DIAZEPAM: THE DETECTION OF A DATE RAPE DRUG.

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The aim of this research was to determine if diazepam can be detected by Gas Chromatography-Mass Spectrometry (GC-MS), ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR), Infrared and Raman Spectroscopy. The results of the ^{1}H-NMR, infrared and Raman spectroscopy suggest that these techniques are more suited for preliminary identification instead of confirmatory identification due to the signal of diazepam appearing to be over-powered by the tablet's fillers. The GC-MS results show that it was possible to detect a pure diazepam tablet which allowed for the estimation for the limit of detection to be 0.001 mg/mL and the limit of quantification to be 0.02 mg/mL. This meant that if a 2 mg diazepam or Valium tablet were to be used in a drug facilitated sexual assault or rape, that the amount of diazepam that could be recovered from the surface of a mobile phone, inside the pocket of a pair of jeans, or inside the wallet, would be in great enough quantity to be detected. This research has shown that should a perpetrator leave a trace of diazepam, it would be forensically possible to link a suspect to the spiking, which could increase the prosecution and conviction rate of rape

Key words: Diazepam; Rape; Facilitated sexual assault; Surface detection; Gas Chromatography - Mass Spectrometry; ^{1}H-Nuclear Magnetic Resonance; Infrared; Raman spectroscopy

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1.0 Introduction

1.1 Background Research

In 2019, it was reported that almost 10% of adults in England and Wales have used or use illicit drugs, with 38% of 15-year-olds using drugs (Public Health England, 2020). The statistics show the need for the ability to identify drugs in small quantities across a multitude of surfaces in an effective and reliable way because when such a proportion of adults in England and Wales are using drugs, they need to be easily identified. It is especially true when the drugs in question need to be identified by a forensic scientist at a crime scene. This can help to prove or disprove their contribution in the crime such as in a death enquiry as well as ruling out the presence of illicit drugs. The constant research into the sensitivity of analytical methods means smaller quantities of trace evidence can be detected, compared to previous techniques. Trace evidence is a vital component of a forensic investigation, due to the importance of determining what happened (Robertson & Roux, 2010). Therefore, reliable, and accurate analysis of a range of common drugs on different surfaces is paramount as every crime scene is different. This paper is going to investigate the limit of detection and quantification of diazepam over a range of surfaces.

The drug was chosen based on a real-world scenario: diazepam, although weak in comparison to Rohypnol, can often be used as a date-rape drug because of the sedative effect and ability to cause amnesia (Gautam et al., 2014). It is estimated that every year 97,000 men and women experience rape in the United Kingdom (Rape Crisis England and Wales, 2020). In 2017, 6% of rape or attempted rape victims reported that they believed they had been drugged or had their drink spiked in order for the perpetrator to be able to abuse them easier (Office for National Statistics UK, 2018). Rape can be considered as one of the hardest

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crimes to prosecute because it can often result in a 'he said, she said' situation, especially as in the year ending March 2020 there was only a conviction rate of 3.57% (BBC News, 2021). Therefore, even when a date-rape drug is used in a tiny quantity, there is the need to be able to detect it on the perpetrator after the attack.

There are numerous papers documenting the research on the presence of diazepam in hair samples and investigating whether they can be detected in drinks after they have been spiked (Gautam, et al., 2014) (Cordero & Paterson, 2007). However, there appears to be a gap in the literature, when swabbing commonly touched surfaces for drugs but there is ^{1}H-NMR, GC-MS, IR, and Raman data readily available for comparison and reference (PubChem, 2020). Previous experimental data suggest that the active ingredients of diazepam would be soluble in ethanol, with 1 g of diazepam dissolving in 15 mL of 95% ethanol (PubChem, 2020). However, the inactive ingredients: anhydrous lactose, corn starch, pregelatinised starch and calcium stearate; are not soluble in ethanol and may prove difficult to determine when the diazepam has dissolved but will likely produce better quality spectra due to minimal interference (RxList, 2020). Regardless of this difficulty, when swabbing the surfaces, ethanol will be used to moisten the swab. Therefore, using a 2 mg diazepam tablet in 2 mL of ethanol would be ample volume for dissolving to allow for spectroscopic analysis.

The decision to use a mobile phone screen, a wallet and a pair of jeans was developed based on information showing the most common surfaces touched. Although there was a gap in literature research, there is an overwhelming amount of news articles on the subject. This is likely because it may not be considered an area that requires research, as it is probably common sense to many people. One news article published by Web MD suggested that the top 15 'germiest' things include a mobile phone screen, purse or wallet and clothing (WebMD, 2020). A second news article also agreed that the mobile phone and a purse or wallet, are some of the dirtiest things (Sharecare, 2020). It was important to choose surfaces that would not be cleaned often, as it increases the chance that trace evidence of drugs would remain on each surface. Furthermore, the three surfaces were chosen based on their different porosity and so it is hypothesised that the drug would likely have a different affinity for each and hence, allow for comparison.

1.2 Characterisation Techniques

1.2.1 GC-MS

There is a considerable amount of research into the different analytical methods used for the detection of drugs in urine, blood and hair, amongst other biological material, however it seems that detecting drugs on surfaces is an under-researched area (Kinani, et al., 2007, Zhang, et al., 2013 and Khajuria & Nayak, 2016). However, literature shows that GC-MS is considered to be the reference technique when trying to identify trace evidence of drugs in biological samples, this is likely because of its high sensitivity and specificity (Qriouet, et al., 2019) (Harris, 2010). Therefore, it is likely to be as good an analytical tool when looking at drugs dissolved in a solvent. Whilst designing the procedure the properties of the drugs needed to be taken into account. Morphine is an alkaloid product from opium and is a naturally occurring compound, alkaloids themselves tend to be volatile and therefore are suitable for GC-MS (DrugBank, 2020). When preparing the trace evidence of the drug for GC-MS, it must be considered whether derivatisation is necessary. Based on the pKa of 3.6 for diazepam, it was determined that derivatisation would not be necessary (PubChem, 2020 and Bawadikji, et al., 2017).

1.2.2 Raman microscopy

Raman microscopy is particularly useful when analysing 'street drugs' because the spectra show characteristic bands of not just the active ingredient but also the cutting agents (Chalmers et al., 2020). Although analysis here is not of 'street drugs', the tablet does not just contain the active ingredient and it is important to be able to identify the fillers and binders of the drug as well. Raman spectroscopy provides information about the

chemical structure, which for diazepam can be seen in figure 1, and the crystallinity of the material being analysed. Diazepam is a known crystalline powder and although the analysis will be of an evaporated liquid, the Raman microscope can be focused on certain areas in order to view specific chosen crystals to analyse (Dundee & Haslett, 1970). Surface enhanced Raman spectroscopy has previously been used to identify trace amounts of different drugs, some even in a quantity as small as a femtogram (Cinta Pinzaru et al., 2004 and Rana et al., 2010). Whereas transmission Raman spectroscopy has been found to quantify levels as low as 0.25-0.71% w/w of the lowest commercially available dose strength of warfarin sodium clathrate (Griffen et al., 2018 and Sultan et al., 2020). The research is applicable to this investigation as it considers a tablet form as opposed to extraction from biological material. These studies with Raman spectroscopy allow detection of the active ingredients, even when it comprises <1% of the tablet so is well suited to trace analysis (Rahman et al., 2015). Furthermore, using Fourier transform-Raman, alongside IR, made it possible to characterise benzodiazepines meaning that diazepam could be distinguished from its substituents (Bumbrah & Sharma, 2016). This means that drugs with extremely similar chemical structures can be differentiated using Raman due to its specificity. Raman is a complementary technique to infrared spectroscopy, but there is not as much investigation into the use of these two techniques together for the analysis of trace drugs. The majority of the spectra obtained can be found on chemical databases instead of in peer reviewed journals.

