THE DEVELOPMENT, VALIDATION AND APPLICATION OF ANALYTICAL METHODS FOR THE ANALYSIS OF DRUGS OF ABUSE

University of Dublin

Trinity College

THE DEVELOPMENT, VALIDATION AND APPLICATION OF ANALYTICAL METHODS FOR THE ANALYSIS OF DRUGS OF ABUSE

A thesis submitted in accordance with the requirements of the University of Dublin for the Degree of

Philosophiae Doctor.

By

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May 2001

Acknowledgements

I would like to thank Dr. Des Corrigan for all his guidance and support over the years. I would also like to thank Mrs. Ingrid Hook and Dr. John Walsh for their help and support. I would like to pay a special tribute to Dr. Mary Meegan and J.J. Keating for all the hard work they have done, without their efforts, completion of this thesis would not have been possible. A big thanks is also due to Dr. Pierce Kavanagh for his help and advice. Thanks also to the technical staff of the School of Pharmacy, but especially to Joe Reilly & Co.

I would also like to thank the State Forensic Science Laboratory, especially Dr. Audrey O'Donnell, for their help. Thanks are also due to the Garda National Drugs Unit, the Department of Health and Children and the Department of Agriculture, Food and Forestry. I would also like to pay tribute to everyone in the Laboratory of the Drug Treatment Centre not only for access to client samples, but also for making me feel welcome since I began working there.

In addition, I would like to thank the Post-grads in the School of Pharmacy but especially the post-grads in the Department of Pharmacognosy James, Emmett and Richie and any other Lincoln affiliates. Special thanks go to Brendan for all his help academic, not to mention his mandolin purchasing, skills.

I also want to thank my friends whom I have had the pleasure of knowing; Conor, John, Kevin Mc., Kevin C., Dave, Fiona and Seamus.

Many thanks to my Family for their support and encouragement.

I would also like to acknowledge all the heartfelt support shown to me and my family by people who were around during the time of my Fathers illness and at the time of his death. I thank you all for just being around.

Finally, HUGE THANKS goes out Sally-Ann, my long suffering girlfriend.

ABSTRACT

There are five chapters presented in this thesis. The first chapter deals with the development and validation of an analytical method, incorporating HPLC, for the analysis of MDMA in illicitly produced ecstasy tablets. In all 100 tablets were analysed and the MDMA content on average was found to be 79.01mg.

The second chapter involved the analysis of route specific compounds (two pyrimidines and *N*-formyl BDB) generated by the synthesis of MBDB that had been synthesised via the Leuckart route. HPLC and GC-MS methods were developed and validated of for the analysis of the three target compounds. Solid phase extraction and liquid-liquid extraction were assessed as potentially suitable methods for the extraction of the three target compounds from MBDB synthesised via the Leuckart route. Street samples of MBDB were analysed and it was discovered that three of the tablets were likely to have been synthesised via Leuckart route.

The most likely routes of synthesis of MDA, MDMA, MDEA, MBDB and 4-MTA were investigated in the Department of Pharmaceutical Chemistry. The third chapter involved subjecting impurities, intermediates and products generated by the routes investigated, to GC-MS analysis. Mass spectral data recorded was incorporated into a software based library which could be used as a forensic tool for the attempted identification of routes of synthesis. Samples licitly prepared Ecstasy and samples of illicitly prepared Ecstasy were subjected to GC-MS analysis. Where possible, routes of synthesis were identified with the aid of the library.

Chapter IV involved the analysis of the 100 urine samples, taken from clients of the methadone programme in the Drug Treatment Centre in Dublin, which had tested positive for amphetamines on EMIT screening. A GC-MS method was used to determine whether the type of amphetamines being abused were non-ring substituted (amphetamine, methamphetamine), ring-substituted propanamines (MDA, MDMA, 4-MTA) or ring-substituted butanamines (MBDB, BDB). Amphetamine and methylenedioxy propanamines were found however, there was no abuse of 4-MTA or ring-substituted butanamines noted.

The final chapter used GC-FID to determine the cannabinoid content of products of the plant *Cannabis sativa* seized and./or grown in the Republic of Ireland. THC, CBD and CBN levels were determined for 62 samples of cannabis resin, 5 samples of herbal cannabis and 21 samples hemp. The average levels of THC, CBD and CBN in resin were 2.11%, 2.19% and 2.13% respectively. The average levels of THC,

CBD and CBN in hemp were 0.06%, 1.61% and 0.06% respectively. The average levels of THC, CBD and CBN in herb were 0.20%, 0.13% and 0.65% respectively.

TITLE:	THE DEVELOPMENT, VALIDATION AND APPLICATION OF ANALYTICAL METHODS FOR THE ANALYSIS OF DRUGS OF ABUSE.
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STUDENT NUMBER:	97166731
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Section 1.1: Background

In the mid-1980's the methylenedioxyamphetamines became the focus of intense scrutiny and controversy in the media, not only because of their association with previously discredited models of psychedelic psychotherapy, but because of their increasing recreational use by young people (Grob, 1998). The compound 3,4-methylenedioxyamphetamine or MDA (**4**), is the parent compound of a series of methylenedioxyamphetamines which have come to be generally known as ecstasy. MDA (**4**) was first synthesised in 1910 by Mannich and Jacobson (Mannich, 1910) and the *N*-methylated analogue of MDA, 3,4-methylenedioxymethamphetamaine or MDMA (**5**) has been known and patented since 1914 (Anon., 1914).

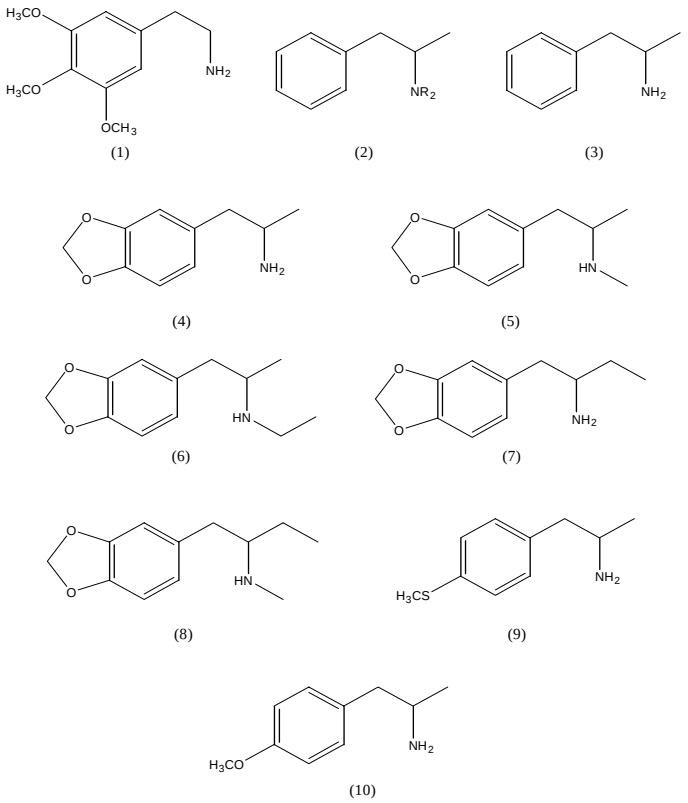


Figure 1.1:Mescaline (1), Phenylalkylamine backbone (2), amphetamine (3), 3,4-Methylenedioxyamphetamine (MDA) (4), 3,4-Methylenedioxymethylamphetamine (MDMA) (5), 3,4-Methylenedioxyethylamphetamine (MDEA) (6), 3,4-Methylenedioxyphenyl-2-butylamine (BDB) (7), N-methyl-1-(3,4-methylenedioxyphenyl)-2-butylamine (MBDB) (8), 4-Methylthioamphetamine (4-MTA) (9) and Para-methoxyamphetamine (PMA) (10).

MDA (**4**) was the first hallucinogenic amphetamine derivative to show popularity as a recreational drug. MDA (**4**) is a phenethylamine resembling amphetamine (**3**) and mescaline (**1**) (Figure 1.1, above). MDA (**4**) is reported to act as a central nervous system stimulant that may be hallucinogenic in large doses (Naranjo, 1967). On methylation the MDMA (**5**) analogue is formed (Figure 1.1). Most of the known psychedelic drugs suffer a major loss of potency on *N*-methylation (Anderson, 1978). MDMA (**5**) is the exception to this rule as it, like amphetamine (**3**), maintains potency as the *N*-methyl homologue (Shulgin, 1986). Methylation does however produce significant changes in the pharmacological effects, resulting in a shorter duration of action, a decrease in potency and elimination of the hallucinogenic properties (Dal Cason, 1990; Shulgin, 1978).

As a result of this observation Nichols *et al.* (1986a) describe these compounds as representing a new pharmacological class, known as the ''Entactogens''. The word ''entactogen'' is derived from the Greek roots ''*en*'' meaning within, ''*gen*'' meaning to produce and the Latin root ''*tactus*'' for touch (to touch within ones self). Entactogens produce their unique behavioural effects without profound sensory experiences or distortion as observed with hallucinogenic or psychedelic drugs, such as Lysergic acid diethylamide (LSD) or Mescaline (**1**) (Naranjo, 1967). Thus, entactogens continue to be proposed as potential therapeutic agents in facilitating psychotherapy (Nichols, 1986a).

Throughout the 1990's, new ring substituted amphetamines appeared on the illicit drug market. The motivation for the supply of the new pharmacologically similar analogues was born out of attempts to elude legislative control. MDMA (**5**) and MDA (**4**) have been subject to control internationally, under the terms of the United Nations Convention on Psychotropic Substances 1971, since 1986 and 1990 respectively (United Nations URL). However N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB) (**8**), methylenedioxyethylamphetamine (MDEA) (**6**) , 4-methylthioamphetamine (4-MTA) (**9**) and para-methoxyamphetamine (PMA) (**10**) have appeared on the international drug market and have subsequently been scheduled as controlled substances. These compounds have not received the same attention in the literature as MDA (**4**) or MDMA (**5**). This observation may be an indication that these drugs have been abused to a lesser extent than MDA **(4)** and MDMA (**5**). However, they are still a major cause of concern for the relevant authorities and it does not seem to matter which of the compounds appears in an illicit tablet as it is still referred to as ecstasy. MDEA (**6**) and MBDB (**8**)

appear to possess pharmacological activities comparable to MDA (**4**) and MDMA (**5**) (Nichols, 1986; Braun, 1980). It is of interest that MBDB (**8**) is found to be only slightly less toxic than MDMA (**5**) in one study (Johnson, 1989) and slightly less potent in another (Nichols, 1986a). There is very little literature available for 4-MTA (**9**) and PMA (**10**), however both drugs have been linked to fatalities (Elliot, 2000; Felgate, 1998) and would therefore be considered toxic under certain conditions.

The acute and chronic toxicity of the methylenedioxyamphetamines is an area of much controversy, however numerous animal studies which have shown toxicity, have been reviewed (Steele, 1994; McCann, 1996). Clinical observations were reported after MDMA (**5**) or MDEA (**6**) intoxications, including loss of appetite, nausea, muscle pain and/or cramps, ataxia (Greer, 1986), mental confusion, depression, anxiety (Strafer, 1985), hypothermia, convulsions and cardiac disrythmias (Johnson, 1989) which dispel any theory that these drugs are harmless. There are also a significant number of accounts of ecstasy related deaths published in the scientific arena involving MDA (**4**) or MDMA (**5**) (Chadwick, 1991; Campkin, 1992; Felgenhauer, 1999 and O'Connor, 1999), MDEA (**6**) (Tsatsakis, 1997) and MBDB (**8**) (Carter, 2000). These fatalities serve to highlight the risks associated with the illegal use of these drugs. A review of 32 deaths related to amphetamine derivatives in Spain from 1993 to 1995 show high blood levels of methylenedioxyamphetamine and paramethoxy amphetamine derivatives (Lora-Tamayo, 1997).

Section 1.2: Qualitative and quantitative considerations

Given that the term ''Ecstasy'' can refer to anyone of a series of drugs including MDA (**4**), MDMA (**5**), MDEA (**6**), and MBDB (**8**) (Figure 1.1), it is important to be able to distinguish between these compounds when analysing tablets purportedly containing ''Ecstasy''. The quantity of active material in these tablets is quite variable, but the dosage is reported to be around 100mg for MDA (**4**)**,** MDMA (**5**) and MDEA (**6**) (King, 1997). The expected MBDB (**8**) content in illicit tablets was also reported to be around 100mg (Van Aerts, 2000). The content of MDMA (**5**) in a number of tablets analysed was reported to vary from trace amounts to 180mg (O'Connell, 1999). A recent communication from the European Monitoring Centre for Drugs and Drug Addiction warned of 4 tablets, seized in France, which contained extremely high levels of MDMA (**5**) (>200mg MDMA) (Wallon, 2000). It is

important to be able to detect, not only, the identity of the main ingredient and/or ingredients but also to accurately estimate the amount of active(s) in the dosage form. Higher doses of MDMA (**5**) seem to be linked with many of the unpleasant side effects reported (Solowij, 1992)

There are many well established techniques available when analysing illicitly prepared tablets for Ecstasy content. Many of the established methods are targeted at some or all of the methylenedioxy compounds, and some also target the non-ring substituted amphetamine type compounds like amphetamine (**3**) itself and methamphetamine, but methods for only the methylenedioxy compounds are described here.

