made, outliers were removed using box-and-whisker plot analysis using ‘Minitab’ version 16.2.2 (Figure 29). An outlier could be caused by a matrix effect within the solution, such as dirt or other contaminants found on banknotes, or a crystallized substance within the injection which would skew the results if injected.

With four samples (on individual banknotes), the overall comparison of results from one end of the banknote to the opposite end gave such differing results that they have been excluded as outliers. It is assumed that these samples in particular do not show an even distribution of cocaine contamination across the entire banknote. This refers to those where the amount extracted appeared less than that obtained through direct analysis i.e. a result which is contradictory to that expected and of the majority of all other tests.
Due to the nature of the TD method, the high throughput of large populations of banknotes helps to reduce any impact of the few banknotes which show uneven distribution. If, however, these results were included, it could be assumed that any uneven distribution (both positive and negative) would cancel each other out. However, a large sample size would be desirable to allow this to occur.

6.3.3 Comparison of Thermal Desorption (TD) with Washing

In comparison with the thermal desorption of the opposite end of the banknotes, it was observed that the amount of cocaine detected on the washed end of the banknote varied quite considerably to that detected when the banknote was analysed directly using TD, indicating a difference in extraction efficiency and analysis. This result was to be expected due to the differing methods and greater losses observed with direct analysis. The difference was further investigated by analysing the extract following the TD process. A discrepancy between the overall values was present (Figure 30)
The average loss over twenty-five individual banknotes tested was 40%, ranging from 4% to 94%, with the majority falling within 40% and 60%. Such variation is most likely caused through random variation in the spread of contamination across individual banknotes, and could also occur through human variation to a lesser extent. This, however, assumes that there is no loss from the injection of the solution into the system. In order to establish if there is a loss, how consistent this may be, and potential reasons for the findings, a comparison with a fully quantitative method is required.

6.3.4 Calculations of Amounts on Banknotes

The response factor obtained using the cocaine and deuterated standards (section 5.5) and the ratio between the internal deuterated standard and banknote peak was used to calculate the final concentration of cocaine from each banknote or batch of banknotes tested.
Using the RF, the concentration of the cocaine on each spiked banknote washing was calculated using the formula:-

\[
R_{x/is} = \frac{(A_x/A_{is})}{(C_x/C_{is})}
\]  

\(A\) = Area
\(C\) = Concentration
\(x\) = Cocaine
\(Is\) = Internal standard

Which is re-arranged to make \(C_x\) (the unknown cocaine concentration on banknotes) the subject of the equation:-

\[
C_x = \frac{(A_x/A_{is})}{(RF)} \cdot C_{is}
\]

Once the concentration of the unknown cocaine had been calculated, the value was adjusted to account for the initial dilution in methanol and that only one quarter of a banknote had been washed. Calculations were made for twenty eight individual banknotes (Figure 31) and ten batches of banknotes (consisting of ten banknotes each) (Figure 32).

![Figure 31. Amount of cocaine extracted from 28 individual banknotes (calculated using the equations above)](image-url)
The amount of cocaine per banknote varied, as expected, with amounts ranging from 0.5 µg to 23.1 µg, with an average of 4.6 µg. The batch analysis (Figure 32) showed less variation in results, as expected when analysing a larger sample. The average amount of the batches was 7.7 µg with a total range of 4.1 to 14.7 µg per banknote. With good extraction efficiencies, it is pertinent to consider the potential errors within the analyses and calculate their impact on the data obtained.

![Bar graph showing amount detected per banknote](image)

**Figure 32.** Amount of cocaine extracted from ten batches of ten banknotes (calculated using the equations above)

### 6.3.5 Errors

There are a number of factors that need to be considered when reviewing the overall results and calculated concentrations. Due to the steps involved in producing a standard, washing banknotes and analysing the sample, there will be a number of propagated errors involved with the final values.

The factors that affect the final result have been produced in a fishbone diagram (Figure 33), which considers the instrument performance and the banknote analysis. The three main factors which could affect the overall results are considered as:

1) the variation in injection of the banknote samples,
2) the variation of the RF injections at the differing ratio concentrations, and
3) the variation of the 1:1 RF injections carried out over time.
Figure 33. Flow diagram showing potential errors which will affect the overall result or instrument response
The last point was considered different to point two since this includes only a 1:1 mix and covers a difference in time, thus accounting for instrument variation and sample degradation. Most other points will not have as significant a bearing on the results and as such have not been considered.

A sum of squares of the points mentioned above were used to propagate the error of the TD-MS/MS banknote analysis, according to the equation:

\[
\frac{\delta Q}{Q} = \sqrt{\frac{\delta a^2}{a} + \frac{\delta b^2}{b} + \frac{\delta c^2}{c}}
\]  

(8)

When the results of the standard deviations were calculated, the overall error involved was approximately 10% (Table 11).