1.2.3 Infrared spectroscopy

Infrared spectroscopy is the analysis of light interacting with a molecule, resulting in the characteristic vibration, stretching, bending and wagging (Smith & Siegel, 2005 and Chemistry LibreTexts, 2020). IR is one of the most commonly used spectroscopic techniques because it assists in the determining and identifying structures of compounds (Chemistry LibreTexts, 2020). Near-infrared (NIR) spectroscopy is a commonly used technique in the analysis of counterfeit drugs, due to the possibility of quantitative and qualitative data to be obtained along with the sensitivity of the analysis and could be applied to trace drug analysis (Olsen et al., 2002). The drug which is already in small amounts may be contaminated with other materials due to the surfaces constantly being in use, for example the mobile phone. This technique has the potential, when operated in the correct manner, to detect trace amounts and produce a unique spectrum, which can show any other drugs present, which can be compared to a library search of known compounds to help confirm what is present. One study showed that near-IR can distinguish between 5 mg and 10 mg tablets of diazepam and flunitrazepam (Ali , 2011). Although this study was looking at an intact tablet, it clearly shows the potential and the suitability of the instrumentation for this investigation, especially as there is limited literature when using IR to detect a substance on a surface.

1.2.4 ^{1}H-NMR

Nuclear Magnetic Resonance, NMR, is a technique that provides information on the structure and the chemical environment of the molecule under analysis, based on the magnetic properties of some nuclei (D'Elia et al., 2015). NMR is a useful analytical technique due to the minimal sample preparation required and the high reproducibility, however, it has limited sensitivity and is not suited for complex mixtures. Research is needed into whether NMR is a suitable technique for trace drug analysis. Although there appears to be limited research available, one study did show that 47 mg of diazepam dissolved in $CDCl_3$ was a large enough quantity to be detected by NMR (MacDonald et al., 1972). However, this research project is going to analyse a maximum of 2 mg of diazepam when using the ^{1}H-NMR, which could be close or passed the detected limit and therefore, the NMR peaks may not be of strong enough intensity for analysis.

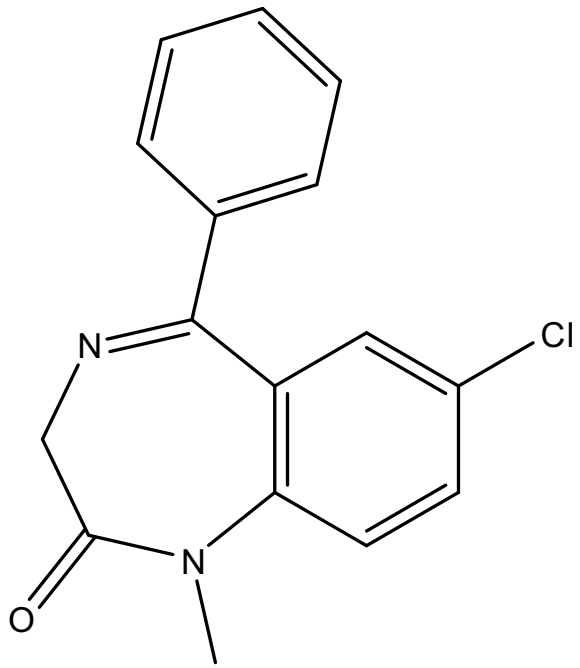

Figure 1.The structure of diazepam

1.3 Aims

The aims of this research are to:

1. Determine if drugs can be detected on a range of surfaces, after being handled, to simulate everyday life.
2. Use three different surfaces (a wallet, a mobile phone screen and a pair of jeans), so that the different porosity of surfaces and their affinity for the drug can be compared.
3. Investigate the use of IR, Raman spectroscopy, GC-MS, and ^{1}H-NMR for diazepam on the different surfaces, allowing for comparison of the different techniques to establish which is most suitable for drug and surface analysis. A critical part of this project is that the analytical method for the identification of the drugs will be developed throughout where necessary.
4. Gain further knowledge and skills in this field of research.

2.0 Experimental

2.1 Materials

Diazepam was used in tablet form containing 2 mg diazepam, with an overall average tablet weight of 0.1741 g (0.1774 g, 0.1724 g, 0.1721 g). A pure standard of ~99% diazepam (supplied by the Central Science Laboratory under Keele University licence) was also used. The solvents ethanol, methanol, acetone and deuterated chloroform were used (Fisher Scientific), and all were of analytical grade and used as received. A risk assessment was also carried out prior to the handling of these chemicals.

2.2 Methodology

In method 1 it was planned that approximately 10 mg of powdered diazepam tablet would be applied to the phone, inside the jeans pocket and a wallet. The tablet contained 2 mg of diazepam making it a dilute system, meaning the active ingredient is in small quantity compared to the inactive ingredients. The drug would then be brushed off using a fingerprint brush to simulate a real-life scenario. The area would then be swabbed with an ethanol moistened swab, which would be dissolved in acetone and then used in a vortex to speed up the dissolving. In regard to infrared and Raman analysis, the solution was placed onto the spectrometer and metal slide, respectively, and allowed to evaporate so only the analyte would remain. Once the solution has evaporated, the slide was analysed three times over three different areas in order to determine if diazepam could be detected. For GC-MS, the sample was evaporated and redissolved in 2 mL ethanol, it would be repeated three times again for validation. The acetone was evaporated off and the analyte was then redissolved in approximately 1 mL of deuterated chloroform in preparation for ^{1}H-NMR.

In method 2, as part of the method development, the method was altered in so that 20 mg of powdered diazepam tablet was applied to the selected surface, as with in method 1, it was brushed off using a fingerprint brush to simulate a real-life scenario. A cotton swab was then moistened

with methanol and the area of interest was swabbed. The cotton swab was then dissolved in 2 mL of methanol and vortexed for five minutes, to increase the dissolvation speed. The solution was then filtered using a syringe and micro-filter cartridge to remove any undissolved solid. The solution was then submitted for GC-MS analysis, but a small amount of the solution was kept allowing a drop to evaporate on a copper slide for Raman analysis and a drop onto the infrared machine for analysis.

Method 3 was then developed with the project outline changing slightly as a result. It was decided that approximately 50 mg of the powdered diazepam tablet would be applied to the surface of a mobile phone, inside a jeans pocket and inside a card slot of a wallet. The surface of each was then brushed to simulate a real-life scenario as before, with a water moistened swab then being used to recover as much of the remaining drug as possible. The surface was weighed before and after so the weight of drug recovered could be determined. An example can be seen in figures 2 – 4, from when the wallet was used. This was repeated three times, for the three different surfaces. This allowed for a mean average of drug recovery to be calculated per surface. This could then be converted to the weight of the active ingredient in the drug that could be recovered to be calculated.

The detection and quantification limit of the GC-MS for diazepam was then determined, to see if in theory the quantity of active ingredient in the drug recovered could be detected. It was important to detect diazepam, the active ingredient, itself because the drug itself contains fillers that may also appear in other drugs. A serial dilution of pure diazepam was completed with the diazepam being dissolved in methanol (see Table 1).

2.3 Instrumentation set-up

The parameters for ^{1}H-NMR, IR and Raman were the standard set-up for each of the instruments. The GC-MS parameters were based on the United Nations Office on Drugs and Crime guidance for the analysis of benzodiazepines (United Nations Office, 2021).