Gas Chromatography

Gas chromatography-mass spectrometry (GC-MS) seems to be the method of choice for the analysis of methylenedioxyamphetamines, where electron ionisation (EI) is the favoured ionisation mode (O'Connell, 1999; Furnari, 1998; Dawson, 1997; de Boer, 1997; King, 1994; Noggle, 1988; Borth, 2000 and de Ruiter, 1995). This is most likely because the mass spectral data allows the possibility of unequivocal identification of an unknown analyte. GC-MS has also been extensively used in the analysis of ecstasy type compounds and their metabolites in biological fluids (Helmlin, 1996; Maurer, 1996; Kintz, 1997; Fallon, 1999; Garret, 1991; Bogusz, 2000 and Valentine, 2000). MDA (**4**), MDMA (**5**), and MDEA (**6**) were successfully separated by Noggle *et al.* (1988), and by O'Connell *et al.* (1999). BDB (**7**) and MBDB (**8**) were the focus of another GC-MS separation (Kintz 1997), while Dawson *et al*. (Dawson, 1997) analysed only MDEA (**6**). The separation of MDMA (**5**) and MBDB (**8**) was the subject of another GC-MS procedure (Noggle, 1991). Enantiomeric separation has also been achieved for MDMA (**5**) (de Boer, 1997). Borth *et al.* analysed a series 2,3-methylenedioxyamphetamines and 3,4-methylenedioxyamphetamines using tandem GC-MS or GC-MS-MS (Borth, 2000).

MDA (**4**) and MDMA (**5**) have been analysed by Gas chromatography with flame ionisation detection (GC-FID) (Gupta, 1977), as have MDA (**4**), MDMA (**5**) and MDEA (**6**) (McAvoy, 1999). MDA (**4**)**,**

MDMA (**5**), MDEA (**6**) and MBDB (**8**) have also been separated by this technique (Furnari, 1998). GC-FID is not as popular as GC-MS, as no spectral information is supplied by this method.

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) has also been used extensively in the analysis of methylenedioxyamphetamine type compounds. The chromatographic system that is normally employed involves the use of a reverse phase system with ultra-violet-visible (UV–Vis) detection (McAvoy, 1999; Sadeghipour, 1997a; Sadeghipour, 1997b; Longo, 1994; Pok Phak Rop, 1995; Clark, 1995; Noggle, 1991 & Noggle, 1988). Some methods focus only on the separation of MDA (**4**) and MDMA (**5**), as these were the first of the methylenedioxyamphetamines to appear on the illicit drug market (Garret, 1991). Others include the separation of MDA (**4**), MDMA (**5**) and MDEA (**6**) (Noggle, 1988; Noggle, 1987; McAvoy, 1999; Sadeghipour, 1997b & Longo, 1994). Other than Sadeghipour *et al.* (1997a), who used a fluorimetric detection system, none of the other workers seem to focus on the separation of MDA (**4),** MDMA (**5**), MDEA (**6**) and MBDB (**8**) from one another, however Clarke *et al.* (1995) describe a method that may be applicable to the analysis of all four compounds. Other methods focus solely on MDEA (**6**) and MDMA (**5**) (Pok Phak Rop, 1995) or MBDB (**8**) and MDMA (**5**) (Noggle, 1991) with the exclusion of all other Ecstasy type compounds.

Other HPLC systems employed cyclodextrin stationary phases which allowed the separation of MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**), and also achieved separation of their enantiomers. These methods are generally used in the analysis of biological fluids (Sadeghipour, 1998). Other workers focused only on the enantiomeric separation of R- and S- MDMA (**5**) (Fallon, 1999).

Many different detection systems have also been employed. In most cases a fixed wavelength UV-Vis detector (Noggle, 1988; Noggle, 1991; Noggle, 1987; Sadeghipour, 1997b; Clark, 1995 and Garret, 1991) was used. Diode Array Detectors (DAD) (McAvoy, 1999; Pok Phak Rop, 1995 and Longo, 1994) and fluorimetric detectors (Sadeghipour, 1997a; Sadeghipour, 1998 and Pok Phak Rop, 1995) have also been used. The fluorimetric detector is possibly the best choice, as the methylenedioxy-bridge is a fluorescent moiety and as such is the detected unit. The background interference compared

to that from a conventional UV-Vis detector is drastically reduced and in essence the sensitivity is increased. These detectors also have the added advantage of allowing more rapid analysis as the selectivity increases dramatically and as such co-eluting interfering compounds are not a problem as they are invisible to the detector unless they fluoresce over the same specific range as the methylenedioxy bridge.

Miscellaneous Chromatographic Methods

Other separation methods that have been proposed as potentially useful techniques for the analysis of the methylenedioxyamphetamines include Capillary Zone Electrophoresis (CZE) and Super Critical Fluid Chromatography (SFC). MDA (**4**), MDMA (**5**) and MDEA (**6**) were analysed using CZE, which showed potential as both a qualitative and quantitative analytical method for these compounds (McAvoy, 1999 and Sadeghipour, 1997b). McAvoy *et al.* also investigated the use of SFC as a qualitative and quantitative method (McAvoy, 1999).

Thin Layer Chromatography (TLC) has found application in the analysis of the ring-substituted amphetamines (Munro, 1995; Martel, 1986 and O'Brien, 1982) but has been overshadowed by HPLC and GC-MS. TLC can be useful however as it is very quick to set up, it is also very cheap, so it can often be used as a preliminary analytical step before other more sophisticated chromatographic techniques are used. Furnari *et al.* employed a TLC system for the analysis of various sugars used as excipients in Ecstasy tablets (Furnari, 1998).

Immunoassays such as Radioimmunoassay, Elisa, Fluorescence polarisation immunoassay and competitive binding assays, (Baker 1995; Ruangyultikarn, 1998; Wu, 1993 and Dasgupta, 1993) have also been employed in the analysis of ring-substituted amphetamines.

Spectroscopic Methods

Nuclear magnetic resonance (NMR), which is the most important tool available with respect to the structural elucidation of unknown molecules, has been used in the analysis of the ring-substituted amphetamines. However it is difficult to obtain good NMR spectra if there is more than one compound in an illicit sample. Because of this, some preparative chromatographic step would be used or else a hyphenated chromatographic technique such as GC-MS would find favour. Dal Cason *et al.* (1997) detailed the proton (H^1) and carbon (C^{13}) NMR assignments for MDA (**4**), MDMA (**5**) and MDEA (**6**) and other analogues of MDA (**4**). NMR has been used in the identification of MDEA (**6**) in an illicit tablet after liquid-liquid extraction (Dawson, 1997) and also in the identification of MDMA (**5**) (Bailey, 1975). Noggle *et al.* (1987) used NMR to good effect for the identification of MDA (**4**), MDMA (**5**), MDEA (**6**) and a variety of *N*-alkyl derivatives of MDA (**4**) after synthesis. Lee *et al.* used NMR to identify MDEA (**6**) and MDMA (**5**) in illicit tablets using solid state NMR, and also to identify a variety of excipients (Lee, 2000).

Raman spectroscopy is a new technique which may well find favour in the future but which is having its progress as a routine method stifled as it has to compete with well established GC-MS techniques. Raman spectroscopy has been successfully used in the analysis of ring-substituted amphetamines (Bell, 2000; Ryder, 1999 and Dawson, 1997) but problems can arise when mixtures of drugs are analysed. However if the excipients are poor Raman scatterers then this technique can be quite useful as it involves little or no sample preparation and the results are rapidly available. Sonderamann *et al.* (1999) used Near Infra-Red (NIR) spectroscopy in the analysis of these compounds. Infra-red and UV-Vis spectroscopy have also been used, but are not considered as useful when compared to GC-MS. HPLC with suitable spectroscopic detection and GC-MS seem to be the mainstay of ring-substituted amphetamine analysis.

Sample Extraction

The extraction of the methylenedioxy amphetamines from illicitly produced tablets prior to chromatographic analysis is not complicated. Some approach the extraction by simply mixing an

aliquot of a homogenised tablet in a polar organic solvent (O'Connell, 2000; Pop Phak Rop, 1995) or dilute acid (Sadeghipour, 1997b; Longo, 1994). The mixture is agitated, by vortex mixing and/or sonication, and any undissolved material is then removed by filtration or centrifugation. This method is suitable for HPLC and GC-MS analysis where the resultant extract can be directly injected into the chromatographic system, however in the case of GC-MS care must be taken as polar organic solvents can sometimes cause bad peak tailing, especially where methanol is used (Furnari, 1998). Sometimes in GC-MS analysis the preferred technique is to dissolve an aliquot of powdered tablet in an alkaline solution and then to extract using an immiscible solvent (Furnari, 1998; Dawson, 1997).

Section 1.3: Objectives

The objective of the work presented in this chapter was to design a HPLC separation for the analysis of a mixture of MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**). The precision and selectivity of this was assessed, in order that a reliable qualitative method could be established for each of the compounds. The linearity was assessed using external standardisation, so that the method could be used for quantitative determinations of MDMA (**5**). Once established the method was used in quantitative determinations of MDMA (**5**) in illicitly produced tablets seized in the Republic of Ireland.

An extraction procedure was investigated and validated for accuracy (i.e. recovery of MDMA (**5**)). Once a suitable extraction procedure was established it was intended that this method be used in conjunction with the previously established quantitative HPLC procedure in the analysis of illicitly prepared tablets.

A batch of 100 illicitly prepared tablets confiscated by the Garda National Drugs Unit (GNDU) bearing a Mitsubishi logo and purported to contain MDMA (**5**) was supplied for analysis (see Figure 1.2). The main active compound in the batch of 100 Mitsubishi tablets was to be identified using retention time data for the already established HPLC method. This method would further be used with the validated extraction protocol for the quantitative analysis of uniformity of MDMA (**5**) content in the batch of tablets. A number of miscellaneous tablets, also supplied by the GNDU, were also analysed qualitatively using the HPLC method. Finally any unusual samples (containing compounds other than

MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**)) which were available were subjected to GC-MS analysis.

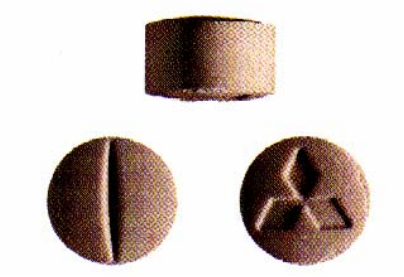


Figure 1.2:Image of Mitsubishi tablets analysed.

Section 2.1: Instrumentation

HPLC-UV

The HPLC system consisted of a Waters 600 controller and pump, a Waters 2487 Dual λ absorbance UV detector. The integrator was a Waters 746 data module. The chromatographic column used was a Waters Spherisorb® 5µm ODS1 (4.6mm × 250mm) analytical column. Samples were introduced into the system using a Rheodyne model 7125.

GC-MS

The GC-MS consisted of a Varian 3800 GC coupled to a Varian Saturn 2000 Ion Trap Mass Spectrometer (mass range 50-650). Varian Saturn Workstation Software was used to control the system. The column used was a 30M Varian DB-35 (35%-phenyl-65%-dimethylsiloxane copolymer), which had a 0.25mm internal diameter and a 0.1µm film thickness.

Miscellaneous Instruments

The centrifuge used was a Sanyo Harrier 18/80 benchtop centrifuge. The ultrasonic bath was a Decon FS4006. The pH meter used was a Metrohm 744 pH meter, equipped with a Metrohm 6.0228.000 glass pH electrode. All weighing was carried out using a Sartorius BP110 S analytical weighing balance.

Section 2.2: Preparation of buffer solutions and mobile phases

Preparation of buffers

The pH 3 phosphate buffer was prepared by adding 34g of KH_2PO_4 to 800 ml of HPLC grade water and adjusting to pH 3.0 using phosphoric acid.

Preparation of Mobile Phases

All organic solvents used for chromatographic purposes were HPLC grade. All buffers were filtered through a Millipore filtration apparatus (42µm cut-off). After the mobile phase was prepared by

adding the correct ratio of each component to the mixture, the solutions were subjected to ultrasonication for 20 minutes, in order to degas.

Section 2.3: Development of a chromatographic system for analysis of MDA (4), MDMA (5), MDEA (6) and MBDB (8) using HPLC-UV.

Preparation of analytical solutions

All reference solutions were prepared by dissolving 0.1 mg in 1 ml of methanol unless otherwise stated.

Reference solution 1: MDA (**4**).

Reference solution 2: MDMA (**5**).

Reference solution 3: MDEA (**6**).

Reference solution 4: MBDB (**8**).

Reference solution 5: A mixture containing 0.1 mg/ml each of MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**) dissolved in methanol.

Chromatographic system

The chromatographic systems investigated are outlined below in Table 2.3.1

System	Mobile Phase Constituents	Proportions	Flow Rate	Wavelength
1	pH 3.0 Phosphate Buffer : MeOH: ACN : Triethylamine	600 : 100 : 25 : 1	1.5 ml/min	280 nm
2	pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	1.5 ml/min	280 nm
3	pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	2.0 ml/min	280 nm

Table 2.3.1: Chromatographic conditions used for the analysis of MDMA (5).

Analysis

An aliquot (20 μl) of each reference solutions was analysed separately under the above chromatographic conditions outlined for system 1, 2 and 3. For results and discussion see section 3.1.

Section 2.4 Assessment of precision of the selected chromatographic method.