<table>
<thead>
<tr>
<th>Banknote injections</th>
<th>Standards at varying ratios</th>
<th>Standards repeated over time at 1:1 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std Dev</td>
<td>All notes tested (n=38)</td>
<td>1.299486</td>
</tr>
<tr>
<td>Average</td>
<td>0.07779</td>
<td>0.058767</td>
</tr>
<tr>
<td>RSD</td>
<td>7.78%</td>
<td>4.52%</td>
</tr>
<tr>
<td>Total</td>
<td>10.04%</td>
<td>3.16%</td>
</tr>
</tbody>
</table>

This does not, however, account for the TD losses within the system when analysing directly, as we have shown the losses previously. It appears that the majority or errors in establishing the TD efficiency and losses are caused by differences in distribution over the banknote area. In order to establish the error and efficiency involved in the TD, it would be recommended to spike notes with known amounts of cocaine and test using the same processes. Alternatively, if enough samples were tested, it could be assumed that the errors involved with distribution would cancel each other out, since the distribution is most likely to be randomly placed across a banknote.
6.4 Summary

Extraction efficiencies (95%) using this method are very good and in line with Esteve-Turrillas et al. (2005) who used similar methods. The errors involved with ‘real’ banknotes (uneven distribution) have implications for the analysis of single banknotes, however, it would be counteracted by using large populations of samples to achieve an overall average. To assist with data and error propagation, even distribution of contamination over a sample would benefit evaluation of certain factors. Experiments using spiked ‘clean’ notes could help to clarify certain errors in relation to instrument performance and capabilities. However, as discussed previously, the spiking of banknotes effectively and realistically is difficult.

The ability to quantify using isotope dilution is successful, with the downside of being time consuming. The amounts detected were in line with Negrusz, Perry and Moore (1998), Luzardo et al. (2011), and Wimmer and Schneider (2011), who report a range of contamination between 1 and 15 µg. The results are also similar to the lower range of Di Donato, Martin and Martinis (2007) and Oyler et al. (1996). Despite these results, it is still somewhat surprising that the results appear to be slightly lower than average, especially considering the prevalence of cocaine on UK currency (Sleeman, Carter and Ebejer, 2005) and that many others have reported values into the tens of micrograms.

Individual banknote washing analysis is helpful when needing to establish specific amounts on every sample, however, to gain an overall view of the contamination, batch testing could be a quicker method to yield higher throughput of samples. In addition, batch testing requires less solvent and fewer repeats, along with less time for multiple samples. Further experiments could involve batch testing to yield better averages of results in less time. Comparison with other techniques would help to establish the reliability of the quantitation of TD-MS/MS, however the results obtained thus far using isotope dilution have proven that the technique is reliable with reproducible results. Thermal desorption-tandem mass spectrometry is a powerful tool in the identification and quantitation of cocaine on banknotes, and isotope dilution of certain samples could assist with supporting the current forensic evidence provided.
7.0 High Performance Liquid Chromatography (HPLC)

7.1 Instrumentation & Standards

An Agilent 1100 Series HPLC with UV detector was used with a ‘Thermo’, hypersil Gold C18 column (150 x 4.6 nm with particle size of 5 µm). ‘ChemStation for LC 3D’ software (Revision A 10.02, Agilent Technologies) was used to view data.

A pH 3 potassium dihydrogen phosphate buffer (75%) with 15% acetonitrile and 10% methanol was used as the mobile phase with a flow rate of 1.2 mL/min at 30ºC. (Mercolini et al., 2008). Certified cocaine hydrochloride powder solids were obtained from Sigma-Aldrich (Missouri, USA), with purity values of >98% (obtained using TLC). Certified deuterated cocaine liquid standards were obtained from Cerilliant (Texas, USA) in 1mg/mL ampoules, with purity values of 99%.

7.2 Experimental

7.2.1 Identifying cocaine

To assist with identifying where the cocaine peak occurred, a banknote sample was spiked with 10 µg/mL cocaine standard. The λ of the peak was expected to occur within the 230 nm region, as established through the fluorescence work.

7.2.2 Testing column performance

Two instruments (both as indicated above) with different columns (1 = Thermo, Hypersil Gold, C18 column, 150 x 4.6 mm with particle size of 5 µm, and 2 = Phenomenex, kinetix C18 column, 75 x 4.6 mm with a particle size of 2.6 µm) were used to establish which column would be best suited to the experiment. It was assumed that the high performance Phenomenex column would work most efficiently due to its selection capability. A mobile phase of pH 3 potassium dihydrogen phosphate buffer (85%) with 15% acetonitrile was used.
7.2.3 Standard Solutions and Calibration Curve

Standard solutions containing between 1 µg/mL and 50 µg/mL cocaine hydrochloride (standards purchased from Cerilliant) were dissolved methanol and further diluted in HPLC mobile phase solution (10% methanol, 15% acetonitrile and 75% phosphate buffer). 100 µL of each standard was injected onto the sample loop (split and 10 µL taken onto the column) to produce a calibration curve. Blanks were run between each sample.

7.2.4 Banknote Washings

One hundred banknotes were washed in batches of ten. The final solutes were each passed through a filter to remove visible detritus, prior to introduction onto the column and were dried and reconstituted in mobile phase, increasing the concentration by five times. The same volumes injected and a buffer was used as with the standards mentioned above. Two instruments were used, with different columns, to establish if any one performed better than another. This was repeated to review its reproducibility. Blanks were run between each sample introduction.