The ^{1}H-NMR instrumentation used was Bruker and the set-up for ^{1}H-NMR was as follows:

Solvent: $CDCl_3$

Temperature: 298.0 K

Spectral width: 8012.820 Hz

Intrinsic digital resn.: 0.122266 Hz

Observation frequency: 400.0424704 MHz

The infrared spectrometer used was Thermo Scientific and the set-up was as follows:

Number of scans: 64

Resolution: 4 cm^{-1}

Accessory: Diamond ATR

Max range limit: 4500 cm^{-1}

Min range limit: 525 cm^{-1}

The Raman spectrometer used was Thermo Scientific and the set-up was as follows:

Collect and preview exposure time: 0.5 seconds

Sample exposures: 100

Laser wavelength: 780 nm / 532 nm

Laser power: 20.0 mW

Estimated resolution: 4.7 – 8.7 cm^{-1}

Estimated spot size: 3.1 µm

Range: 100 cm^{-1} – 3400 cm^{-1}

The GC-MS equipment used was Agilent and the set-up for GC-MS was as follows:

Column: 10 – 15 m x 0.53 mm 1D; film thickness of 1.5 – 3 µm

Phase: Fused silica, chemically boned and cross-linked methylsilicone

Oven: Temp prog. 4 min at 135 °C, 13 °C/min to 200 °C, 6 °C to 312 °C, 6 minute final hold

Carrier gas: Helium, 1 mL/min; constant flow

Injector: Splitless, 250 – 300 °C

Serial Dilution Number	Concentration of diazepam
1	1 mg/mL
2	0.5 mg/mL
3	0.25 mg/mL
4	0.125 mg/mL
5	0.0625 mg/mL

Table 1. The serial dilution concentrations prepared for the calibration curve to allow for the GC-MS limit of detection to be determined.

Ionisation mode: EI mode

Transfer line temp: 280°C

Ion source temp: 230°C

Scan parameters: TIC (SIM if required), scan range: to 500 amu

3.0 Results

3.1 Method 1

3.1.1 GC-MS

After the completion of method 1 within the laboratory, it was discovered that ethanol was an inappropriate solvent to use on the GC-MS and so it was decided that methanol would be used instead, as shown in method 2. This was because ethanol can be damaging to the stationary phase in the column and when ethanol is vaporised, the volume of gas is much more than if, for example, hexane was vaporised. This means than 1 µl of ethanol in the gas-phase is much bigger than 1 µl of hexane. This cause problems because the liner in the GC is then too small to fir the vapour of hexane. Therefore, there is no GC-MS data from method 1.

3.1.2 ^{1}H-NMR

The ^{1}H-NMR spectrum as completed in method 1, can be seen in figure 2. The reference spectrum, figure 3, obtained from a database shows that diazepam would have 3 peaks at 3.4, 3.8 and 4.8 ppm (PubChem, 2020) (see Table 2). The three main peaks at 0.0, 1.8 and 7.2 ppm were likely because of the solvent used because the same three peaks were also

Peak	Corresponding structure
3.8 ppm	R-CH_2Cl
4.8 ppm	$R_2C=CH_2$

Table 2 – showing the corresponding structures, which are present in diazepam as shown in figure 1

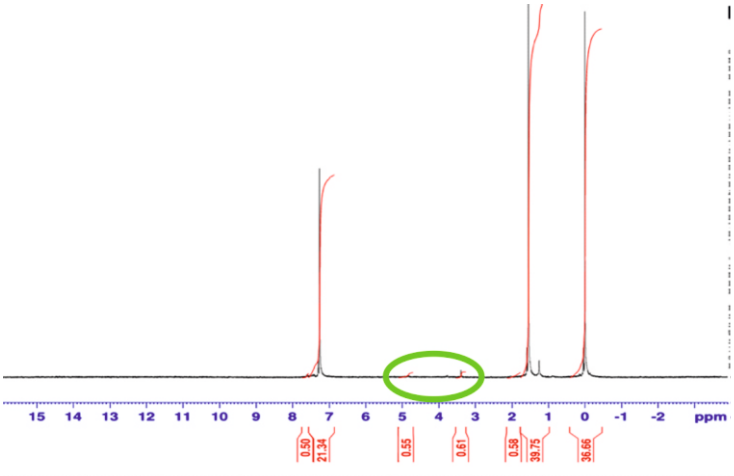

Figure 2 – showing the ^{1}H-NMR spectrum for diazepam. The green circle highlights the low intensity peaks.

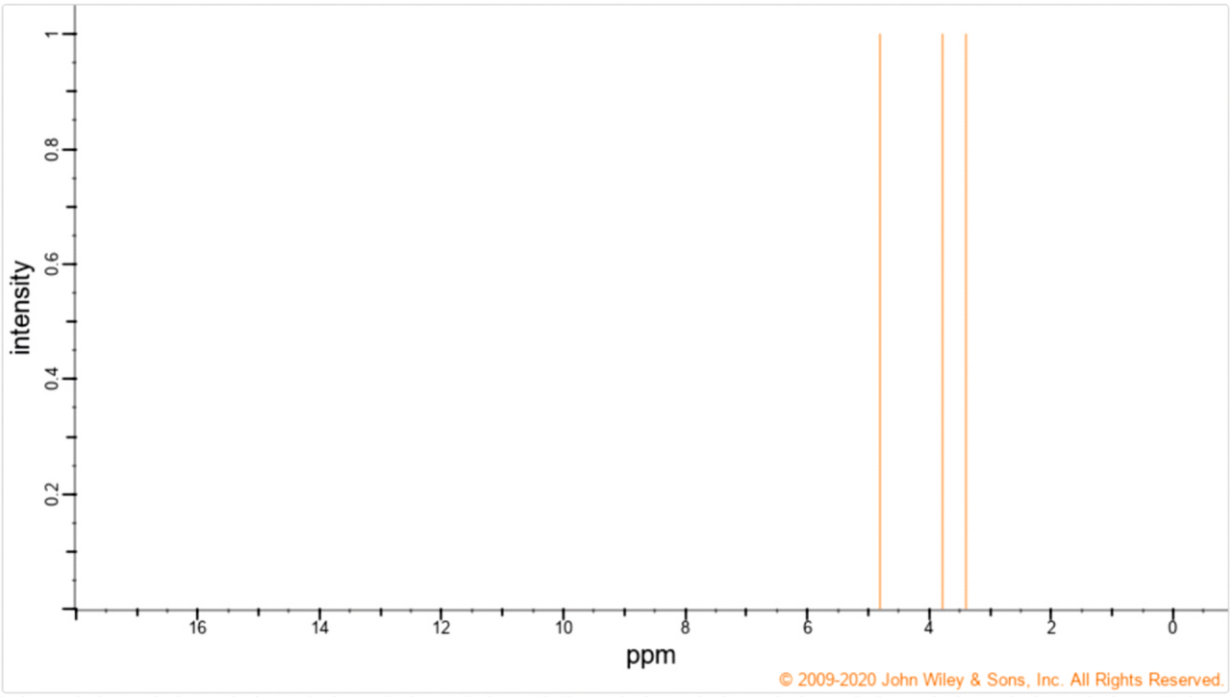

Figure 3 – the reference spectrum for the ^{1}H-NMR of diazepam (PubChem, 2020).

present in a blank sample that was run under the same conditions. There are 3 very low intensity peaks, circled in green on figure 2, which are in the region of 3.4, 3.8 and 4.8 ppm.