Preparation of analytical solutions

Reference solution 5: A mixture containing 0.1 mg/ml each of MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**) dissolved in methanol.

Chromatographic conditions

The chromatographic system used is outlined below in Table 2.4.1

Mobile Phase Constituents	Proportions	Flow Rate	Wavelength
pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	2.0 ml/min	280 nm

Table 2.4.1: Chromatographic conditions used for the analysis of MDMA (5).

Analysis

Six replicate injections (20µl) were chromatographed. This data generated was then used to test the precision of the method. For results and discussion see section 3.2.

Section 2.5 Assessment of selectivity of the selected chromatographic method.

Preparation of analytical solutions

All standard solutions 7-12 of potentially interfering compounds were prepared by dissolving 1.0mg aliquots of the respective compound in 1ml of acetonitrile, unless otherwise stated.

Reference solution 7: BDB (**7**).

Reference solution 8: 4-MTA (**9**).

Reference solution 9: Ketamine (**27**).

Reference solution 10: Amphetamine (**3**).

Reference solution 11: Caffeine (**28**).

Reference solution 12: Paracetamol (**29**).

Chromatographic conditions

The HPLC conditions used in the analysis are outlined in Table 2.5.1.

Mobile Phase Constituents	Proportions	Flow Rate	Wavelength
pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	2.0 ml/min	280 nm

Table 2.5.1: Chromatographic conditions used for the analysis of MDA (4), MDMA (5), MDEA (6) and MBDB (8).

Analysis

The selectivity of the above chromatographic system was tested by a series of 5µl injections of reference solutions 7-12 inclusive. For results and discussion see section 3.3.

Section 2.6: Assessment of linearity of response for MDMA in the selected chromatographic system.

Preparation of the analytical solutions

Reference solution 13: A 0.25 mg/ml MDMA (**5**) standard was prepared by dissolving 2.97mg of MDMA•HCl in methanol.

Reference solution 14: A 0.50 mg/ml MDMA (**5**) standard was prepared by dissolving 5.95mg of MDMA•HCl in methanol.

Reference solution 15: A 0.75 mg/ml MDMA (**5**) standard was prepared by dissolving 8.92mg of MDMA•HCl in methanol.

Reference solution 16: A 1.0 mg/ml MDMA (**5**) standard was prepared by dissolving 11.90mg of MDMA•HCl in methanol.

Chromatographic conditions

The chromatographic system used is outlined below in Table 2.6.1

Mobile Phase Constituents	Proportions	Flow Rate	Wavelength
pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	2.0 ml/min	280 nm

Table 2.6.1: Chromatographic conditions used for the analysis of MDMA (5).

Analysis

Each of the solutions 13, 14, 15 and 16, were injected (20μl) in duplicate, into the chromatographic system with aid of a microlitre syringe. The peak areas, determined by integration, were averaged and plotted against concentration in order to assess the linearity of the method. For results and discussion see section 3.4.

Section 2.7: GC-MS analysis of reference MDMA (5) standard.

MDMA (5) Standard

MDMA (**5**) synthesised in the Department of Pharmaceutical Chemistry in the School of Pharmacy was used as standard (Keating, 2001).

Preparation of solution 17

Powdered MDMA•HCl (10mg) was dissolved in 1 ml of water, in a test tube. Aliquots of conc. ammonia (100 μl) and petroleum ether (1 ml) were added and the contents of the test tube were shake for circa (ca.) 1minute. The supernatant petroleum ether was transferred to a glass vial and evaporated under a stream of nitrogen. The residue was re-dissolved in acetonitrile (500 μl) and labelled test solution 17.

Chromatographic conditions

The chromatographic conditions are outlined in Table 2.7.1.

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Temperature Program	90°C(1 min) up to 300°C (@ 15°C/min) hold for 10 minutes
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1 μl
Injection Solvent	Methanol

Table 2.7.1: GC-MS conditions used for the qualitative analysis of Standard MDMA (5).

Analysis

Test solution 17 was analysed using the above chromatographic conditions. The results and discussion can be found in Section 3.5.

Section 2.8: Determination of the active compound contained in ‘’Mitsubishi’’ tablets

HPLC analysis of ‘’Mitsubishi’’ tablet

Tablet Analysed

One ‘’Mitsubishi’’ tablet from a batch of 100 tablets supplied by the GNDU was analysed. The tablets were bi-convex, slightly off white tablets bearing a ‘’Mitsubishi’’ logo on one side.

Extraction

Powdered tablet (10mg) was dissolved in 1 ml of methanol. The supernatant was removed and labelled test solution 18.

Chromatographic conditions

The chromatographic system used is outlined below in Table 2.8.1

Mobile Phase Constituents	Proportions	Flow Rate	Wavelength
pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	2.0 ml/min	280 nm

Table 2.8.1: Chromatographic conditions used for the analysis of ''Mitsubishi'' tablet.

Analysis

A volume (20 µl) of test solution 18 was introduced into the chromatographic system outlined above.

For results and discussion see section 2.8.1.

GC-MS analysis of ''Mitsubishi'' Tablet

Tablet Analysed

One ''Mitsubishi'' tablet from a batch of 100 tablets supplied by the GNDU was analysed.

Extraction

Powdered tablet (10mg) was dissolved in 1 ml of water, in a test tube. Aliquots of conc. ammonia (100 µl) and petroleum ether (1 ml) were added and the contents of the test tube were shaken for ca. 1minute. The supernatant petroleum ether was transferred to a glass vial and evaporated under a stream of nitrogen. The residue was re-dissolved in acetonitrile (500 µl) and labelled test solution 19.

Chromatographic conditions

The chromatographic conditions are outlined in Table 2.8.2

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35

Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Temperature Program	90°C(1 min) up to 300°C (@ 15°C/min) hold for 10 minutes
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Impact
Filament Delay	4 minutes
Injected Volume	1 μl
Injection Solvent	Methanol

Table 2.8.2: GC-MS conditions used for the qualitative analysis of ‘’Mitsubishi’’ tablet.

Analysis

Test solution 19 was analysed using the above chromatographic conditions. The results and discussion can be found in Section 10.6.

Section 2.9: Validation of extraction of MDMA (5) from illicitly produced tablets using liquid extraction.

Extraction protocol

To 25 mg of powdered homogenised ‘’Mitsubishi’’ tablet in a centrifuge tube, was added 8mls of methanol. This solution was vortex mixed for 10 seconds before being sonicated for 10 minutes. The tubes were then centrifuged at 1000 rpm for 10 minutes. After centrifugation the supernatant was added to a 25ml volumetric flask, the contents made up to volume with methanol and the flask was marked test solution 20. The pellet remaining in the centrifuge tube after removal of the supernatant was re-extracted in 8mls of methanol and vortex mixed. The solution was sonicated for a further 10 minutes, after which time it was centrifuged for 10 minutes at 1000rpm. The supernatant was then transferred to a 25ml volumetric and made up to volume with methanol and the flask was marked test solution 21. The pellet was re-extracted in 8mls of methanol. Homogenisation was achieved by vortex mixing. The solution was sonicated and then subjected to centrifugation (1000rpm, 10minutes). The supernatant was then transferred to a 25ml volumetric flask. The volumetric flask was made up to volume with methanol. The flask was labelled solution 22.

The above procedure was repeated so the analysis could be carried out in duplicate giving test solutions 23-25 respectively.

Chromatographic conditions

The chromatographic system used is outlined below in Table 2.9.1

Mobile Phase Constituents	Proportions	Flow Rate	Wavelength
pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	2.0 ml/min	280 nm

Table 2.9.1: Chromatographic conditions used for the analysis of MDMA (5).

Analysis

Test solution 20,21, 22, 23, 24 and 25 were analysed, in duplicate, by injecting them into the HPLC system (20µl). The peak areas estimated by the integrator were recorded and graphed against extraction number. For results and discussion see section 3.7.

Section 2.10: Determination of uniformity of content in suspected MDMA (5) containing tablets using HPLC-UV.

Preparation of the analytical solutions

Reference solution 13: A 0.25 mg/ml MDMA (**5**) standard was prepared by dissolving 2.97mg of MDMA•HCl in HPLC.

Reference solution 14: A 0.50 mg/ml MDMA (**5**) standard was prepared by dissolving 5.95mg of MDMA•HCl in HPLC.

Reference solution 15: A 0.75 mg/ml MDMA (**5**) standard was prepared by dissolving 8.92mg of MDMA•HCl in HPLC.

Reference solution 16: A 1.00 mg/ml MDMA (**5**) standard was prepared by dissolving 11.90mg of MDMA•HCl in HPLC.

Tablets analysed

A batch of 100 tablets supplied by the Garda National Drugs Unit were extracted and analysed as outlined below. The tablets were bi-convex, slightly off white tablets bearing a 'Mitsubishi' logo on one side (see Figure 2.10.1 below). The tablets were well made and not chipped to any extent on inspection. Tablet weights were recorded for the tablets.

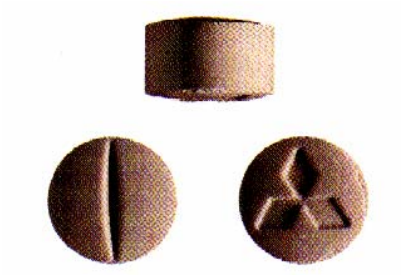


Figure 2.10.1: Image of Mitsubishi tablets analysed.

A further twenty miscellaneous tablets were analysed. Physical descriptions of these tablets are outlined in Table 2.10.1 below.

Tablet Number	Logo/Shape	Tablet Number	Logo/Shape
1a	No logo/Cross shaped	11a	Bird/round
2a	No logo/Cross shaped	12a	Bird/round
3a	Mitsubishi/round	13a	Bird/round
4a	Mitsubishi/round	14a	Bird/round
5a	Mitsubishi/round	15a	Bird/round
6a	$/round	16a	Bird/round
7a	$/round	17a	Bird/round
8a	Bird/round	18a	Bird/round
9a	Bird/round	19a	Bird/round
10a	Bird/round	20a	Bird/round

Table 2.10.1: Description of miscellaneous tablets analysed by HPLC

Extraction protocol

To 25 mg of powdered homogenised tablet in a centrifuge tube, was added 8mls of methanol. This solution was vortex mixed for 10 seconds before being sonicated for 10 minutes. The tubes were then centrifuged at 1000 rpm for 10 minutes. After centrifugation the supernatant was added to a 25ml volumetric flask. The pellet remaining in the centrifuge tube after removal of the supernatant was re-dissolved in 8mls of methanol and vortex mixed. The solution was sonicated for a further 10 minutes, after which time it was centrifuged for 10 minutes at 1000rpm. The supernatant was then added to the previously extracted supernatant in the 25ml volumetric flask. The flask was then made up to volume with methanol. This procedure was carried out in duplicate for each of the tablets analysed.

Chromatographic conditions

The chromatographic system used is outlined below in Table 2.10.2

Mobile Phase Constituents	Proportions	Flow Rate	Wavelength
pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	2.0 ml/min	280 nm

Table 2.10.2: Chromatographic conditions used for the analysis of MDMA (5).

Analysis

Each of the reference solutions 13, 14, 15 and 16, were injected in duplicate (20µl). Peak areas were calculated by the integrator. The average of the areas for duplicate injections were calculated and plotted against concentration. Each of the tablet extracts was injected in duplicate in the chromatographic system outlined above (20 µl). The peak areas were estimated and concentrations of MDMA were calculated from the standard curve. For results and discussion see Section 3.8.

Section 2.11 Analysis of tablet 3a by GC-MS

Preparation of analytical solutions

Reference solution 26: 0.1 mg/ml solution ketamine (**27**) in methanol.

Chromatographic conditions

The chromatographic conditions are outlined in Table 2.11.1.

GC-MS Parameter	GC-MS Condition

Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Temperature Program	90°C(1 min) up to 300°C (@ 15°C/min) hold for 10 minutes
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1 μl
Injection Solvent	Methanol

Table 2.11.1: GC-MS conditions used for the qualitative analysis of illicitly prepared tablets.

Analysis

Reference solution 26 was analysed under the above chromatographic conditions. The extract of sample 3a extracted by the method described in section 9.8 was chromatographed in the system outlined above. The mass spectrum for the most significant peak was compared to the Varian reference library, which is part of the Varian Saturn software. The unknown was also compared to the spectrum obtained from reference solution 26. For results and discussion see section 3.9.

Section 3.1: Development of a chromatographic system for analysis of MDA (4), MDMA (5), MDEA (6) and MBDB (8) using HPLC-UV.

Three chromatographic systems were investigated in the separation MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**) (Table 3.1.1).

System	Mobile Phase Constituents	Proportions	Flow Rate	Wavelength
1	pH 3.0 Phosphate Buffer : MeOH: ACN : Triethylamine	600 : 100 : 25 : 1	1.5 ml/min	280 nm
2	pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	1.5 ml/min	280 nm
3	pH 3.0 Phosphate Buffer : ACN : Triethylamine	600 : 100 : 1	2.0 ml/min	280 nm

Table 3.1.1: Chromatographic conditions used for the analysis of MDMA (5).