7.3 HPLC Results

7.3.1 Identifying cocaine (Section 7.2.1)

When running samples initially, the peak clarity was poor. The samples were dried and reconstituted in the same volume of buffer, to enhance peak clarity.

The spiked sample identified where the cocaine peak occurred, however, it was observed that the λ of the signal was not at the expected 233 nm. This was resolved once the baseline signature wavelength had been removed.

7.3.2 Testing column performance (Section 7.2.2)

During repeat analyses, column drift occurred on both instruments, most significantly with the Phenomenex column. Initially it was thought that the
increasing concentrations of standards affected this. However after leaving overnight on slow flow to combat any effects, the column drift was not rectified.

Mercolini et al., (2008) use a slightly different mobile phase to that initially employed, wherein the phosphate buffer is reduced to 75% by substituting with 10% methanol and the flow rate is slightly slower. It is thought that the pKa of cocaine could have caused some interaction with the pH of the buffer, reducing its efficiency on the column. These settings reduced the column drift of the ‘Hypersil Gold’ significantly. The ‘Phenomenex’ column was still problematic and therefore further experiments were conducted using the ‘Hypersil Gold’.

7.3.3 Standard Solutions and Calibration Curve (Section 7.2.3)

Initial experiments had established that the cocaine responses on real banknotes were smaller than expected and therefore the concentration of the standards was reduced accordingly. A linear calibration curve ($R^2$ value of 0.999) was produced within the range of concentrations tested (Figure 34). This was repeated to review its reproducibility. Blanks were run between each sample introduction.

![Figure 34. Calibration Curve of Cocaine Hydrochloride Using HPLC and Fluorescence Detection ($m = 37.067, c = -6.1695$)]
7.3.4 Banknote Washings & Calculations (Section 7.2.4)

Using the calibration curve, concentrations of the batches of banknotes were calculated using \( y = mx + c \) model. The calculation also considered:

- a scaling factor to account for the extract dilution of 70 mL on the banknotes,
- only a quarter of each of the ten notes (in batches) were tested,
- the concentration step wherein the samples were reconstituted in buffer,
- banknotes were tested in batches of ten.

Upon review, it was observed that the values obtained for the cocaine concentrations were too high (Table 12), compared with published examples of expected ranges of concentrations (see Table 2). The filtering of the solute could have concentrated the samples through absorption of some solvent onto the filter paper. However, the large apparent increase in concentration could not be accounted for solely through filtering. Alternatively, the matrix effects of the banknote samples could cause interference, or the samples could have become contaminated.

<table>
<thead>
<tr>
<th>Extract</th>
<th>RT</th>
<th>Peak Area</th>
<th>Conc. From Calib. Curve</th>
<th>Ave conc. Per whole banknote (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9.694</td>
<td>90.98</td>
<td>2.62</td>
<td>14676</td>
</tr>
<tr>
<td>B</td>
<td>9.74</td>
<td>58.76</td>
<td>1.75</td>
<td>9808</td>
</tr>
<tr>
<td>C</td>
<td>9.708</td>
<td>61.30</td>
<td>1.82</td>
<td>10193</td>
</tr>
<tr>
<td>D</td>
<td>9.808</td>
<td>176.00</td>
<td>4.91</td>
<td>27470</td>
</tr>
<tr>
<td>E</td>
<td>9.828</td>
<td>67.16</td>
<td>1.98</td>
<td>11078</td>
</tr>
<tr>
<td>F</td>
<td>9.904</td>
<td>178.09</td>
<td>4.97</td>
<td>27836</td>
</tr>
<tr>
<td>G</td>
<td>-</td>
<td>111.92</td>
<td>3.19</td>
<td>17840</td>
</tr>
<tr>
<td>H</td>
<td>9.946</td>
<td>49.76</td>
<td>1.51</td>
<td>8450</td>
</tr>
<tr>
<td>I</td>
<td>9.946</td>
<td>80.76</td>
<td>2.35</td>
<td>13132</td>
</tr>
<tr>
<td>J</td>
<td>9.964</td>
<td>49.49</td>
<td>1.50</td>
<td>8409</td>
</tr>
</tbody>
</table>

7.1 Summary

Using appropriate settings, the HPLC was able to resolve cocaine peaks from other compounds within the sample. The analysis time was significantly shorter than that
of GC/MS, with less sample preparation required. The calibration curve proved linear within critical range, however, it does not include the matrices contained on banknotes themselves. The issues identified when initially analysing were overcome by choosing an appropriate column and mobile phase.

The final problem identified was that of the banknote analysis response, as the overall calculations yielded a final concentration value which was too high. In this circumstance, it could be pertinent to use a clean-up step involving solid phase extraction or an internal standard. It is most likely that the issue has occurred through operational error (contamination of the samples), or through matrix effects, (matrices on banknotes can be complex).

Based upon other research and the straight line calibration curve, along with its specificity and speed, this method appears to be an excellent source of corroboration for the purposes of this study. I would recommend further research to establish how to continue its use, and determine whether any interferents exist.