Although these three peaks are in the correct position when compared to those of diazepam due to the low intensity it cannot be said with confidence whether these are in fact diazepam. Moreover, an extremely small amount of diazepam would have been used on the NMR after the sample preparation. It is likely that as a result of this, the concentration of diazepam is very close to the detection limit of the instrument. This technique would likely have a much greater success if it was used on a pure standard compound than a tablet mixture. However, it may be possible that greater success may have been achieved had the tablet contained 10 mg of diazepam, as opposed to the 2 mg tablet used.

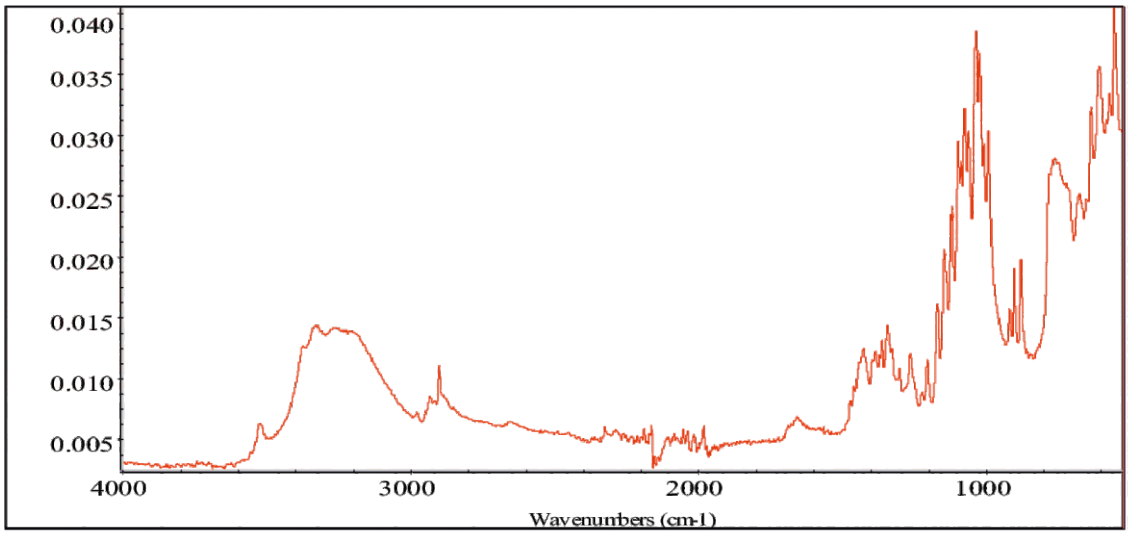

Figure 4 – the spectrum showing the IR data of diazepam in method 1.

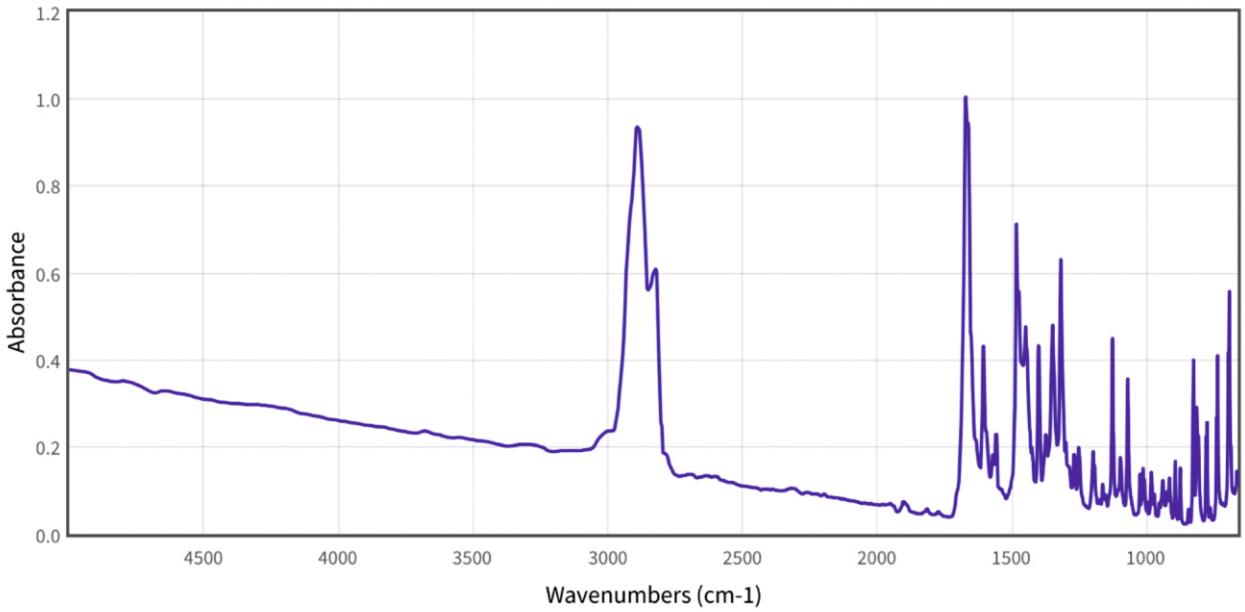

Figure 5 – the reference infrared spectrum for diazepam (WebBook, n.d.).

3.1.3 IR

The IR spectrum from method 1 can be seen in figure 4, with the reference spectrum in figure 5 (WebBook, n.d.). Based on the location of the peaks on the reference spectrum, there should be a strong corresponding peak at 2900, 1750, 1500 and 1300 cm^{-1}, with multiple weaker peaks between 500 and 1500 cm^{-1}. There is a lot of noise and non-sharp peaks present in figure 7. However, there does appear to be a peak at around 2900 cm^{-1} and 1300 cm^{-1}, but there are no clear peaks at 1750 and 1500 cm^{-1}. However, based on the inconsistencies between the reference spectrum and that in figure 4, it cannot be said with confidence that diazepam has been detected but could also be fillers present as well.

3.1.4 Raman

The Raman spectrum for method 1 can be found in figure 6, with the reference spectrum shown in figure 7 (PubChem, 2020). However, as shown in figure 6, the blue line from the

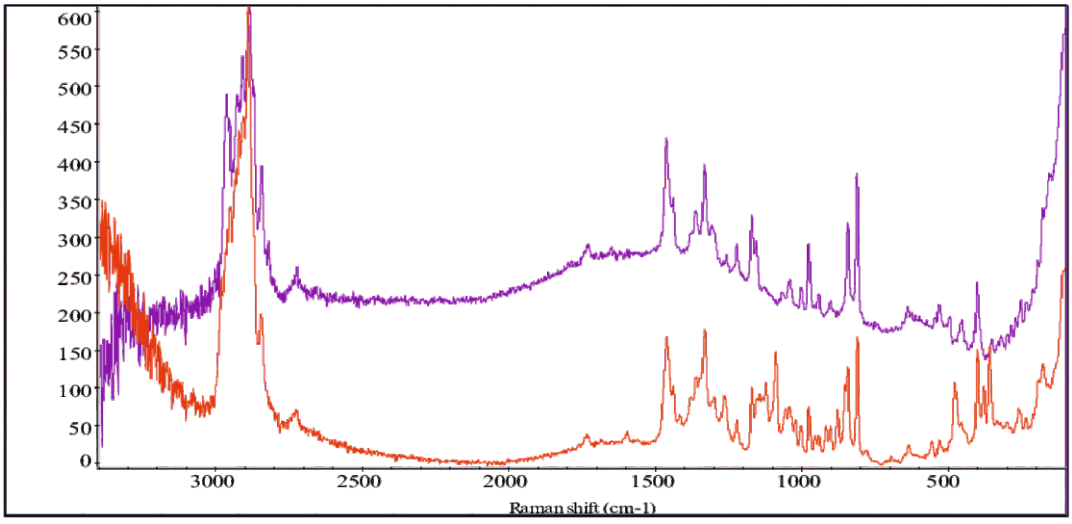

Figure 6 – the Raman spectra for diazepam (red) and tape used to hold the powder in place (purple).