Clark *et al.* used system 2 as a means of separating MDA (**4**), MDMA (**5**) and MDEA (**6**) and in a separate experiment used the same system to separate a series of methylenedioxybutanamines such as BDB (**7**) and MBDB (**8**) (Clark, 1995). Clark *et al* did not attempt a separation of MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**). By injecting aliquots of reference solution 5 (containing all four compounds) chromatographic data was acquired for each of the three systems. A chromatogram obtained under the conditions outlined in system 3 (Table 3.1.1) is shown below (Figure 3.1.2).

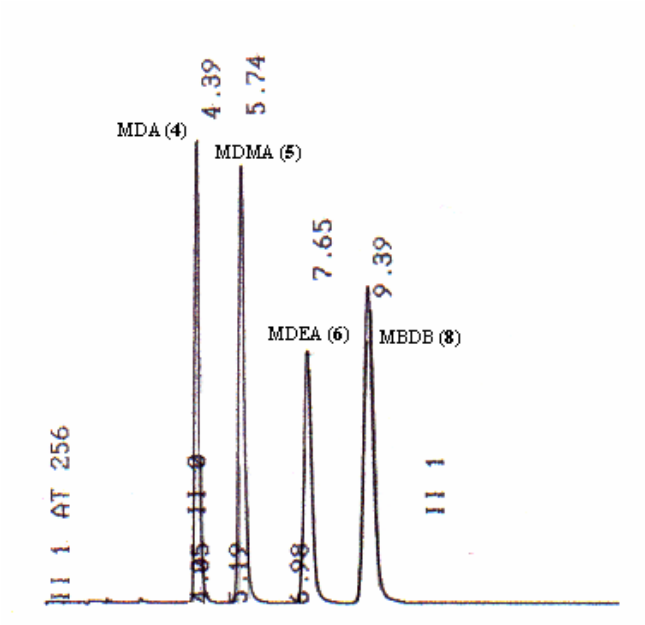


Figure 3.1.2: Chromatographic profile obtained for a 0.1 mg/ml mixture of MDA (4), MDMA (5), MDEA (6) and MBDB (8).

Chromatographic parameters such as capacity factors, resolution, peak tailing and column efficiency were calculated manually and are tabulated below (Table 3.1.3). For details of the formulae used see appendix I. Visual inspection of a typical chromatogram shows separation was achieved and the peaks are symmetrical. The order of elution was determined by sequentially injecting reference solutions 1-4 containing MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**) respectively. The retention times were compared with those of the chromatographed mixture and the identities of the peaks were determined. The retention time relative to MDMA (**5**) were also calculated (Table 3.1.3). The first compound to elute was MDA (**4**) at ca. 4.37 minutes followed by the more polar MDMA (**5**) at ca. 5.72 minutes. MDEA (**6**) eluted at ca. 7.66 minutes and its isomer MBDB (**8**) at ca. 9.43 minutes was the last compound to elute.

Peak	Peak Identity	t_R	t_R Relative to MDMA	Width	Capacity Factor	Resolution	Asymmetry	Theoretical Plates
1	MDA (**4**)	4.37 mins	0.76	0.15 mins	3.37	--	1.1	13,580
2	MDMA (**5**)	5.72 mins	1.00	0.15 mins	4.72	$9.00_{MDA/MDMA}$	1.1	23,266
3	MDEA (**6**)	7.66 mins	1.34	0.25 mins	6.66	$11.08_{MDMA/MDEA}$	1.1	19,397
4	MBDB (**8**)	9.43 mins	1.65	0.30 mins	8.43	$6.60_{MDEA/MBDB}$	1.1	15,809

Table 10.1.3 Table of chromatographic parameters calculated from the HPLC trace recorded for the separation of MDA (4), MDMA (5), MDEA (6), MBDB (8).

In each case the tailing factors were within acceptable limits (Dolan, 2000) and the resolution between the peaks was excellent, where all calculated resolution values exceeded 6.60. The calculated theoretical plate values were high enough to indicate the column was performing satisfactorily. The capacity factors of the analytes were within acceptable limits (2-10 ideally; Dolan, 2000). The wavelength of 280 nm, a common wavelength used in the analysis of aromatic compounds seemed to be suitable, as there was a relatively good response from the detector and little or no background at the 0.10 mg/ml level investigated.

The conclusion, that system 3 was the best, was arrived at on the basis that the chromatography was acceptable (i.e. tailing, resolution and column efficiency). Another reason for this conclusion was that the compounds eluted from the column under the conditions of system three more quickly that from either of the other two chromatographic systems, which would keep the run-time to a minimum.

Section 3.2: Assessment of precision of the chromatographic method

Chromatographic parameters, such as, retention time and area (for six replicate injections) were determined using the integrator (see Table 3.2.1) for the four peaks in the chromatograms resulting from injections of reference solution 5. Chromatographic parameters such as retention times and peak areas, from the replicate injection of a standard mixture containing MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**), were acquired via electronic integration for all of the peaks (see Table 3.2.1)

Compound	Average Retention Time	Relative Standard Deviation	Average Peak Area	Relative Standard Deviation
MDA (minutes)	4.38 mins	0.52 %	1554207	7.36 %
MDMA (minutes)	5.79 mins	0.71 %	1948065	7.24 %
MDEA (minutes)	7.74 mins	0.74 %	1535068	6.99 %
MBDB (minutes)	9.51mins	0.75 %	2289160	6.94 %

Table 3.2.1: Chromatographic parameters for peaks in six replicate injections of reference solution 5

The relative standard deviations (R.S.D) (see Appendix I) of the retention times for all four compounds were low enough for the precision to be considered good. The relative standard deviation is a term often used to describe the precision (Riley, 1996). Ideally one strives to minimise the relative standard deviation as much as possible, but values of less than two are usually considered very good (Riley, 1996). The peak area deviated more than the retention time, however the deviation was acceptable. This deviation could have been reduced by the use of an internal standard and it is advisable that any

future work would use a suitable internal standard. The precision of the method was considered to be good, as there was little deviation in the retention time and the peak areas as estimated by the integrator.

Section 3.3: Assessment of selectivity of the chromatographic method.

The selectivity of chromatographic system 3 (see Section 2.3) was assessed by injecting various potential interfering compounds, such as caffeine (**28**), paracetamol (**29**), ketamine (**27**), amphetamine (**3**), BDB (**7**) and 4-MTA (**9**) (reference solutions 7-12) into the system. If one includes MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**) were 10 compounds. The resolution between the chromatographic peak of these 10 compounds should ideally be greater than 1. The retention time and resolution between all of these compounds, with respect to each other was calculated and is shown below (see Figure 3.3.1).

Compound	Retention Time (minutes)	Resolution
Paracetamol	2.87 minutes	10.27 (Paracetamol, MDA)
MDA	4.31minutes	5.19 (MDA, MDMA)
MDMA	5.63 minutes	3.36 (MDMA, BDB)
BDB	6.77minutes	2.18 (BDB, MDEA)
MDEA	7.51 minutes	1.70 (MDEA, Caffeine)
Caffeine	8.09 minutes	0.94 (Caffeine, Ketamine)
Ketamine	8.41 minutes	2.09 (Ketamine, MBDB)
MBDB	9.21 minutes	1.52 (MBDB, 4-MTA)
4-MTA	9.84 minutes	
Amphetamine	No peak recorded at 280nm	

Figure 3.3.1: Retention times, and resolution values estimated for the analysis of selectivity of chromatographic system 3.

The selectivity of the method with respect to other methylenedioxyamphetamines, (MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**)) was adequate, this was clearly shown in section 3.1 where the resolution values were acceptable. Amphetamine (**3**) did not give a signal at 280 nm over the 15 minute period monitored. Caffeine (**28**) did not interfere with the method as it eluted before MBDB (**8**). 4-MTA (**9**) eluted just after the MBDB (**8**) peak at ca. 9.84 minutes. The resolution between the two peaks was calculated to be circa 1.52, which would indicate, that the peaks did not interfere with each other and that this method would be suitable for the analysis of 4-MTA (**9**)**.** Ketamine (**27**) eluted between MDMA (**5**) and MDEA (**6**) in the chromatogram but as there was particularly good resolution between these compounds to begin with ketamine (**27**) did not interfere. Hence the method is considered selective for the methylenedioxyamphetamines and also may be an appropriate method for the analysis of ketamine (**27**) and 4-MTA (**9**) also.

Section 3.4: Assessment of linearity of the chromatographic method for MDMA (5).

The linearity of the method for MDMA **(5)** was tested by performing external standardisation. Using a series of standards, reference solutions 13-16, varying in concentration (0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml and 0.10 mg/ml respectively) the peak area was used to plot a standard curve. The average peak area for two injections of each of the aforementioned standards can be seen in Table 3.4.1. A plot of the average peak area for two injections versus the concentration can also be seen below. (see Figure 3.4.1).

Solution Number	Standard Concentration	Average Peak Area
4	0.25 mg/ml	5600990
5	0.50 mg/ml	9904739
6	0.75 mg/ml	1408798
7	0.10 mg/ml	19738837

Table 3.4.1: Average peak area for two injections of MDMA (5) standards

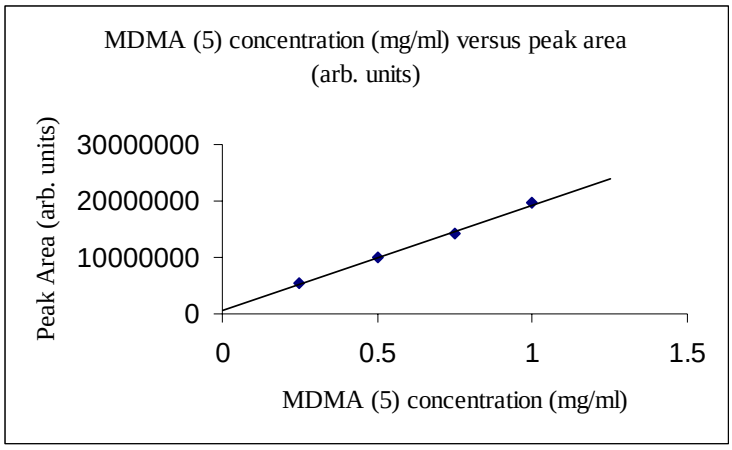


Figure 3.4.1: Plot of average peak area versus concentration of MDMA (5) *standard.*

One can clearly see from the above plot that the relationship between MDMA (**5**) concentration and peak area is linear. The R^2 value as calculated with the aid of Microsoft Excel was found to be 0.99

(Microsoft®, 1997). The R^2 parameter is a correlation coefficient which is a measure of goodness of fit of a set data points to a linear trend. A value of +1 indicates perfect correlation and a positive slope (Riley, 1996). Values of greater than 0.9 are considered very good. The equation of the line was estimated to be y = 18647x + 683941. The linearity test conducted indicated that relationship between the absorbance of MDMA (**5**) and concentration obeyed Beer's law (Appendix I) and hence it was acceptable to use this method in the quantitation of MDMA (**5**) in illicit tablets provided that a suitable extraction technique could be established.

Section 3.5: GC-MS analysis of reference MDMA (5) standard

A GC-MS of the standard MDMA (**5**) was run. This would help prove the identity and purity of the standard MDMA (**5**) which had been synthesised in the Department of Pharmaceutical Chemistry in the School of Pharmacy, T.C.D. It was found that only one peak eluted for MDMA (**5**) in the chromatogram at 6.4 minutes, indicating that there were no other impurities present in the standard (see Figure 3.5.1).

Figure 3.5.1: GC-MS trace for the MDMA (5) standard.

Section 3.6: Determination of the active compound contained in ''Mitsubishi'' tablets using HPLC and GC-MS

The GC-MS procedure outlined in section 2.8 was used to assess the type of compound contained in the 'Mitsubishi'' tablets. The tablet analysed was found to contain MDMA (5**) as the peak eluted at 6.5 minutes (the same retention time as that of the standard MDMA, discussed above, Section 3.5). The GC-MS chromatogram of the tablet extract is shown below (Fig 3.6.1a). As can be seen MDMA (**5**) was the main peak in the chromatogram.**

Fig 3.6.1a:GC-MS chromatogram of the ''Mitsubishi'' tablet extract.

The mass spectrum was also a good match with that of the standard MDMA (5**) when searched against the National Institute of Standards and Technology (NIST) mass spectral library (see Figure 3.6.1b) (NIST, 1995). The NIST library is an electronic database containing thousands of reference EI mass spectra.**

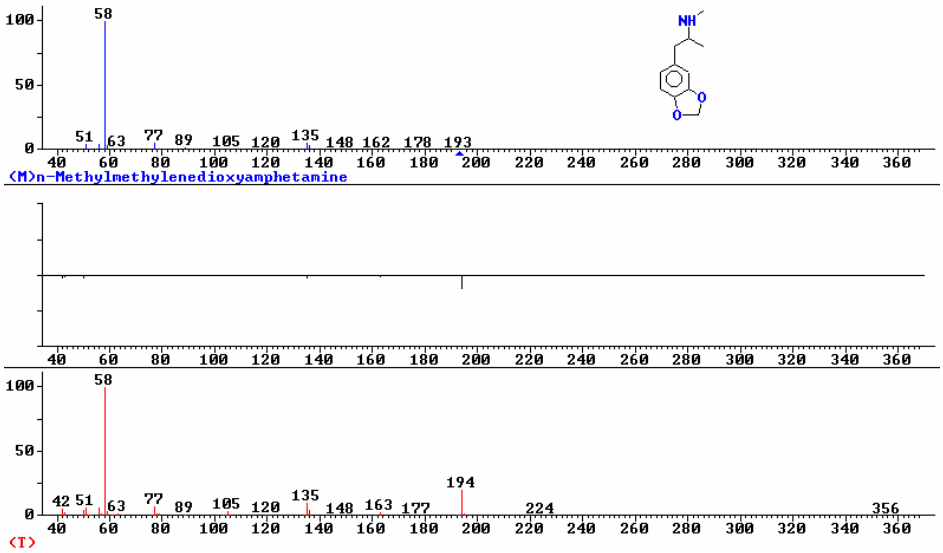


Figure 3.6.1b: NIST library comparison of standard MDMA (5) from library and compound found in ''Mitsubishi'' tablet. The uppermost spectrum represents the NIST library spectrum, including the structure of MDMA (5). The lowermost spectrum was the spectrum acquired when the standard extract was chromatographed The middle spectrum represents the difference spectrum between the uppermost and the lowermost spectrum in the figure.