### 8.0 Gas Chromatography Mass Spectrometry (GC/MS)

#### 8.1 Instrumentation

A Perkin Elmer Clarus 600/Clarus 500A, GC/MS was used with a 30m, 250 µm internal diameter, DB-5ms capillary column. Parameter settings were altered throughout the experimental phase, resulting in final settings as detailed in Table 13.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Set-up</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier gas</td>
<td>Helium</td>
<td>20 mL/min</td>
</tr>
<tr>
<td>Oven Temp.</td>
<td>Ramp 15 °C min⁻¹</td>
<td>60 - 300 °C</td>
</tr>
<tr>
<td>Split</td>
<td>Off</td>
<td></td>
</tr>
<tr>
<td>Injection</td>
<td>2 µL</td>
<td></td>
</tr>
<tr>
<td>Run</td>
<td>23 mins</td>
<td></td>
</tr>
<tr>
<td>Selected Ion Monitoring (SIM)</td>
<td>Deuterated cocaine</td>
<td>306, 185, 85</td>
</tr>
<tr>
<td></td>
<td>Cocaine</td>
<td>303, 182, 82</td>
</tr>
</tbody>
</table>
8.2 Preliminary Experiments

8.2.1 Banknote Analysis

Banknotes were prepared in accordance with the methodology set out in section 3.0. In order to increase the amount of cocaine within the sample, batch testing was also employed. Experiments were conducted using unadulterated samples and also when 1mL of the sample was dried under a stream of nitrogen and reconstituted in 200 µL C\textsubscript{24}.

To further assist with sample preparation, dried banknote samples were reconstituted in the deuterated standard and ethyl acetate, rather than the normal internal standard of C\textsubscript{24}. Samples were injected in split mode and splitless mode. Due to the time constraints that GC/MS has over the other techniques employed, SPE extraction was not employed in the first instance, since the other experiments conducted did not include this step.

8.2.2 Standard Solutions and Internal Spike

Deuterated standards at varying concentrations were run to establish the retention time with varying parameters. Mixtures of known concentrations of cocaine hydrochloride and deuterated cocaine were used as reference standards, establishing a response factor and further clarification of retention times for each. Internal deuterated standard spikes (section 5.4.1) were used within banknote samples and SIM employed to further assist with peak identification.

8.3 Preliminary GC/MS Results

8.3.1 Banknote Analysis (8.2.1)

Initial tests established that without concentrating the sample, the peak was difficult to identify. Also without any clean-up step, the baseline engulfed the majority of the peaks. Samples containing one banknote wash and a ten banknote batch wash
(after drying and reconstituting in C$_{24}$ – analysed in split mode) only identified a peak with the more concentrated ten banknote batch sample.

Despite the absence of solid phase extraction, when the samples had been dried and reconstituted in deuterated standard, the cocaine peak could be readily identified amongst the other matrices within the sample (Figure 35). As the banknote peak was smaller than expected, the deuterated spike was reduced accordingly, to allow better comparisons within similar ranges.

![Figure 35. Chromatograms showing the cocaine response from a real banknote sample with deuterated spike. Good resolution and the ability to distinguish between (b) the deuterated and standard cocaine was apparent.](image)

The batch sample was re-analysed using splitless mode wherein an identifiable peak became much more apparent. These results indicated that a simple drying and
reconstituting step can assist with sample clean up and enable identification of contamination in splitless mode.

### 8.3.2 Standard Solutions and Internal Spike (Section 8.2.2)

The peaks of the two cocaine standards were identified readily within the chromatogram using the spectra to confirm the molecule. Both had a retention time of approximately 14.55 mins.

![Chromatograms of a 2:1 mix of (a) standard cocaine and (b) deuterated standard](image)

Figure 36. Chromatograms of a 2:1 mix of (a) standard cocaine and (b) deuterated standard

The standard mix was run using identical parameters to that of the banknote sample (splitless mode, SIM), as detailed in Table 13.
8.4 Response Factor

Using the peak areas of the cocaine hydrochloride and deuterated cocaine mixtures, the ratio response was calculated (Table 14). It was observed that the 1:1 mix appeared to show anomalous results and was therefore excluded as an outlier. When the remaining three points were plotted, the ratio responses gave a curved graph, however the log values provided a straight line fit, with an $R^2$ value of 0.9995 (Figure 37). Further corroboration of the three points could assist with confirming the straight line fit, and is recommended as part of further research.