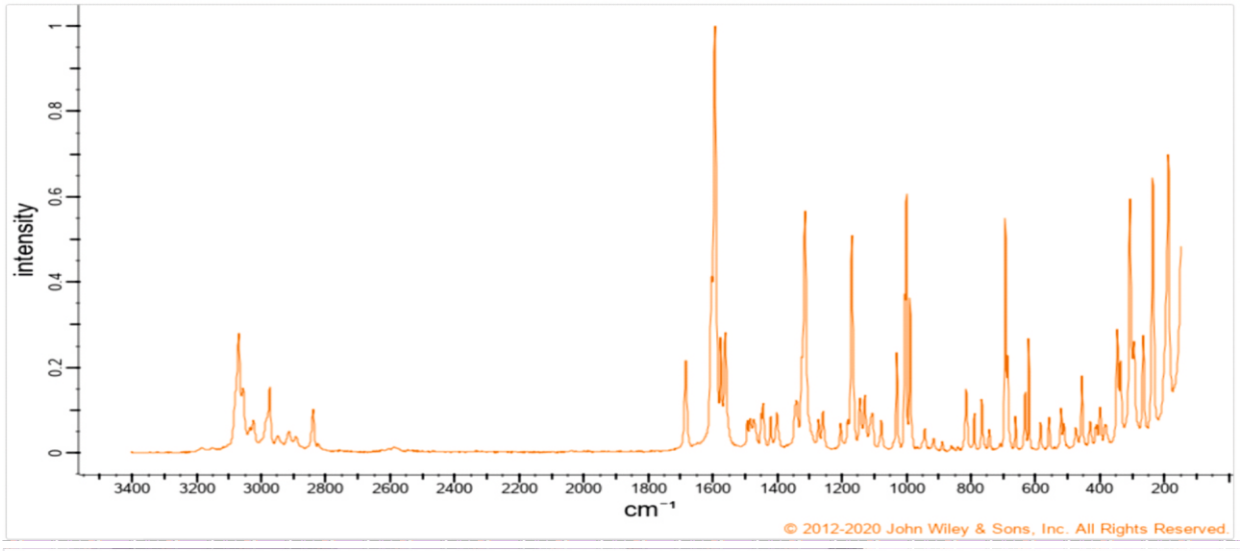

Figure 7 – the reference Raman spectrum for diazepam (PubChem, 2020).

Raman analysis of just Sellotape, which was used to prevent the powder from entering the machine, is similar which suggests that there has been drastic interference with the tape and the diazepam drug in the analysis. Further to this, as the whole tablet was used in the analysis, it is possible that the fillers are also interfering or over-powering diazepam. Further to this, based on the reference spectrum, diazepam should have its main peak at 1600 cm^{-1}. However, this was not present in the Raman spectrum, supporting the conclusion that the Raman machine had detected the Sellotape and fillers in the drug, not the active ingredient.

3.2 Method 2

3.2.1 GC-MS

In method 2, due to the change in solvent, it was possible to carry out GC-MS analysis on the diazepam tablet. The chromatogram can be seen in figure 8, there is no mass spectrum because there were no peaks to analyse. This could possibly be due to the fact that no diazepam was collected during the extraction process and it was in essence a blank run.

3.2.2 IR

The spectrum for IR for method 2 can be seen in figure 9. Based on the location of the peaks on the IR reference spectrum, there should be a strong corresponding peak at 2900, 1750, 1500 and 1300 cm^{-1}, with multiple weaker peaks between 500 and 1500 cm^{-1}. When comparing this to figure 6, there is a lot of noise and broad peaks. However, there does appear to be peaks at around 2900, 1750 and 1300 cm^{-1}, but there is no clear peak at 1500 cm^{-1}. Based on the inconsistencies between the reference spectrum, that in figure 4 and figure 3, it cannot be concluded if diazepam has been detected. If this cannot be done with the up-most confidence, it cannot be used in a Court of Law.

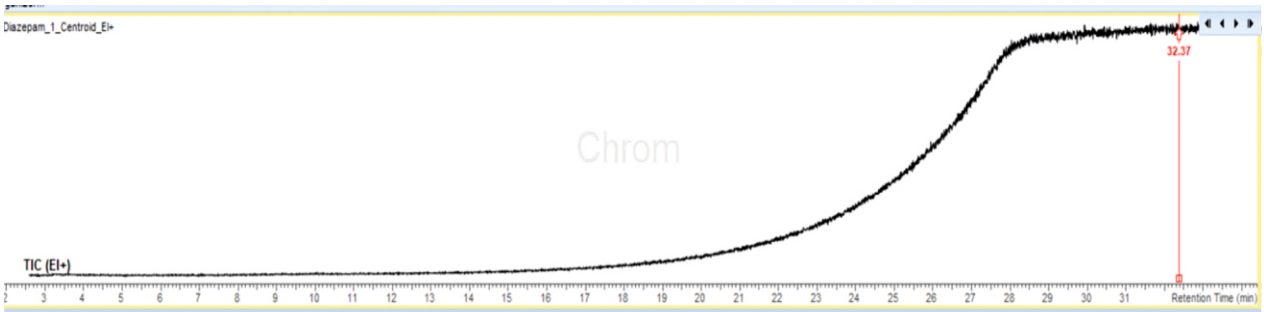

Figure 8 – the gas chromatogram based on method 2.

3.2.3 Raman

The Raman spectra for method 2 can be seen in figures 10 and 11, at a wavelength of 720nm and 532 nm, respectively. Figure 10 shows copious amounts of noise at the higher wavelengths, with the only arguably potential peak at 1350 cm^{-1}. As a result of this, the laser was changed to that of a wavelength of 532 nm instead of 780 nm to help with the overpowering fluorescence. Based on the reference spectrum, the main peak should be at around 1600 cm^{-1}, with many other multiple peaks to the right of this main peak, alongside 3 other peaks of interest between 2800 and 3100 cm^{-1}. Based on the spectrum in figure 10, it cannot be concluded because of the noise, whether diazepam had been detected.

However, the spectrum shown in figure 11 and the zoomed in version in figure 12, has the potential for peak identification. The main area for diazepam peaks are in the 1600 – 200 cm^{-1} region with 3 other peaks in the 3000 to 2800 cm^{-1} area. Due to the fluorescence, it is impossible to detect any peaks in the 3000 to 2800 cm^{-1} region. There appears to be peaks at 1450 and 1400 cm^{-1} which does match the reference spectrum in figure 7. There also could be peaks at 1300, 1050, 900 and 700 cm^{-1}. These could match those from the reference spectrum. These bonds match up with the structure of diazepam, figure 1, hence providing support that it was probable that diazepam was detected but due to the fluorescence impossible to confirm.