A tablet extract was also chromatographed using the HPLC system described earlier (see Section 2.3). It was found that the only peak to elute under the chromatographic conditions described in section 2.8 did so at 5.72 minutes, which was the same time as the standard (see Figure 3.6.2).

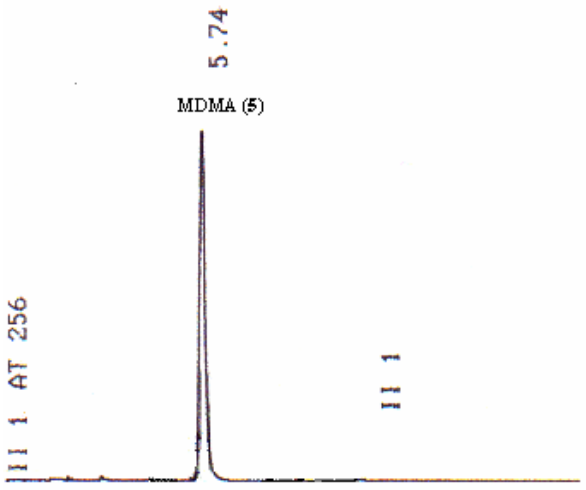


Figure 3.6.2: HPLC chromatogram of ''Mitsubishi'' tablet extract.

Section 3.7: Extraction of MDMA (5) from illicitly produced tablets using liquid extraction.

It was established that the tablet to be used in the validation of the extraction of MDMA (**5**) actually contained MDMA (**5**) by a simple methanol extraction followed by HPLC analysis and GC-MS see Section 2.8). In order to determine the recovery of MDMA (**5**) from illicitly prepared tablets an experiment was designed. This experiment involved the extraction of MDMA (**5**) from an illicitly prepared tablet with methanol with the aid of sonication and centrifugation. The supernatant was removed (test solution 20). The pellet left after the first extraction was re-extracted (test solution 21). Finally the pellet from the second extraction was re-extracted again (test solution 22). This procedure was carried out in duplicate (test solutions 23, 24 and 25 respectively). Each extract was made up to 25mls and subjected to HPLC analysis. The averaged peak areas returned for the duplicate procedure were plotted and are shown below (see Figure 3.7.1).

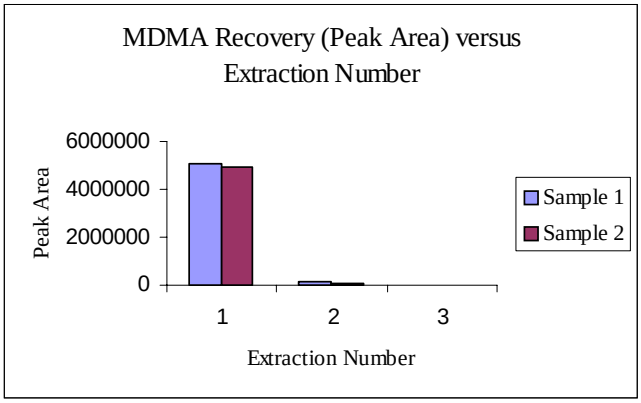


Figure 3.6.1: Plot of peak area versus MDMA (5) recovery (peak area) used to establish the best method for the extraction of MDMA (5) form illicitly prepared tablets.

As can be seen from the above graph the majority of MDMA (**5**) is extracted after the first extraction but there is still MDMA (**5**) extracted with the second extraction. The third extraction seemed unnecessary as there did not seem to be any MDMA (**5**) left to extract. Hence it was decided that

maximum recovery of MDMA (**5**) from illicitly prepared tablets could be achieved with two extractions using methanol.

Section 3.8: Analysis of suspected MDMA (5) containing tablets using HPLC-UV.

A new standard curve was prepared each day and as sample extracts became available they were subjected to the HPLC analysis outlined in system 3, section 2.3. Samples, which had been extracted in duplicate, were chromatographed in duplicate and average peak areas were calculated from integrator data. These peak areas were then interpolated from the standard curve and the amount of MDMA (**5**) per tablet was estimated by substituting the values in the formula below with experimental values.

$$\text{MDMA(mg)} = \left(\frac{\text{interpolated value}}{40} \right) \div \text{weight in mg of tablet taken)} \times \text{Weight of whole tablet}$$

The estimated amounts of MDMA (**5**), as calculated from HPLC data for the 100 'Mitsubishi' tablets are shown below (see Table 3.8.1).

Tablet Number	MDMA (mg)	Tablet Number	MDMA (mg)	Tablet Number	MDMA (mg)	Tablet Number	MDMA (mg)
1	85.67mg	26	75.74mg	51	76.40mg	76	86.90mg
2	74.40mg	27	76.90mg	52	76.13mg	77	85.00mg
3	73.62mg	28	70.56mg	53	87.89mg	78	84.95mg
4	72.36mg	29	78.16mg	54	83.93mg	79	89.91mg
5	79.02mg	30	82.39mg	55	75.90mg	80	85.70mg
6	59.24mg	31	73.91mg	56	76.95mg	81	98.18mg
7	73.31mg	32	69.63mg	57	78.16mg	82	66.51mg
8	77.30mg	33	74.31mg	58	72.04mg	83	83.43mg
9	90.94mg	34	70.41mg	59	76.51mg	84	77.29mg
10	89.73mg	35	82.44mg	60	79.65mg	85	78.01mg
11	79.62mg	36	75.17mg	61	79.41mg	86	77.96mg
12	88.07mg	37	83.66mg	62	62.05mg	87	82.00mg
13	68.54mg	38	77.84mg	63	68.12mg	88	78.94mg
14	76.06mg	39	76.77mg	64	70.44mg	89	76.92mg
15	80.21mg	40	77.43mg	65	79.01mg	90	81.63mg
16	97.64mg	41	80.92mg	66	76.29mg	91	76.87mg
17	84.09mg	42	78.83mg	67	75.23mg	92	76.51mg
18	77.69mg	43	78.40mg	68	79.17mg	93	82.15mg
19	63.39mg	44	76.52mg	69	78.25mg	94	71.95mg
20	76.64mg	45	94.88mg	70	74.30mg	95	72.09mg

21	80.33mg	46	76.46mg	71	75.96mg	96	81.36mg
22	78.04mg	47	87.88mg	72	84.65mg	97	78.01mg
23	80.05mg	48	89.02mg	73	78.72mg	98	79.47mg
24	90.17mg	49	80.82mg	74	90.41mg	99	72.24mg
25	83.79mg	50	84.01mg	75	89.72mg	100	78.93mg

Table 3.8.1: MDMA (5) content (mg) estimated for a batch of 100 Mitsubishi tablets analysed by HPLC

The average amount of MDMA (**5**) calculated for the 100 tablets analysed was 79.01mg, with a standard deviation of 6.91mg. The relative standard deviation was calculated and found to be 8.74%. The lowest concentration was 59.24 mg and the highest concentration was 98.18 mg. The tablets were obviously not made to pharmaceutical grade but it was of interest to compare the British Pharmacopoeia (BP) requirements for Uniformity of Content of tablets to the batch analysed in this case. The BP general monograph (BP, 2000) concerning uniformity of content states, that if one or more tablets out of 10 analysed are outside the limit of 85%-115% of the stated amount but within the limit 75%-125%, then 20 more tablets should be analysed. Then if out of the 30 analysed there is not more than one individual tablet outside the 85%-115% and none are outside the limits 75%-125% the batch can be accepted, otherwise the batch must be rejected. If we consider the batch here and say the target content is the average we can see whether the tablets conform to the BP monograph. Table 3.8.2 shows the uniformity of content values calculated with 79.01 mg as the target concentration, solving the equation below arrived at the percentage values.

$$\left(\frac{\text{Average value}}{\text{Observed value}}\right) \times 100/1$$

None of the tablets were outside the wider interval, 75%-125%. However tablets 6 (75%), 16 (124%), 19 (80%), 45 (120%) and 81 (124%) were outside the narrower range.

Tablet Number	MDMA (%)	Tablet Number	MDMA (%)	Tablet Number	MDMA (%)	Tablet Number	MDMA (%)
1	108	26	96	51	97	76	110
2	94	27	97	52	96	77	108
3	93	28	89	53	111	78	108
4	92	29	99	54	106	79	114
5	100	30	104	55	96	80	108
6	75	31	94	56	97	81	124
7	93	32	88	57	99	82	84
8	98	33	94	58	91	83	106

9	115	34	89	59	97	84	98
10	114	35	104	60	101	85	99
11	101	36	95	61	101	86	99
12	111	37	106	62	79	87	104
13	87	38	99	63	86	88	100
14	96	39	97	64	89	89	97
15	102	40	98	65	100	90	103
16	124	41	102	66	97	91	97
17	106	42	100	67	95	92	97
18	98	43	99	68	100	93	104
19	80	44	97	69	99	94	91
20	97	45	120	70	94	95	91
21	102	46	97	71	96	96	103
22	99	47	111	72	107	97	99
23	101	48	113	73	100	98	101
24	114	49	102	74	114	99	91
25	106	50	106	75	114	100	100

Table 3.8.2: Uniformity of content for batch of 100 Mitsubishi tablets analysed by HPLC

If it was a pharmaceutical product the batch would therefore be rejected. It is worth noting however that in this instance the majority of the tablets, 95%, are within the limits, indicating that the tablets are reasonably well made.

The average weight of tablets was 0.305g, with a standard deviation of 0.017%. The relative standard deviation was 5.73%. The BP also has a monograph for the uniformity of weight of uncoated tablets. The tablets analysed were uncoated. The monograph states that out of 20 tablets, randomly chosen and weighed, not more than two tablets may deviate from the target weight by more than 5%, and no tablets at all should deviate by more than 10%. Table 3.8.3 shows the uniformity of weight data for tablets.

Tablet Number	MDMA (%)	Tablet Number	MDMA (%)	Tablet Number	MDMA (%)	Tablet Number	MDMA (%)
1	110	26	99	51	99	76	105
2	108	27	101	52	97	77	100
3	96	28	99	53	106	78	103
4	93	29	93	54	105	79	109
5	95	30	109	55	105	80	106
6	87	31	91	56	104	81	99
7	100	32	88	57	104	82	100
8	96	33	91	58	96	83	103
9	96	34	85	59	103	84	103
10	103	35	103	60	97	85	99
11	105	36	101	61	106	86	101
12	105	37	95	62	80	87	106

13	103	38	102	63	90	88	99
14	97	39	93	64	99	89	100
15	104	40	98	65	100	90	105
16	105	41	98	66	98	91	98
17	103	42	100	67	100	92	98
18	99	43	99	68	106	93	104
19	90	44	96	69	107	94	91
20	105	45	105	70	98	95	96
21	106	46	91	71	93	96	102
22	98	47	105	72	99	97	102
23	104	48	110	73	101	98	99
24	107	49	97	74	106	99	97
25	101	50	95	75	108	100	105

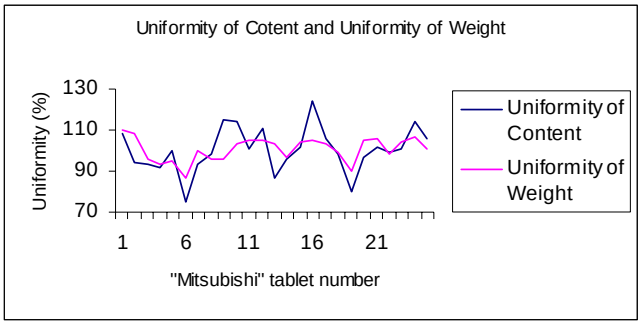
Table 3.8.3: Uniformity of weight for batch of 100 Mitsubishi tablets analysed by HPLC

Solving the equation below arrived at the values in Table 3.8.3.

$$\left(\frac{\text{Average value}}{\text{Observed value}}\right) \times 100/1$$

Looking at the uniformity of content (Table 3.8.3) it appears that the samples vary significantly in weight and would certainly not comply with the monograph. Graphs of Uniformity of Content and Uniformity of Weight were plotted versus ‘’Mitsubishi’’ tablet number (see Figure 3.8.4).