Table 14 – Ratio responses of varying mixtures of cocaine and deuterated standards

<table>
<thead>
<tr>
<th>Cocaine Standard % Content</th>
<th>Ratio</th>
<th>Peak Area Ratio 182/185 Ions</th>
<th>Log$_{10}$ Data of Ratio</th>
<th>Calculated Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>33%</td>
<td>1:2</td>
<td>0.208</td>
<td>-0.682</td>
<td>0.416</td>
</tr>
<tr>
<td>50%</td>
<td>1:1</td>
<td>1.718</td>
<td>0.235</td>
<td>1.718</td>
</tr>
<tr>
<td>67%</td>
<td>2:1</td>
<td>1.359</td>
<td>0.133</td>
<td>0.679</td>
</tr>
<tr>
<td>83%</td>
<td>5:1</td>
<td>3.802</td>
<td>0.580</td>
<td>0.951</td>
</tr>
</tbody>
</table>

Using this information, the relative response factor (RRF) was considered (Table 14) using the equation given in section 5.5. However, when the equation was applied, the average values could not be plotted due to variation. This can be observed in GC/MS where cross contribution of ions can have an effect upon the standard’s response at certain concentrations (Whiting et al., 2001).

Figure 37. Log$_{10}$ data from the ratio comparisons of peak areas of differing amounts of cocaine:deuterated standard
It has been reported by Wu et al. (2006) that cross contribution can occur in SIM from $^2$H analogues to its non-isotopic counterpart, to a greater extent than occurs vice versa. They report that the best method of overcoming this is to employ hyperbolic or polynomial calibration, but response can also vary with the choice of ions.

### 8.5 Banknote calculations

During the initial banknote analysis, the quantitative information was calculated using the RRF from the cocaine:deuterated mix with the closest ratio response, helping to take into account any cross contribution from the deuterated sample. Using the calculations as detailed in section 6.3.4, the amount of cocaine on the sample of banknotes was calculated: 

\[
C_x = \frac{(A_x/A_{is})}{(RF)} \cdot C_{is}
\]  

(9)

The peak areas of the cocaine and deuterated internal standard were used, as well as the response factor from the 5:1 mix (Table 14). When these were applied, the average amount detected on an individual banknote from the tested batch was 19 µg.

\[
C_x = \frac{(148901/34896)}{(0.950581)} \cdot 0.909 \mu g/mL = 5.10 \mu g/mL
\]  

(10)

Values input into equation (9) to provide initial concentration upon which to calculate the final amount per banknote

The dilution factors and sample size were then calculated to provide a final approximate value per note of 23 µg.

Without further injections of the standard mixes or repeats of the sample, a further calculation was made to compare the RRF calculated result with a single calibration point (11). The calculated amount per note using this method was approximately 18 µg.

\[
C_x = \frac{A_x}{A_{is}} \cdot C_{is}
\]  

(11)
Whilst the result differs to that using a RRF, this is still in line with the other value and is more prone to error given the ratio contributions involved. Both results are slightly higher than those obtained using TD-MS/MS, however could be due to random variation in banknote contamination. In order to establish the average values using both techniques, further samples need to be analysed, and tested simultaneously for a direct comparison to be made.

### 8.1 Summary

Due to time constraints, further experiments, repeats and more complex review of data were not conducted. The initial indications showed promising results with minimal sample clean-up.

Through obtaining the isotope dilution data from the TD-MS/MS it could be possible to cross reference these values as being ‘typical’ of banknotes in general circulation, despite being lower than originally anticipated. Initial findings indicate that limited sample clean up could be adopted to reduce the overall analysis time. In SIM mode, the chromatogram appeared to show good resolution with identification of the ions selected. Further work into the quantitation and its reproducibility is required.

Further experiments will need to establish the relative errors involved with this type of analysis, and can also consider cross contribution of isotopes and find the best transitions to use. Alternatively, a polynomial calibration approach could be adopted, or the amount of deuterated spike altered to produce a 1:1 peak area with the unknown solute. By applying a 1:1 peak area response for each sample, the cross contribution of ions at varying concentrations is eliminated, however this requires an additional analysis step to establish the amount of deuterated standard required, thus adding to an already protracted method.

The method used is supported by the many other researchers who have used this type of technique to quantify cocaine traces on banknotes and is worth exploring further. However, given that HPLC could also involve less sample preparation and
involve shorter analysis times, it would be advisable to consider these factors when choosing an appropriate method.

9.0 Overall Summary

The extraction method, of twice washing batches of banknotes in methanol, appears to be efficient in extracting the majority of cocaine from them, and is similar to that used by many other researchers. Overall, TD-MS/MS has been shown to be a reliable and quick quantitative technique without any sample clean-up required. Using targeted analysis in SRM mode helps to eliminate any matrix interference, and the TD inlet allows high throughput of samples. Further repeats will allow more statistical evaluation of the results on banknotes and help to further establish the efficiency of the thermal desorption unit.

GC/MS with SIM testing without a sample clean-up step (such as SPE), but with simple drying and reconstitution appear sufficient to provide suitable concentration values on banknotes. Other researchers, however, have found SPE to provide good results and reduce any matrix effects.

The overall values obtained and calculated using both TD-MS/MS and GC/MS appeared consistent with that found by the majority of published data using a variety of techniques and banknotes. A comparison of the range of values identified and the averages are plotted in Figure 38. To assist with comparison, the results have been logged to allow the data to be shown on a smaller scale. It is apparent from this information that Jourdan et al. (2013) and Bones, Macka and Paull (2007) produce values much lower than the remaining reported figures, however in the case of Jourdan et al. (2013), this may be due to the technique applied (vacuuming) and is not particularly comparable.