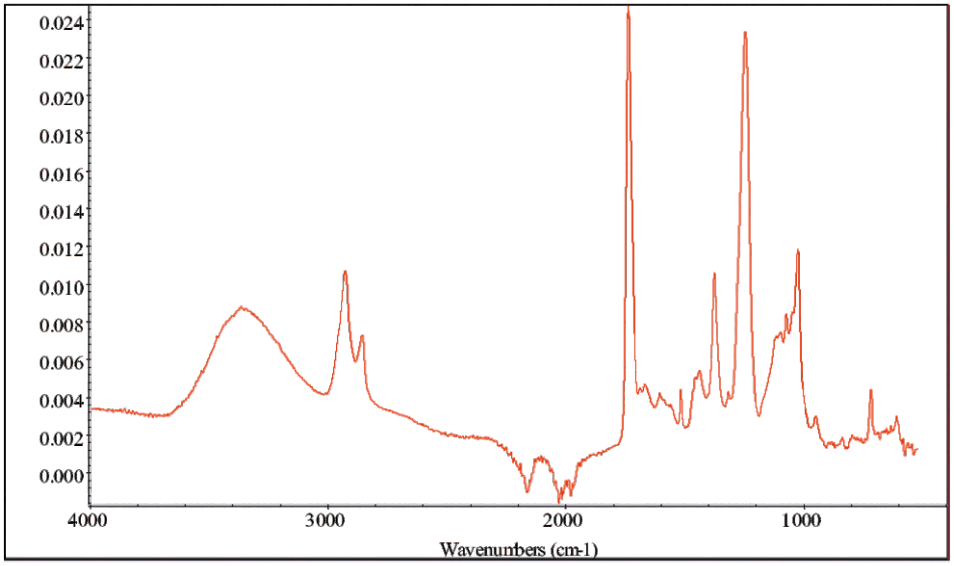

Figure 9 – the spectrum showing the IR data for diazepam from method 2.

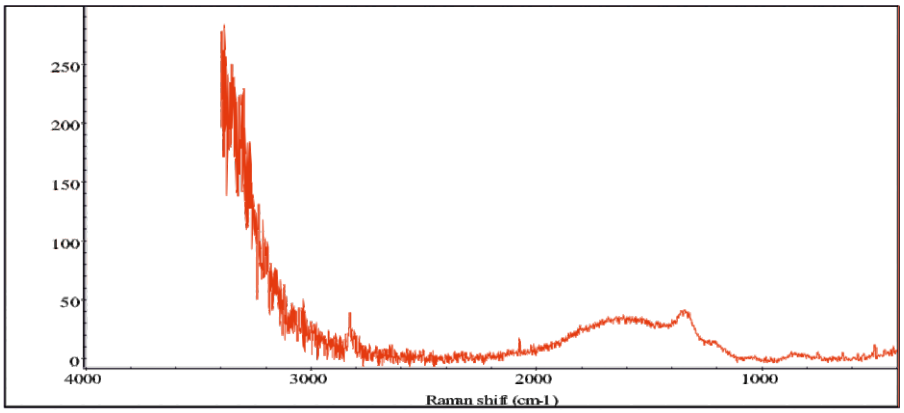

Figure 10 – the Raman spectrum for diazepam at wavelength of 720 nm.

Peak	Corresponding structure
1400 and 1450 cm^{-1}	CH_3 and CH_2 deformations
1300 cm^{-1}	CH deformation
1050 cm^{-1}	Aromatic ring
900 cm^{-1}	CNC bond
700 cm^{-1}	Ring vibration

Table 3. The peaks could correspond to those from the reference spectrum.

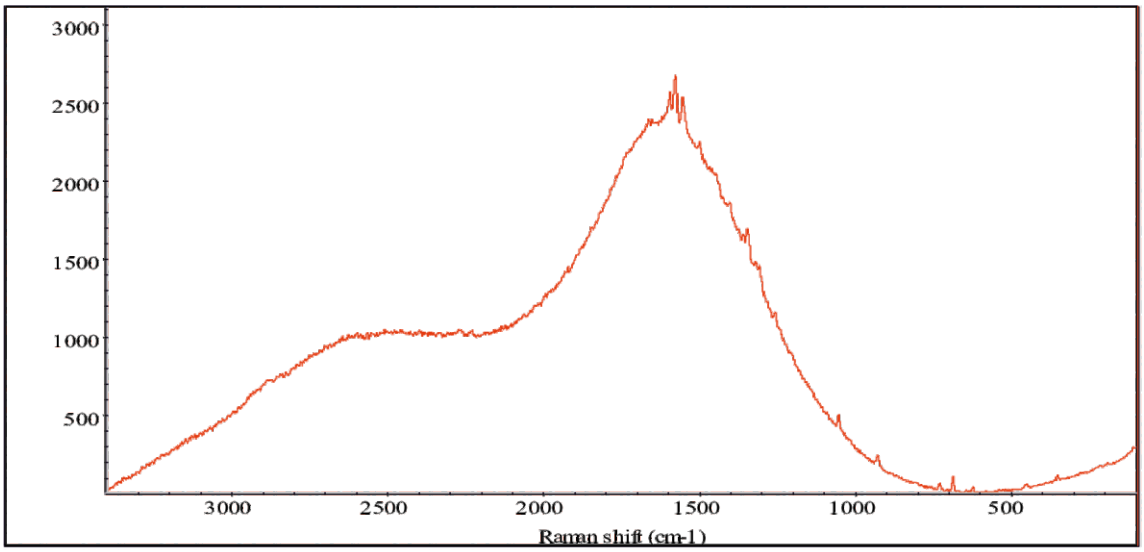

Figure 11. The Raman spectrum for diazepam at a wavelength of 532 nm.

3.3 Method 3

When determining the amount of diazepam that could be recovered from each surface in method 3, 3 repeats of each surface were conducted so a mean average and a standard deviation could be determined. The diazepam concentration was determined from converting the amount of diazepam in the whole tablet weight, to the probable concentration of diazepam in the powder recovered. It was determined that for the phone, the mean average amount of pure diazepam recovered was 0.22 mg ± 0.04 mg after 47.9 mg of the powdered tablet had been applied to the phone. The jeans pocket had a mean average recovery of 0.09 mg ± 0.10 mg after 20 mg of the powdered tablet had been applied to the surface, whilst the wallet had a mean average recovery of diazepam of 0.19 mg ± 0.03 mg after 42 mg had been applied to

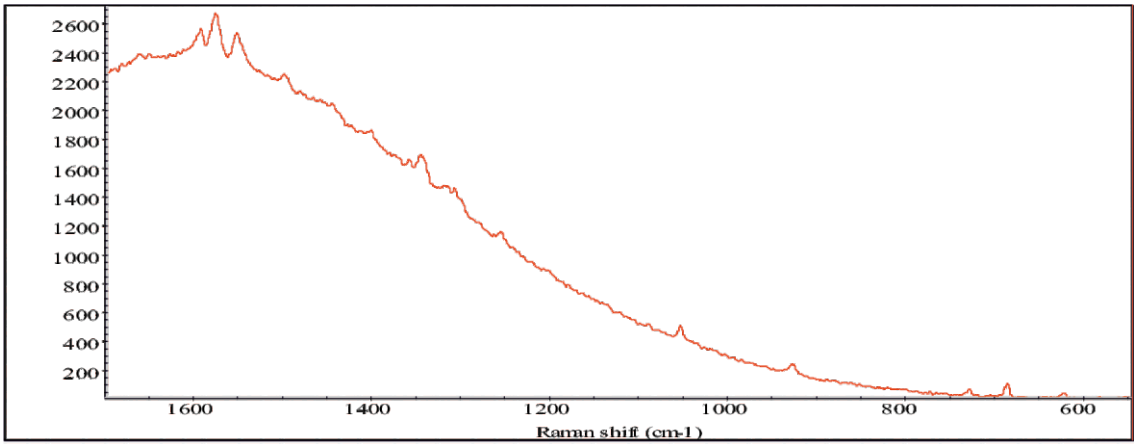

Figure 12. The Raman spectrum for diazepam at a wavelength of 532 nm, with the area of interest zoomed in on.

the wallet. An example of the calculations for this can be found in table 4. The same calculations were completed for the pocket of the jeans and inside the wallet.