(a)



(b)

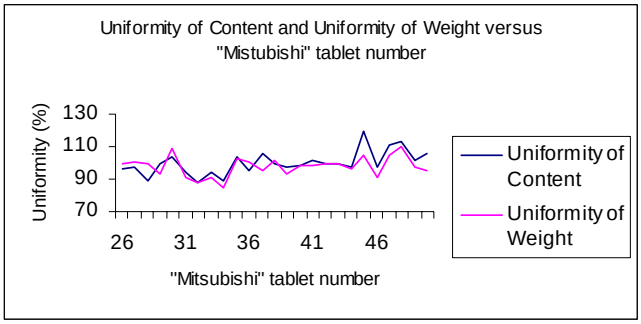
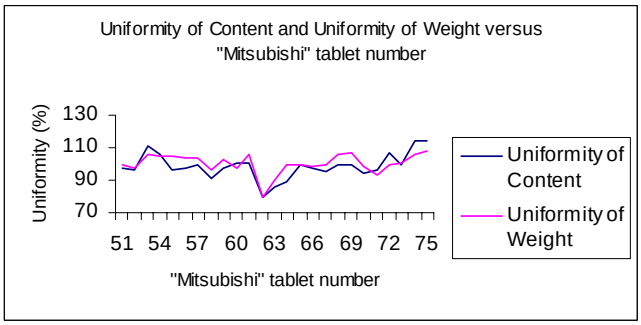


Figure 3.8.4 continued: Graphs of Uniformity of Content and Uniformity of Weight plotted against ''Mitsubishi'' Tablet number.

(a) tablets 51-75, (b) tablets 76-100.

(c)



(d)

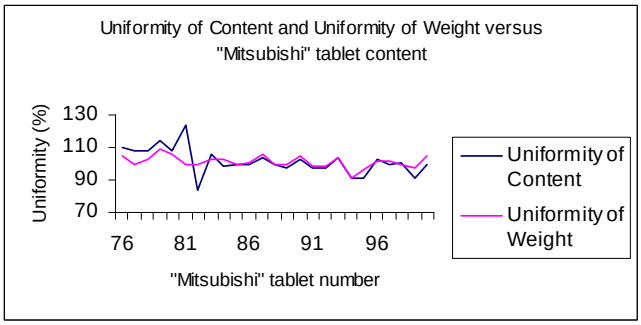


Figure 3.8.4 continued: Graphs of Uniformity of Content and Uniformity of Weight plotted against ''Mitsubishi'' Tablet number. (c) tablets 51-75, (d) tablets 76-100.

The plots were made for every 25 samples such that the spread could be seen. As can be seen from visual inspection of the graphs there seems to be a link between the uniformity of content and the uniformity of weight.

In conclusion the tablets analysed would appear to be reasonably well made based on the appearance and the uniformity of content data. An MDMA (**5**) concentration of 79.01mg is the low end of the scale, if one considers the average concentration to be ca. 100mg. The underground text 'Secrets of Methamphetamine Manufacture' (Uncle Fester, 1996) advocates the use of 100mg. Shulgin experimented with 100mg and 120mg (Shulgin, 1991) and many laboratory investigations have proved that the concentration of MDMA (**5**) in illicitly prepare tablets range from trace (O'Connell, 2000)-300mg (Wallon, 2000)

The miscellaneous samples, which were analysed (Table 2.10.1) had the concentration of MDMA (**5**) calculated from a standard curve and the above equation in the same way as the batch of aforementioned 'Mitsubishi' tablets. Table 2.8.4 below details the results of the analysis.

Tablet Number	Logo/Shape	Tablet Weight (g)	MDMA (mg)
1a	No logo/Cross shaped	0.3758g	77.60mg
2a	No logo/Cross shaped	0.3882g	77.14mg
3a	Pink mottled/round	0.5471g	-----
4a	Mitsubishi/round	0.2910g	78.45mg
5a	Mitsubishi/round	0.3020g	82.68mg
6a	Mitsubishi/round	0.3099g	80.44mg
7a	$/round	0.3070g	----
8a	$/round	0.2866g	----
9a	Bird/round	0.2617g	108.75mg
10a	Bird/round	0.2525g	114.93mg
11a	Bird/round	0.2135g	114.80mg
12a	Bird/round	0.2343g	115.39mg
13a	Bird/round	0.2135g	97.88mg
14a	Bird/round	0.2343g	99.68mg
15a	Bird/round	0.2701g	111.77mg
16a	Bird/round	0.2612g	123.56mg
17a	Bird/round	0.2751g	117.56mg
18a	Bird/round	0.2711g	110.51mg
19a	Bird/round	0.2703g	111.16mg
20a	Bird/round	0.2550g	93.15mg

Table 3.8.4: Physical appearance & MDMA (5) content of miscellaneous tablets analysed by HPLC-UV.

Tablets 1a and 2a were cross-shaped tablets, which were nicknamed *shamrocks* when introduced to the Irish market (see Figure 3.8.5).

Figure 3.8.5: Image of ''Shamrock'' MDMA tablet.

These tablets were reported by the Irish media to contain twice the normal amount of MDMA (**5**) (Williams, 1999). In the case of our analysis the two tablets which were analysed were found to contain ca. 77mg of MDMA (**5**) in each. The Mitsubishi tablets analysed, which were not part of the batch that was analysed earlier, contained a similar level to the amount observed in the larger batch. The average of the three tablets was 80.52mg, with a standard deviation of 1.73mg. Whether these tablets were from the same batch or not, isn't known, but this result backs up the theory that the tablets may have been intended to contain ca. 80mg MDMA (**5**). It was not known whether the tablets bearing the bird logo were from the same batch or not (see Figure 3.8.6).

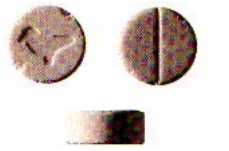


Figure 3.8.6: Image of MDMA tablet bearing a Bird logo.

These tablets varied greatly in MDMA (**5**) content where the average was 109.92mg MDMA (**5**), with standard deviation of 8.47mg. The relative standard deviation was 7.7%. The MDMA (**5**) concentration is certainly higher in the case of these tablets when compared to the 'Mitsubishi' tablets.

The other tablets analysed did not contain MDMA (**5**). Tablets 7a and 8a contained MBDB (**8**), which was confirmed in section 3.8. The amounts of MBDB (**8**) in found in tablets were discussed in section 3.9 as estimated by HPLC-UV analysis.

Section 3.9: Analysis of tablet 3a by GC-MS

The large pink speckled tablet, 3a, did not appear to contain any of the methyelendioxyamphetamines when analysed by HPLC, it did however have a compound eluting from the chromatographed extract at ca. 4.7minutes which may indicate that the compound was ketamine (see Section 3.3 above). The tablet extraction mixture was subjected to GC-MS analysis and was found to contain ketamine (**27**) eluting at 8.8 minutes, when compared to the NIST mass spectral library. The library spectrum and the sample spectrum are shown below (Figure 3.9.1). The difference between the two spectra is also included and one can see that there is little difference between the two spectra from Figure 3.9.1. The quality of the hit in the NIST library was found to be 793, which is reasonable. The structure of ketamine (**27**) is shown below (Figure 3.9.2). A reference solution of ketamine (**27**) was analysed, where ketamine was found to elute at 8.8 minutes. The spectrum of the standard and tablet extract were also a good match which confirm that the tablet 3a contained ketamine (**27**).

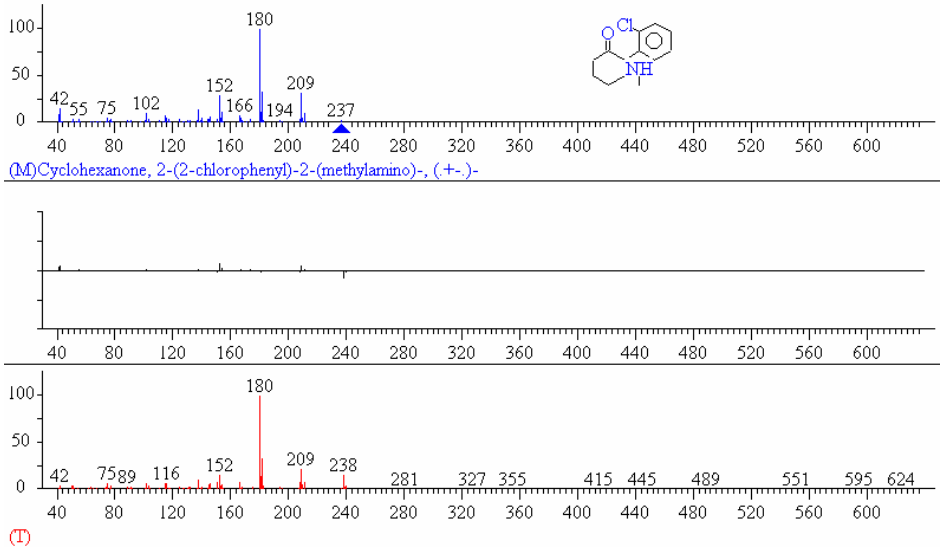


*Figure 3.9.1: The uppermost spectrum represents the NIST library spectrum, including the structure of ketamine **(27)**. The lowermost spectrum was the spectrum acquired when the sample of 3a extract was chromatographed The middle spectrum represents the difference spectrum between the uppermost and the lowermost spectrum in the figure.*

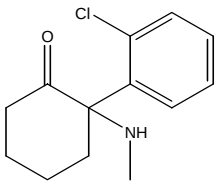


*Figure 3.9.2: Structure of ketamine **(27)**, the compound found to be eluting at 8.8 minutes in the GC-MS trace of the extract of sample 3a.*

Section 3.10: Conclusion

A valid method for the extraction of MDMA (**5**) from illicit tablets followed by analysis via HPLC-UV was successfully established. The HPLC-UV method was considered to be useful as a qualitative method for the analysis of MDA (**4**), MDMA (**5**), MDEA (**6**), MBDB (**8**), 4-MTA (**9**) and ketamine (**27**), however this was not fully validated. The method may well also be suitable for the quantitation of the aforementioned compounds but again this was not the subject of validation. The batch of 100 tablets bearing the Mitsubishi logo appeared to be well made and would possibly pass the some of the BP requirements for manufactured tablets. The miscellaneous tablets for the most part contained MDMA (**5**), however samples containing ketamine (**27**) and MBDB (**8**) were also identified.

Section 5.1: Instrumentation

HPLC

The HPLC consisted of a Shimadzu LC-6A isocratic pump and a Shimadzu SPD-M10AVP diode array detector (DAD). The chromatographic system was controlled using Shimadzu CLASS-VP software (version 5.032). The chromatographic column used was a Waters Spherisorb® 5µm ODS1 (4.6mm × 250mm) analytical column. Samples were introduced into the system using a Rheodyne model 7125.

GC-MS

The GC-MS consisted of a Varian 3800 GC coupled to a Varian Saturn 2000 Ion Trap Mass Spectrometer (mass range 50-650 m/z). Varian Saturn Workstation software was used to control the system. The column used was a 30M Varian DB-35 (35%-phenyl-65%-dimethylsiloxane copolymer), which had a 0.25mm internal diameter and a 0.1µm film thickness.

Miscellaneous Instruments

The centrifuge used was a Sanyo Harrier 18/80 benchtop centrifuge. The ultrasonic bath was a Decon FS4006. The pH meter used was a Metrohm 744 pH meter, equipped with a Metrohm 6.0228.000 glass pH electrode. All weighing was carried out using a Sartorius BP110 S analytical weighing balance.

Section 5.2: Preparation of buffer solutions and mobile phases

pH 3.0 buffer

The pH 3 phosphate buffer was prepared by adding 34g of KH_2PO_4 to 800mls of HPLC grade water and adjusting to pH 3.0 using phosphoric acid.

pH 4.0 buffer

The pH 4.0 buffer was prepared by dissolving 5.04 g Na_2HPO_4 and 3.01 g of KH_2PO_4 in sufficient HPLC grade water to produce 1000ml. The solution was then adjusted to pH 4.0 with glacial acetic acid.

pH 4.9 buffer

The pH 4.9 buffer was prepared by dissolving 40 g Na_2HPO_4 and 1.2 g of NaOH in sufficient HPLC grade water to produce 100 ml. If necessary the pH was adjusted to 4.9 with 1*M* H_2SO_4 or 1*M* NaOH as required.

pH 6.0 buffer

Phosphate buffer of pH 6.0 was prepared by mixing 50 ml of 0.2*M* potassium dihydrogen (volumetric solution) orthophosphate with 5.70 ml of sodium hydroxide 0.2*M* (volumetric solution).

pH 7.0 buffer

Phosphate buffer of pH 7.0 was prepared by mixing 50 ml of 0.2*M* potassium dihydrogen orthophosphate (volumetric solution) with 29.6 ml of sodium hydroxide 0.2*M* (volumetric solution).

Preparation of Mobile Phases

All organic solvents used were HPLC grade. All buffers were filtered through a Millipore filtration apparatus (42µm cut-off). After the mobile phase was prepared by adding the correct ratio of each component to the mixture the solutions were subjected to ultrasonication for 20 minutes, in order to degas them.

Section 5.3: Liquid chromatographic separation of seven Leuckart specific impurities derived from the synthesis of MBDB (8)

Preparation of Analytical Solutions:

All standard impurity solutions 1-7 were prepared by dissolving 0.5 mg aliquots of the relevant compound(s) in 1ml of acetonitrile (ACN) unless otherwise stated.

Reference Solution 1: *N*-formyl BDB (**17**).

Reference Solution 2: 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**).

Reference Solution 3: 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**).

Reference Solution 4: 2,6-diethyl-3,5-di(3,4-metyhylenedioxyphenyl)pyridine (**22**).