The values obtained in this current study (averages of approximately 6 µg per note with a range of 0.8 – 23 µg) fall in line with the majority of other reported values. Such responses corroborate that the methods employed are successful in quantifying cocaine on banknotes. Further repeats, larger sample sizes and expansion of
comparative studies with different instruments would assist with statistical evaluation and establishing errors.

![Figure 38. A log$_{10}$ plot of the range of cocaine levels detected by different researchers and the average (log) values (red), with the addition of results obtained in this research (Johnson)](image)

By applying this method to analyse seized samples of banknotes which have already been found to bear greater than typical levels of cocaine on them, it could assist the courts by providing grounds for the notes having an association with drug related activities. It could also assign a particular value to the cocaine levels, which could also help lay persons in court interpret the information gathered more easily (since comparisons of larger and smaller numbers are often more straightforward than pattern or chromatogram analysis). Such numerical values could also then be used for scientific review by other laboratories to corroborate the findings independently, which is particularly useful within the justice system.

Fluorescence analysis appears to show difficulties in distinguishing the cocaine peak in ‘real’ samples, most probably due to matrix effects. Its ability to quantify using standards is adequate, however must take into account any quenching factors. Due to these limitations, fluorescence detection on banknote washings, and their associated complex matrices, is inadequate without further sample clean-up and additional steps to combat quenching.
Straight line calibration curves were created using HPLC and showed good resolution. However, it appears that HPLC with fluorescence detection also suffers from interferents within the banknote sample matrix. Sample clean-up could be employed or alternative analytical detection technique twinned with the HPLC (e.g. MS) could offer better selectivity and specificity.

10.0 Recommended Further Research

Some degree of degradation of standards and liquid samples was observed. In order to establish the rate of deterioration of samples, it would be useful to conduct more studies. This should allow the timescale for analysis to be determined and reduce the error caused by differences in the reproducibility.

The variation in response factor of the internal standard could be considered when conducting further experiments using TD-MS/MS. This was shown to vary slightly between different experiments and on different days, which should be accounted for if variation is large. Furthermore, the gradual change in response factor using GC/MS should be explored further and a suitable method of calibration assigned.

Further HPLC quantitation studies could help to corroborate the cocaine concentration of samples. If sample clean-up is required, research could be conducted into the simplest form of clean-up step, such as wire wool, which is unlikely to absorb much of the solvent. Research could also show the extraction efficiencies once full quantitation has been satisfied using HPLC. HPLC-MS or alternative detectors should also be explored. Repeats of more samples using GC/MS and a direct comparison with another method (for example TD-MS/MS) could help to compare the different techniques directly, in particular if combined with studies through spiking banknote directly to help establish TD efficiency.

With the introduction of plastic banknotes, further research could help to show the cocaine affinity for different materials, such as linen and plastic. In addition, this may favour new methods of analysis and detection, especially since plastic notes can be more easily washed without risk of damage, but would perish in the TD.
References


83


86


Images and Structures References

Images and structures were drawn using specialist software:


Autodesk Inc. (AutoCAD) (2010). Available from Autodesk, Inc. San Rafael, CA, United States of America (USA)
Appendix A. Fluorescence Background Subtraction

Manual background subtractions using the total sum areas of the fluorescence response were calculated:

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>Area with background</th>
<th>Area with background removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4529</td>
<td>3005</td>
</tr>
<tr>
<td>100</td>
<td>6112</td>
<td>4588</td>
</tr>
<tr>
<td>200</td>
<td>8337</td>
<td>6813</td>
</tr>
<tr>
<td>400</td>
<td>17000</td>
<td>15476</td>
</tr>
<tr>
<td>600</td>
<td>19411</td>
<td>17887</td>
</tr>
<tr>
<td>800</td>
<td>23790</td>
<td>22266</td>
</tr>
<tr>
<td>1000</td>
<td>23532</td>
<td>22008</td>
</tr>
<tr>
<td>1250</td>
<td>30058</td>
<td>28534</td>
</tr>
<tr>
<td>1500</td>
<td>32374</td>
<td>30850</td>
</tr>
</tbody>
</table>
Appendix B. Dilution Error Analysis

Dilution errors were estimated for each concentration of cocaine and the number of dilutions involved. Below is an example of the errors considered for the 1000 ng/mL sample.

Initial Concentration Error

<table>
<thead>
<tr>
<th>Mass (mg)</th>
<th>Error</th>
<th>Flask Volume (mL)</th>
<th>Error</th>
<th>Conc. (mg/mL)</th>
<th>Error</th>
<th>Relative Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.1</td>
<td>100</td>
<td>0.05</td>
<td>0.1</td>
<td>0.1</td>
<td>0.01001</td>
</tr>
</tbody>
</table>

First Dilution Error

<table>
<thead>
<tr>
<th>Conc. (mg/mL)</th>
<th>Error</th>
<th>Pipette Volume (mL)</th>
<th>Error</th>
<th>Flask Volume (mL)</th>
<th>Error</th>
<th>Conc. (mg/mL)</th>
<th>Error</th>
<th>Relative Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.00101</td>
<td>1</td>
<td>0.007</td>
<td>100</td>
<td>0.05</td>
<td>0.001</td>
<td>1.2227</td>
<td>0.012227</td>
</tr>
</tbody>
</table>
Appendix C. Fluorescence Regression Analysis

Regression analysis when considering the dilution errors (Appendix B. Dilution Error Analysis) was plotted to review whether a straight line fit could be observed when taking into account the propagation of errors involved with serial dilution. The regression fit was still linear, and therefore further investigation into the curved results was needed.