3.3.1 GC-MS

An example of the GC-MS data from pure diazepam can be found in the figure 13. It was determined that the peak at 17.400 minutes was diazepam, this was because the mass spectra from the samples that were run matched. The main matching mass spectrum peaks have been colour coded by circles to illustrate this. Further to this, in-depth analysis of the fragments was conducted, which matched a reference spectrum for diazepam as shown in figure 14 (National Institute of Standards and Technology, n.d.). Once it had been confirmed that all five spectra contained diazepam at the peak in question, the area under the peaks was taken to produce the calibration curve. The points were plotted as a calibration curve and the equation of the line was determined, which can be seen in figure 15.

The calibration curve produced based on the

Item	Weight of repeat 1	Weight of repeat 2	Weight of repeat 3
Weighing boat	0.6473 g	0.6516 g	0.6499 g
Weighing boat and drug	0.6983 g	0.7002 g	0.6944 g
Drug	0.0510 g	0.0483 g	0.0445 g
Phone	109.8463 g	109.8505 g	109.8514 g
Phone and drug	109.8784 g	109.8972 g	109.8939 g
Phone and drug after 'real life' simulation	109.8691 g	109.8842 g	109.8788 g
Phone and drug after swab	109.8497 g	109.8601 g	109.8633 g
Drug recovered (tablet recovered)	0.0194 g	0.0241 g	0.0155 g
Percentage drug recovered	38%	50%	35%
Pure diazepam present	0.220 mg	0.270 mg	0.170 mg

Table 4. Calculations for the amount of diazepam estimated to be present in the drug recovered from the phone. The same were completed for the pocket of the jeans and the wallet.

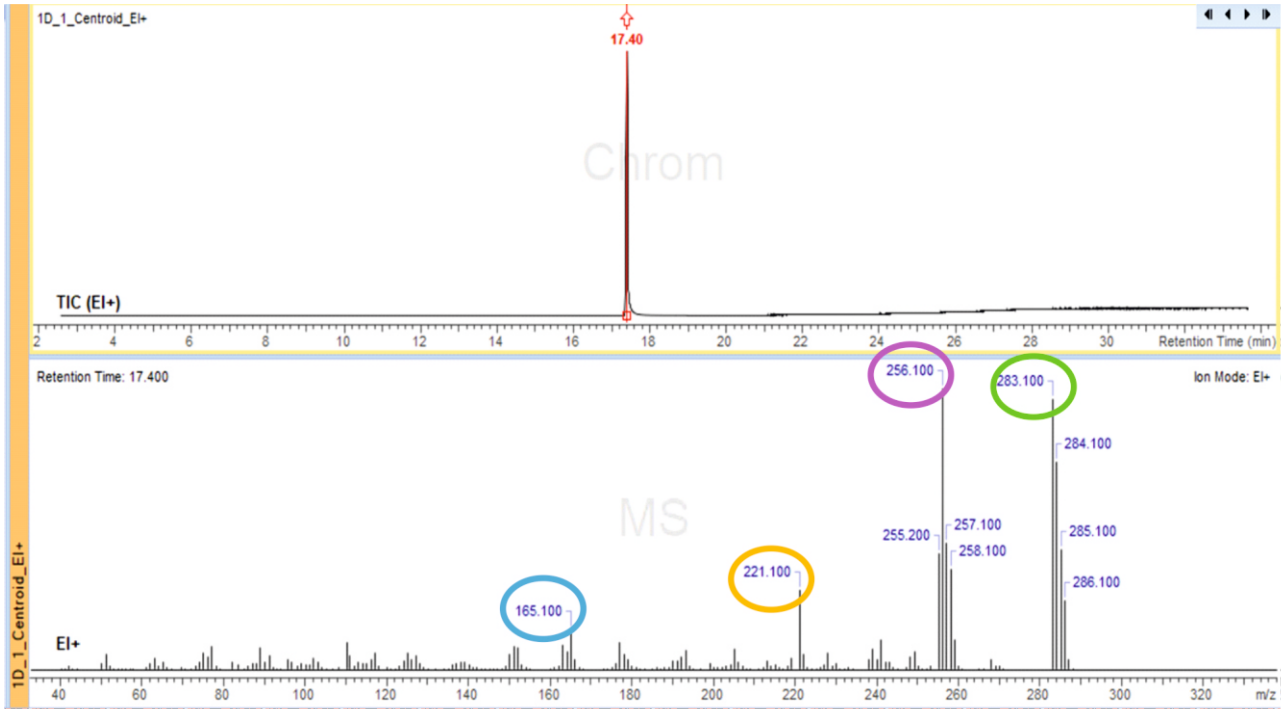

Figure 13 – the GC-MS spectrum for 1.0 mg/mL of diazepam.

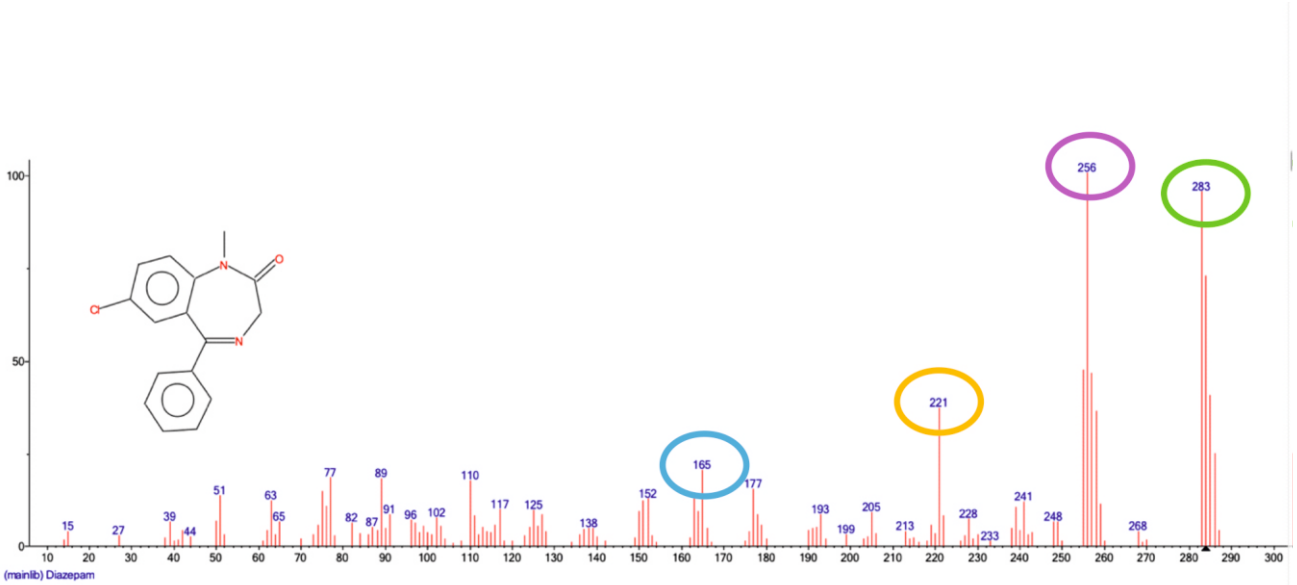

Figure 14 – the reference spectrum for the GC-MS of diazepam (National Institute of Standards and Technology, n.d.).