Reference Solution 5: 2,6-dimethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine (**23**).

Reference Solution 6: 2-ethyl-5-methyl-3-(3,4-methylenedioxyphenyl)-6-(3,4-methylenedioxybenzyl) pyridine (**25**).

Reference Solution 7: 2,4-diethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine (**26**).

Reference Solution 8: A combined standard solution (ca. 0.5mg/ml) of compounds **17**, **20**, **21**, **22**, **23**, **25** & **26** in acetonitrile.

Chromatographic conditions

The chromatographic conditions used are outlined in Table 5.3.1 below. A waters 5 ODS1column (4.6mm × 250mm) was used in all HPLC analysis.

System	Mobile Phase Constituents	Proportions	Flow Rate	Wavelength Range
1	pH 3.0 Phosphate Buffer : ACN	60:40	1.0 ml/min	190-370 nm
2	pH 3.0 Phosphate Buffer : ACN : THF*	66 : 25 : 13	1.0 ml/min	190-370 nm
3	pH 3.0 Phosphate Buffer : ACN : THF*	66 : 25 : 13	1.5 ml/min	190-370 nm

*Table 5.3.1: Chromatographic conditions used in the analysis of seven Leuckart impurities (17, 20, 21, 22, 23, 25 & 26) derived from the synthesis of MBDB (8). (*Tetrahydrofuran (THF).*

Analysis

The mixture containing all of the standards (reference solution 8) was analysed using the mobile phase conditions shown in Table 5.3.1. Each of the individual peaks was identified using reference solutions 1-7. The retention times, column efficiency, resolution and tailing factor were determined using the equations in appendix I. The results of these experiments are shown in section 6.1.

Section 5.4: HPLC-DAD separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Three experiments were carried out including, section 5.4.1 the development of the liquid chromatographic separation, section 5.4.2 the assessment of the precision of the most suitable system and section 5.4.3 assessment of the selectivity of the most suitable system.

Section 5.4.1 Development of HPLC-DAD separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Preparation of analytical solutions

All standard impurity solutions 9-11 were prepared by dissolving 0.15 mg aliquots of the respective compound in 1ml of acetonitrile unless otherwise stated.

Reference Solution 9: *N*-formyl BDB (**17**).

Reference Solution 10: 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**).

Reference Solution 11: 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**).

Reference Solution 12: A combined standard containing three impurities, *N*-formyl MBDB **(17)**, 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared by dissolving 0.15 mg of each in 1 ml of acetonitrile.

Chromatographic conditions

The various chromatographic systems investigated are outlined below in Table 5.4.1.1. In all cases a Waters 5 ODS1column (4.6 mm × 250 mm) and a wavelength range of 190 nm-370 nm. The results are shown in section 6.2.

System Number	Constituents	Proportions	Flow Rate
1	ACN : H_2O : TE*	50 : 50: 1	1.0 ml/min
2	ACN : H_2O : TE	50 : 50: 1	1.5 ml/min
3	ACN : H_2O : TE	50 : 50: 1	2.0 ml/min
4	ACN : H_2O : Formic Acid	50 : 50: 1	2.0 ml/min
5	MeOH : H_2O : TE	45 : 55 : 1	1.0 ml/min
6	ACN : H_2O : THF	45 : 50 : 5	1.0 ml/min
7	pH 3.0 Phosphate Buffer : ACN	66 : 30	1.0 ml/min
8	pH 3.0 Phosphate Buffer : ACN :THF	66 : 33 : 1	1.0 ml/min
9	pH 3.0 Phosphate Buffer : ACN : THF	66 : 33 : 1	2.0 ml/min
10	pH 3.0 Phosphate Buffer : ACN : THF	66 : 30 : 3	1.5 ml/min
11	pH 3.0 Phosphate Buffer : ACN : THF	66 : 25 : 13	1.5 ml/min
12	pH 3.0 Phosphate Buffer : ACN : THF	66 : 30 : 5	2.0 ml/min

*Table 5.4.1.1: Chromatographic conditions used in the attempted separation of N-formyl BDB (17) and pyrimidine (20, 21) Leuckart impurities derived from the synthesis of MBDB (8). (* Triethylamine (TE))*

Analysis

A composite mixture containing all three standards, *N*-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylene dioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) (reference solution 12) was analysed using the mobile phase conditions shown in Table 5.4.1.1. Each peak was identified using reference solutions 9-11. The results are discussed in section 6.2.

Section 5.4.2: Assessment of precision of HPLC-DAD separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Preparation of analytical solutions

Reference Solution 12: A combined standard containing the three impurities, N-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared by dissolving 0.15mg of each in 1ml of acetonitrile.

Chromatographic conditions

The high pressure liquid chromatographic conditions used in the analysis are outlined in Table 5.4.2.1

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength	Injection Volume
pH 3.0 Phosphate Buffer : ACN : THF	66 : 30 : 5	5 ODS (4.6 mm × 250 mm)	2.0 ml/min	190nm-370nm	20 µl

Table 5.4.2.1: Chromatographic conditions used for the assessment of precision of HPLC-DAD separation of three Leuckart impurities (17, 20 & 2 1) derived from the synthesis of MBDB (8).

Analysis

Five replicate injections, 20µl each, of reference solution 12 were introduced into the chromatographic system outlined above. The results are shown in section 6.2.

Section 5.4.3: Assessment of selectivity of HPLC-DAD separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Preparation of analytical solutions

All standard impurity solutions 13-23 were prepared by dissolving 1.0mg aliquots of the respective compound in 1ml of acetonitrile, unless otherwise stated.

Reference solution 13: MBDB (**8**).

Reference solution 14: BDB (**7**).

Reference solution 15: MDA (**4**).

Reference solution 16: MDMA (**5**).

Reference solution 17: MDEA (**6**).

Reference solution 18: 4-methylthioamphetamine (**9**).

Reference solution 19: Ketamine (**27**).

Reference solution 20: Amphetamine (**3**).

Reference solution 21: Caffeine (**28**).

Reference solution 22: Paracetamol (**29**).

Reference solution 23: Piperonyl Ethyl Ketone (**16**).

Chromatographic conditions

The high pressure liquid chromatographic conditions used in the analysis are outlined in Table 5.4.3.1.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength	Injection Volume
pH 3.0 Phosphate Buffer : ACN : THF	66 : 30 : 5	5 ODS (4.6 mm × 250 mm)	2.0 ml/min	190nm-370nm	20 µl

Table 5.4.3.1: Chromatographic conditions used in the assessment of selectivity of HPLC-DAD separation of three Leuckart impurities (17, 20 & 2 1) derived from the synthesis of MBDB (8).

Analysis

The selectivity of system 12 (section 5.4.1) was tested by a series of 5µl injections of reference solutions 13-23 inclusive, into that particular chromatographic system. The results are shown in section 6.2.

Section 5.5: Construction of UV-Vis spectral library of Leuckart impurities, derived from the synthesis of MBDB (8), and related compounds.

The software that controlled the diode array detector, was entitled Shimadzu CLASS-VP version 5.032. This software allowed for the construction of a customised UV-Vis spectral library. Compounds were entered into the spectral library from chromatographic data files generated during the experiments described in Section 6.3 and 6.4. The following compounds were included in the library; amphetamine (**3**), MDA (**4**), MDMA (**5**), MDEA (**6**), BDB (**7**), MBDB (**8**), 4-MTA (**9**), PEK (**16**), *N*-formyl BDB (**17**), *N*-formyl MBDB (**18**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**), 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**), 2,6-diethyl-3,5-di(3,4-metyhylenedioxyphenyl) pyridine (**22**), 2,6-dimethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine (**23**), 2-ethyl-5-methyl-3-(3,4-methylene

dioxyphenyl)-6-(3,4-methylenedioxybenzyl) pyridine (**25**), 2,4-diethyl-3,5-di(3,4-methylene dioxyphenyl)pyridine (**26**) , ketamine (**27**), caffeine (**28**) and paracetamol (**29**).

Library creation using CLASS-VP is fully explained in the help tool, which is part of the software (Varian 1997). An electronic copy of this library, called LW3.lib, can be found on the supplementary disk at the back of this thesis (see Appendix II). This electronic copy requires Shimadzu CLASS-VP software or some other compatible software in order to function. The results are shown in Section 6.3.

Section 5.6 GC-MS separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Three experiments were carried out including, section 5.6.1 the development of the liquid chromatographic separation, section 5.6.2 the assessment of the precision of the chosen system and section 5.6.3 the assessment of the selectivity of the chosen method.

Section 5.6.1 Development of GC-MS separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Preparation of analytical solutions

All standard impurity solutions (solutions 9-11) were prepared by dissolving 0.15 mg aliquots of the respective compound in 1ml of acetonitrile unless otherwise stated.

Reference Solution 9: N-formyl BDB (**17**).

Reference Solution 10: 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**).

Reference Solution 11: 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**).

Reference Solution 12: A combined standard of the three impurities, N-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared by dissolving 0.15 mg of each impurity in 1ml of acetonitrile.

Chromatographic conditions

The chromatographic conditions are outlined in Table 5.6.1.1

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35 (35%-phenyl-65%-dimethyl siloxane-copolymer)
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1μl
Injection Solvent	ACN

Table 5.6.1.1: Chromatographic conditions used in the development of a GC-MS separation of N-formyl (17) and pyrimidine Leuckart impurities (20 & 21) derived from the synthesis of MBDB (8).

The temperature programme conditions were varied in an attempt to optimise the separation. The various temperature programmes investigated are outlined in Table 5.6.1.2.

System No.	Initial Temperature	Hold Time	Final Temperature	Ramp Rate	Final Hold Time	Total Run Time
1	90°C	1 minute	300°C	15°C/min	10 minutes	25 minutes
2	200°C	25 minutes	200°C	0°C/min	0 minutes	25 minutes
3	250°C	25 minutes	250°C	0°C/min	0 minutes	25 minutes
4	90°C	1 minute	300°C	10°C/min	15 minutes	37 minutes
5	90°C	1minute	200°C	5°C/min	10 minutes	33 minutes
6	90°C	5 minutes	300°C	25°C/min	5 minutes	18.4 minutes
7	90°C	1minute	150°C	10°C/min	10 minutes	17 minutes

Table 5.6.1.2: Various temperature programs used in the attempted development of a separation of N-formyl (17) and pyrimidine Leuckart impurities (20 & 21) derived from the synthesis of MBDB (8).

Analysis

An aliquot (1µl) of the composite mixture of *N*-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) i.e. reference solution 12, was injected into the chromatographic system and separation was attempted using the various temperature programme, outlined in table 5.6.1.2 above. Reference solutions 9, 10 and 11 were used to identify the retention times of compounds **17**, **20** and **21** respectively, for the various temperature program runs attempted. The results of the analysis are shown in section 6.4.

Section 5.6.2 Assessment of precision of GC-MS separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Preparation of analytical solutions

Reference Solution 12: A combined standard of the three impurities, N-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared by dissolving 0.15mg of each in 1ml of acetonitrile.

Chromatographic conditions

The chromatographic conditions are outlined in Table 5.6.2.1.

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1μl
Injection Solvent	ACN
Initial Temperature (Hold Time)	90°C (1minutes)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	300°C (10minutes)
Total Run Time	25 minutes

Table 5.6.2.1: Chromatographic conditions used for the assessment of the precision of GC-MS separation of three Leuckart impurities (17, 20 & 21) derived from the synthesis of MBDB (8).

Analysis

Five replicate injections of reference solution 12 were injected, under the conditions outlined in system 1, in order to assess the precision of the method. The results are shown in section 6.4.

Section 5.6.3 Assessment of selectivity of GC-MS separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Preparation of analytical solutions

All standard impurity solutions 13-23 were prepared by dissolving 1.0mg aliquots of the respective compound in 1ml of acetonitrile unless otherwise stated.

Reference solution 13: MBDB (**8**).

Reference solution 14: BDB (**7**).

Reference solution 15: MDA (**4**).

Reference solution 16: MDMA (**5**).

Reference solution 17: MDEA (**6**).

Reference solution 18: 4-methylthioamphetamine (**9**).

Reference solution 19: Ketamine (**27**).

Reference solution 20: Amphetamine (**3**).

Reference solution 21: Caffeine (**28**).

Reference solution 22: Paracetamol (**29**).

Reference solution 23: Piperonyl Ethyl Ketone (**16**).

Chromatographic conditions

The chromatographic conditions are outlined in Table 5.6.3.1 (overleaf).

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1μl
Injection Solvent	ACN
Initial Temperature (Hold Time)	90°C (1minutes)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	300°C (10minutes)
Total Run Time	25 minutes

Table 5.6.3.1: Chromatographic conditions used for the assessment of the selectivity of the GC-MS separation of three Leuckart impurities (17, 20 & 21) derived from the synthesis of MBDB (8).

Analysis

The selectivity of programme 1 was checked by injecting a series of 1μl aliquots of solutions 13-23 inclusive into the GC-MS system. The results are shown in section 6.4.

Section 5.7: Construction of Mass spectral library of Leuckart impurities, derived from the synthesis of MBDB (8), and related compounds.

The Saturn Workstation version 5.2.1 software that controlled the mass spectral detector allowed for the construction of mass spectral libraries (Varian 1989-1998). Compounds were entered into the custom spectral library from chromatographic data generated in section 5.6.