Equation: $E = ax + b$

<table>
<thead>
<tr>
<th></th>
<th>sigma x</th>
<th>Obs</th>
<th>sigma Obs</th>
<th>W</th>
<th>wx</th>
<th>wx2</th>
<th>wObs</th>
<th>wObsx</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>7.99609E-07</td>
<td>3005.65</td>
<td>0</td>
<td>1.56403E+12</td>
<td>7.82014E+13</td>
<td>3.9101E+15</td>
<td>4.70092E+15</td>
<td>2.35046E+17</td>
<td>3165.578</td>
</tr>
<tr>
<td>100</td>
<td>1.34443E-06</td>
<td>4588.46</td>
<td>0</td>
<td>5.5325E+11</td>
<td>5.5325E+13</td>
<td>5.5325E+15</td>
<td>2.53857E+15</td>
<td>2.53857E+17</td>
<td>4367.885</td>
</tr>
<tr>
<td>400</td>
<td>5.01697E-06</td>
<td>15476.99</td>
<td>0</td>
<td>39729837108</td>
<td>1.58919E+13</td>
<td>6.3568E+15</td>
<td>6.14898E+14</td>
<td>2.45959E+17</td>
<td>11581.72</td>
</tr>
<tr>
<td>600</td>
<td>7.55447E-06</td>
<td>17887.97</td>
<td>0</td>
<td>17522340985</td>
<td>1.05134E+13</td>
<td>6.308E+15</td>
<td>3.13439E+14</td>
<td>1.88063E+17</td>
<td>16390.95</td>
</tr>
<tr>
<td>800</td>
<td>1.00339E-05</td>
<td>22266.57</td>
<td>0</td>
<td>9932459277</td>
<td>7.94597E+12</td>
<td>6.3568E+15</td>
<td>2.21162E+14</td>
<td>1.76929E+17</td>
<td>21200.17</td>
</tr>
<tr>
<td>1000</td>
<td>1.2227E-05</td>
<td>22008.82</td>
<td>0</td>
<td>6688963211</td>
<td>6.68896E+12</td>
<td>6.689E+15</td>
<td>1.47216E+14</td>
<td>1.47216E+17</td>
<td>26009.4</td>
</tr>
<tr>
<td>1250</td>
<td>1.43538E-05</td>
<td>28534.06</td>
<td>0</td>
<td>4853632641</td>
<td>6.06704E+12</td>
<td>7.5838E+15</td>
<td>1.38494E+14</td>
<td>1.73117E+17</td>
<td>32020.93</td>
</tr>
<tr>
<td>1500</td>
<td>1.65869E-05</td>
<td>30850.02</td>
<td>0</td>
<td>3634711495</td>
<td>5.45207E+12</td>
<td>8.1781E+15</td>
<td>1.12131E+14</td>
<td>1.68196E+17</td>
<td>38032.46</td>
</tr>
</tbody>
</table>
Appendix D. Calculating an outlier in the Fluorescence calibration results

The 1000 ng/mL sample appeared to be an outlier when plotted and compared with the other results. Calculations showed that it varied from more than 3 standard deviations and so it was formally excluded to allow better evaluation of the calibration curve data.

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Observed fluorescence</th>
<th>Predicted fluorescence</th>
<th>(Pred. - obs.)^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4529</td>
<td>4100</td>
<td>184176</td>
</tr>
<tr>
<td>100</td>
<td>6112</td>
<td>6091</td>
<td>455</td>
</tr>
<tr>
<td>200</td>
<td>8337</td>
<td>9661</td>
<td>1752797</td>
</tr>
<tr>
<td>400</td>
<td>17001</td>
<td>15505</td>
<td>2236527</td>
</tr>
<tr>
<td>600</td>
<td>19412</td>
<td>20087</td>
<td>456882</td>
</tr>
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<td>23790</td>
<td>23777</td>
<td>171</td>
</tr>
<tr>
<td>1250</td>
<td>30058</td>
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<td>18310</td>
</tr>
<tr>
<td>1500</td>
<td>32374</td>
<td>32468</td>
<td>8939</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Sum of squares</th>
<th>Standard error</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>4658259</td>
<td>965</td>
<td>0.997</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Observed fluorescence</th>
<th>Predicted fluorescence</th>
<th>Standard deviation of result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>23532</td>
<td>26811</td>
<td>3.397</td>
</tr>
</tbody>
</table>
Appendix E. Initial Experiments (Fluorescence and TD-MS/MS)

**TD-MS/MS Experiments**

1) To review the differences in extraction from various materials, liquid samples of cocaine were injected onto pieces of cotton paper, pieces of standard printer paper and pieces of foil, all cut to the size of a banknote. Once dried, the samples were inserted directly into the thermal desorption region, and then inserted again. The majority of response was removed from the foil during the first analysis stage, however much more remained on the paper and cotton samples.