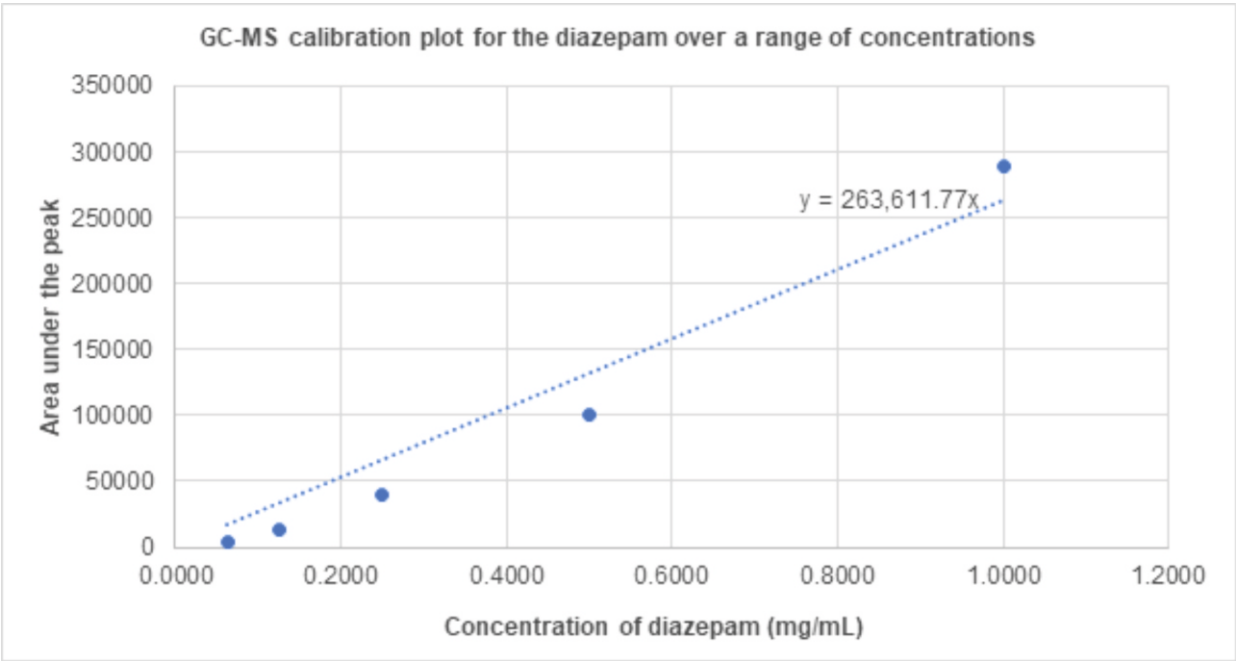

Figure 15 – showing the calibration curve to allow for the GC-MS of diazepam

GC-MS data cannot be used to accurately determine the limit of detection and limit of quantification. In order to do so, the calibration data needs to be reproduced. This is because the line on the calibration plot would not be considered straight if it went through all the points, further to this, it does not go through 0, 0 without being forced. However, it is possible to estimate the limit of detection and quantification based on the GC-MS data, using the smallest concentration from the serial dilution.

Assuming that the detection limit will correspond to the peak height which is approximately three times greater than the noise level, it is possible to estimate the LOD from the smallest concentration of diazepam (Harris, 2010). Therefore, it can be estimated that the LOD will have an intensity poorer than the smallest concentration detected (0.0625 mg/mL) and so can be estimated to be 0.001 mg/mL. As with the limit of detection, it was also possible to approximately determine the

limit of quantification. It was possible to quantify the smallest concentration used again. Therefore, it can be suggested that the LOQ is approximately 0.02 mg/mL. However, additional calibration would be required to confirm this.

4.0 Discussion

^{1}H-NMR could be used to suggest the presence of diazepam when a 2 mg tablet of diazepam was used. However, it would likely be easier to detect a more concentrated sample which could either be a 10 mg diazepam tablet or pure diazepam powder. Infrared and Raman spectroscopy could be a more useful tool for preliminary identification rather than conclusive identification. Raman spectroscopy is very susceptible to significant interferences particularly because of the fluorescence as shown in method 1 and 2 (Vankeirsbilck, et al., 2002). ^{1}H-NMR, IR and Raman are all excellent methods for the analysis of pure compounds because the signal in the associated spectra can be easily over-powdered. As a result of this, hyphenated techniques, such as GC-MS, are preferred. GC-MS is shown to be the most powerful technique for this research because as the results have shown it was the only technique that could identify diazepam. This is also shown in literature, with GC-MS often described as the reference technique for the analysis of drugs (Qriouet, et al., 2019) (Harris, 2010).

The calculations and estimation for a whole tablet and for the amount used in this research project both have a diazepam concentration greater than the GC-MS limit of detection and limit of quantification. This is extremely important because it provides evidence for the theory that if a perpetrator had used crushed diazepam to spike a drink, for example, then it appears possible that trace evidence of the tablet could be left on the person. This could be crucial evidence in a date-rape drugging crime because it means that there is direct evidence linking a suspect to diazepam and could help to provide the police with more support when prosecuting because it could allow for the 'he said, she said' situation to be diminished.

Diazepam can be prescribed to be taken at a dosage of 2 – 10 mg to be taken 2 to 4 times daily (NHS, n.d.). Even at a dose of 2 mg, the person consuming diazepam can experience drowsiness and tiredness (NHS, n.d.). This research project has looked at the detection when one 2 mg tablet is used. This is important because if one 2 mg tablet can be detected in a great enough quantity, any amount greater than this could be detected. This is vital to casework because if diazepam can be detected on a suspect where the presence of diazepam cannot be reasonably explained, it could help to convict a perpetrator and allow the rape victim to receive justice for the heinous crime committed against them. This is especially true if the suspect does not have a diazepam prescription, suggesting that it had been obtained illegitimately.

This project was somewhat successful in determining if drugs can be detected on a range of surfaces, after being handled. It was possible to confidently determine the presence of diazepam using GC-MS when using a pure diazepam standard. It was only possible to preliminary identify diazepam when using IR, Raman and ^{1}H-NMR for the detection of drugs over the range of surfaces. In addition, there was consistent analytical method development throughout, which meant that critical skills relating to this project have been improved as well. The final aim was to gain further knowledge and skills in this field of research. This aim was definitely achieved, not only as a result of the consistent method development but also due to the practical element of the research, with the hands-on element allowing for the improvement of equipment use and laboratory skills as well.

5.0 Conclusion and Future Work

In conclusion, GC-MS analysis resulted in the confident detection of diazepam and allowed for the estimation of the limit of detection and quantification. The limit of detection and quantification were both small enough that, in theory, they would be able to detect

diazepam in the remains of a 2 mg tablet. This is vital to forensic work because it reduces the pressure placed on a victim in a date-rape case because this analysis offers another avenue of forensic analysis. IR, Raman and ^{1}H-NMR are most practically used for preliminary identification due to the diazepam signal often being over-powered by the tablet's fillers.

In future work, it would be beneficial to conduct IR, Raman and ^{1}H-NMR analysis on the pure standard of diazepam to allow for further confirmation and detection of the diazepam. This was not possible in this project due to time restrictions as a result of coronavirus. In addition to this, once the standard spectra have been produced, it would be ideal to develop a method of extracting diazepam only from the original Valium tablet. This would mean that the theory could be implemented into real-life scenarios without the risk of analysing a mixture where the signal of diazepam could be easily over-powered by the fillers as demonstrated in method 1 and 2.

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