2) The number of extractions from real banknote samples was established by testing multiple banknotes with a variety of washing steps. Once a third step was introduced, the banknote was dried and analysed directly using TD-MS/MS, and the first, second and third washing solutes were injected individually. The third wash was found to show very little signal detection, yet the dried banknote showed some degree of response. The response was much smaller than that of wash one and two.

   It was postulated that the remnants of cocaine could be due to that found in the wash solution and retained by the banknote itself and therefore a ‘rinse’ step after wash two was conducted. This rinse step helped to prove that the final third wash was unnecessary and merely diluted the final solute down to a lower level.

3) The syringe used to inject the liquid samples was washed between each analysis (except where multiples or repeats were carried out on the same sample). Initial tests found that rinsing 15 times in methanol and then using the syringe to inject a blank into the TD-MS/MS still found detectable traces of residue. Whilst the traces were very small and should not have had a significant impact upon most samples (especially due to multiple injections), since the concentration of the banknote samples was unknown, the cleaning was essential to avoid carry over from a highly contaminated sample to a low one. Repeat tested found that
approximately 30 rinses of the syringe cleared the residue of all cocaine samples tested.

4) During GC/MS analysis, sample extraction and clean up steps were considered. However due to the additional time taken to employ SPE or similar clean up steps, the sample was simply dried and reconstituted in a number of solvents. The solvent was dried and reconstituted in ethyl acetate (to try and dissolve the cocaine, but not the grease and contaminants), and a variety of internal standards. The drying and reconstitution step found that detection was much improved upon direct injection without any clean up.

**Fluorescence**

5) Cotton paper was handled ten times for approximately three minutes per time, placed onto desks and subjected to general environmental conditions. This paper was tested using the fluorimeter to establish if there were any interferents. Whilst no response was observed, it cannot be confirmed that such little handling compared with banknotes could not cause interference. Based upon the results from the real banknote samples, it is advisable that such interferent studies be conducted, or indeed methods into sample clean-up considered.
Appendix F. Degradation of samples in solvents

A review of the peak areas from samples made in methanol only showed reproducible peak areas with very little peak splitting. Samples made in acetonitrile produced split peaks, which in turn provided a wide range of responses. This may have been due to the volatility of the acetonitrile within the thermal desorber.

Comparisons of the peak area responses when injected into the TD-MS/MS on day one, fourteen and thirty of production were made. The same comparisons were made between the ratio of the ions (cocaine:deuterated). After thirty days of refrigeration, the ratio of the samples in methanol and acetonitrile appeared to change, and a T-Test indicating that the change was significant. Water, however, showed no significant signs of change in ratio response.

The use of water was considered due to the indication that the cocaine ratios remain more stable. However with peak areas being important to the overall calculations, methanol was chosen as the optimum solvent and analyses conducted within suitable timescales. Further research and repeats should be conducted to further clarify if any significant degradation occurs, in particular if other solvents are chosen.

<table>
<thead>
<tr>
<th>Week 1 ratio</th>
<th>Week 2 ratio</th>
<th>Week 3 ratio</th>
<th>Week 4 ratio</th>
<th>T-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>182:185 in MeOH</td>
<td>182:185 in MeOH</td>
<td>182:185 in MeOH</td>
<td>182:185 in MeOH</td>
<td>Week 1 to Week 4</td>
</tr>
<tr>
<td>0.899</td>
<td>0.905</td>
<td>0.905</td>
<td>0.867</td>
<td>0.0056</td>
</tr>
</tbody>
</table>
Appendix G. Testing varying parameters for TD-MS/MS optimisation

A review of the peak areas from samples made in methanol only showed reproducible peak areas with very little peak splitting.

<table>
<thead>
<tr>
<th>Injection Speed μl/sec</th>
<th>Temp °C</th>
<th>Std Dev 105 ions</th>
<th>Std Dev 182/7 ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>285</td>
<td>0.04579</td>
<td>0.03886</td>
</tr>
<tr>
<td>30</td>
<td>285</td>
<td>0.07323</td>
<td>0.08871</td>
</tr>
<tr>
<td>50</td>
<td>250</td>
<td>0.06260</td>
<td>0.06108</td>
</tr>
<tr>
<td>80</td>
<td>250</td>
<td>0.04266</td>
<td>0.05474</td>
</tr>
<tr>
<td>80</td>
<td>200</td>
<td>0.07121</td>
<td>0.04826</td>
</tr>
<tr>
<td>50</td>
<td>225</td>
<td>0.06386</td>
<td>0.06717</td>
</tr>
<tr>
<td>80</td>
<td>225</td>
<td>0.06161</td>
<td>0.09047</td>
</tr>
</tbody>
</table>

Correlation of the ratio values for Cocaine 304/105 to 307/105 (parameters: temp 285, inj 50)