The compounds that were included in the library were: amphetamine (**3**), MDA (**4**), MDMA (**5**), MDEA (**6**), BDB (**7**), MBDB (**8**), 4-MTA (**9**), PEK (**16**), *N*-formyl BDB (**17**), *N*-formyl MBDB (**18**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**), 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**), 2,6-diethyl-3,5-di(3,4-metyhylenedioxyphenyl) pyridine (**22**), 2,6-dimethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine (**23**), 2-ethyl-5-methyl-3-(3,4-methylene dioxyphenyl)-6-(3,4-methylenedioxybenzyl) pyridine (**25**), 2,4-diethyl-3,5-di(3,4-methylene dioxyphenyl)pyridine (**26**) , ketamine (**27**), caffeine (**28**), paracetamol (**29**).

Library creation using the Saturn Workstation is fully explained in the software help tool (Varian 1989-1998). An electronic copy of this library called LW3.msp is available on the supplementary disk at the back of this thesis (see Appendix II). This electronic copy requires Varian Saturn software or some other compatible software in order to function. The results are shown are shown in section 6.5.

Section 5.8: Solid Phase Extraction of Leuckart impurities from MBDB (8)

The following sections, 5.8.1, 5.8.2, 5.8.3 and 5.8.4, detail the experimental procedures used in order to develop an extraction protocol for the extraction of *N*-formyl BDB (**17**), ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**) from MBDB (**8**).

Preparation of MBDB (8)

A batch MBDB•HCl (**8**) was prepared, by J.J. Keating, in the Department of Pharmaceutical Chemistry (for details of the synthesis, vacuum distillation and steam distillation see appendix III).

Preparation of analytical solutions

Test solutions 24: A 50mg/ml MBDB•HCl solution was prepared in pH 7.0 phosphate buffer.

Reference solution 25: A composite solution, 0.15 mg/ml in each of N-formyl BDB (**17**), ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**), was prepared in a solution which contained 95% water and 5% methanol.

Reference solution 26: A 1 mg/ml MBDB (**8**) solution was prepared by dissolving the appropriate amount of MBDB•HCl in H_2O.

Solid phase extraction cartridges

Lida manufactured the solid phase extraction cartridges used. The two phases investigated were endcapped Octylsilyl (C8) and endcapped Octadecylsilyl (C18) solid phase extraction cartridges. Each had a sorbent bed volume of 100mg and an average particle size of 50 μm. The average pore size was 60Å and the silica surface area was 480 m^2/g.

Section 5.8.1: Development of a suitable system for the elution of interfering components from a matrix containing Leuckart impurities, derived from the synthesis of MBDB (8), from C8 and C18 solid phase extraction cartridges.

Column Conditioning

In the case of each solid phase extraction cartridge (C8 and C18), the cartridges were conditioned with 300 μl of methanol. The supernatant methanol was drawn off under a slight vacuum (circa. 3ml/min). The vacuum was removed in time to avoid drying out the column, the eluate was then discarded. A 300 μl aliquot of H_2O was then added and drawn through the column, taking care not to dry the column bed.

MBDB (8) Elution experiment

Once conditioned a 1 ml aliquot of test solution 24 was then added to the column and drawn through under a slight vacuum. As this volume exceeded the bed volume by a factor of ten the excess was collected in a glass test tube and labelled appropriately. At this point the elution of MBDB (**8**) from the beds of the respective solid phase extraction cartridges could be tested. In the case of each system, 1-4, 2 ml of the relevant solvent (see Table 5.8.1.1) was added, 100 μl at a time. Each 100 μl aliquot was collected in a glass vial and labelled appropriately.

System	C8 solid phase cartridge	C18 solid phase cartridge
System 1	H_2O	H_2O
System 2	pH 4.0 Phosphate Buffer	pH 4.0 Phosphate Buffer
System 3	H_2O : MeOH* (95% : 5%)	H_2O : MeOH (95% : 5%)
System 4	pH 4.0 : ACN (6:1)	pH 4.0 : ACN (6:1)

*Table 5.8.1.1: Table of various solvents investigated as potential MBDB (8) elution solvents from both C8 and C18 solid phase extraction (* Methanol (MeOH)).*

Chromatographic conditions

The chromatographic conditions are outlined for in Table 5.8.1.2.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength	Injection Volume
pH 3.0 Phosphate Buffer : ACN : THF	66 : 25 : 13	5 ODS (4.6 mm × 250 mm)	1.5 ml/min	289 nm	20 μl

Table 5.8.1.2: Chromatographic conditions used in the development of a solid phase extraction protocol for the elution of MBDB (8) from C8 and C18 solid phase extraction cartridges.

Analysis

Solution 25, which contained, *N*-formyl BDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**) was injected (20 μl) into the above system for the purpose of system suitability (retention time, peak tailing and column efficiency). Each

of the 100 μl aliquots collected from the C8 and C18 stationary phases was then subjected to HPLC analysis as outlined above. The peak area of the MBDB (**8**) peak was recorded for each aliquot. The results of this experiment are shown in section 6.6.

Section 5.8.2: Assessment of the retention of impurities when subjected to MBDB (8) elution protocol.

Impurity retention experiment

Using H_2O, the ability of the C8 and C18 cartridges to retain *N*-formyl BDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**), was assessed. In the case of each solid phase extraction cartridge (C8 and C18), the cartridges were conditioned as described above (Section 5.8.1). A 100 μl aliquot of reference solution 25 was then added to each of the two columns (C8 and C18) and drawn through under a slight vacuum. A volume of H_2O (2ml) was then passed through each column in 100 μl aliquots. Each 100 μl aliquot was collected in a glass vial and labelled appropriately.

Chromatographic conditions

The chromatographic conditions are outlined for in Table 5.8.2.1.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength	Injection Volume
pH 3.0 Phosphate Buffer : ACN : THF	66 : 25 : 13	5 ODS (4.6 mm × 250 mm)	1.5 ml/min	289 nm	20 μl

Table 5.8.2.1: Chromatographic conditions used in the development of a solid phase extraction protocol for the elution of MBDB (8) from C8 and C18 solid phase extraction cartridges.

Analysis

Reference solution 25, which contained, *N*-formyl BDB (**17**), ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**)

was injected (20 µl) into the above system for the purpose of system suitability. A solution containing each of the aliquots was subjected to HPLC analysis as outlined above. The results of this experiment are shown in Section 6.6.2.

Section 5.8.3: Development of a suitable system for the elution of Leuckart impurities, derived from the synthesis of MBDB (8), from C8 and C18 solid phase extraction cartridges

Impurity Elution Experiment

The solvents, acetonitrile and tetrahydrofuran, were used in an attempt to elute *N*-formyl BDB (**17**), ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) from the respective solid phase extraction cartridge. Firstly 100 µl of reference solution 25 were added to both C8 and C18 cartridges which had been conditioned with methanol (300 µl) and then H_20 (300 µl), as described above (Section 5.8.1). In the case of each solvent, acetonitrile and tetrahydrofuran, 1 ml of the relevant solvent was added, 100 µl at a time. Each 100 µl aliquot was collected in a glass vial and labelled appropriately. Each of the aliquots was then subjected to HPLC analysis as outlined below. The peak area each of the impurity peaks was recorded for each injected aliquot.

Chromatographic conditions

The chromatographic conditions are outlined in Table 5.8.3.1.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength	Injection Volume
pH 3.0 Phosphate Buffer : ACN : THF	66 : 25 : 13	5 ODS (4.6 mm × 250 mm)	1.5 ml/min	289 nm	20 µl

Table 5.8.3.1: Chromatographic conditions used in the development of a solid phase extraction protocol for the elution of MBDB (8) from C8 and C18 solid phase extraction cartridges.

Analysis

Reference solution 25, which contained, *N*-formyl BDB (**17**), ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**)

was injected (20 µl) into the above system for the purpose of system suitability. A solution containing each of the aliquots was subjected to HPLC analysis as outlined above. The peak area of each of the impurity peaks was recorded for each aliquot. The results of this experiment are shown in section 6.6.

Section 5.8.4: Extraction of Leuckart impurities from a sample of MBDB (8) prepared via the Leuckart reaction.

Extraction protocol

The following protocol was adopted for the extraction of compounds **17**, **20** and **21** from pure MBDB (**8**). Either a 100 mg C8 or 100 mg C18 (both phases were tested separately) solid phase extraction cartridge was conditioned as described above (Section 5.8.1). At this point, 1ml of a test solution 24 containing 50mg/ml MBDB (**8**) in pH 7 buffer was added and pulled through the bed at a rate of 3ml/min. Again the vacuum was removed before drying out the column. An aliquot of 1ml of HPLC grade H_2O was added at this point and pulled slowly through the column (3ml/min). The vacuum was applied at this point to dry the column and remove any traces of H_2O. The retained mass was removed from the column by applying 100µl of tetrahydrofuran to the column and then collecting the eluent in a test tube. This was repeated five times to a final collection volume of 500µl, which was collected in a V-vial. The tetrahydrofuran was then removed under a gentle stream of nitrogen. The residue in the V-vial was reconstituted in 60µl of acetonitrile.

Chromatographic Conditions

The high pressure liquid chromatographic conditions used in the analysis are outlined Table 5.8.4.1.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength
PH 3.0 Phosphate Buffer : ACN	66 : 30 : 5	5 ODS1 (4.6 mm × 250 mm)	2.0 ml/min	289 nm

Table 5.8.4.1: Chromatographic conditions used for the analysis of solid phase extracts of impurities derived from the synthesis of MBDB (8) via the Leuckart reaction.

The GC-MS conditions used in the analysis are outlined in Table 5.8.3.2.

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1μl
Injection Solvent	ACN
Initial Temperature (Hold Time)	90°C (1minutes)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	300°C (10minutes)
Total Run Time	25 minutes

Table 5.8.4.2: Chromatographic conditions used for the analysis of solid phase extracts of impurities derived from the synthesis of MBDB (8) via the leuckart-wallach reaction.

Analysis

Reference solution 25 was injected into both the HPLC-DAD (20μl) and GC-MS (1 μl) system in order to assess the system suitability (retention time, tailing factor and column efficiency). Each of the extracts that were collected from the respective solid phase extraction cartridges was subjected to HPLC-DAD (20 μl) and GC-MS (1 μl) analysis as described above.

Section 5.9 Development of liquid-liquid extraction for the isolation and enrichment of Leuckart-Wallach impurities from MBDB (8)

Preparation of analytical solutions

Reference Solution 12: A composite standard 0.15 mg/ml in each of the three impurities *N*-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared in acetonitrile.

Extraction protocol

The following procedure was carried out using buffers pH 3, 4, 4.9, 6 and 7. A 50 mg quantity of homogeneous powdered MBDB•HCl (**8**) was added to 10 ml of buffer and the pH was adjusted back to the correct pH with either 0.2*M* NaOH or 0.2*M* HCl. The resulting solution was quantitatively transferred to a 20 ml clean dry centrifuge tube and sonicated for 20 minutes. The tubes were then centrifuged at 3000 rpm for 5 minutes. The supernatant was removed and quantitatively transferred to a clean dry separating funnel. The contents of the funnel were then extracted with petroleum ether (10 ml). The aqueous layer was discarded and then the petroleum ether layer was filtered through anhydrous sodium sulphate and evaporated at reduced pressure using a rotary evaporator. The residue in the flask was reconstituted with acetonitrile (600 µl). The acetonitrile was then transferred to a V-vial and the acetonitrile was removed at 40°C, under a stream of nitrogen. The V-vials containing the residue were stored at -18°C until HPLC and GC-MS analysis. Immediately before analysis the residue in each vial was reconstituted in acetonitrile (60 µl).

Chromatographic Conditions

The high pressure liquid chromatographic conditions used in the analysis are outlined Table 5.9.1.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength
pH 3.0 Phosphate Buffer : ACN	66 : 30 : 5	5 ODS (4.6 mm × 250 mm)	2.0 ml/min	289 nm

Table 5.9.1: Chromatographic conditions used for the analysis of solid phase extracts of impurities derived from the synthesis of MBDB (8) via the Leuckart reaction.

The GC-MS conditions used in the analysis are outlined in Table 5.9.2.

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1μl
Injection Solvent	ACN
Initial Temperature (Hold Time)	90°C (1minutes)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	300°C (10minutes)
Total Run Time	25 minutes

Table 5.9.2: Chromatographic conditions used for the analysis of solid phase extracts of impurities derived from the synthesis of MBDB (8) via the leuckart-wallach reaction.

Analysis

In each case reference solution 12 was injected (in duplicate) for the purpose of system suitability (column efficiency, retention time and peak tailing). The samples, each of which had been extracted at different pH values, were then analysed into both GC-MS (1 μl) and HPLC (20 μl) systems. The results can be seen in section 6.7.

Section 5.10: Identification of MBDB (8) in illicitly prepared tablets

Preparation of Analytical Solutions

Reference solution 27: MBDB (**8**) synthesised in the Department of Pharmaceutical Chemistry (Section 5.8) was used to prepare a solution of 1 mg/ml MBDB (**8**) in acetonitrile.

Tablets Analysed

Tablets, #6600, #6840, #A6765, #6660, #6758 and #6758, believed to contain MBDB (**8**) were supplied by Dr. Les King of the Forensic Science Service, Lambeth Road, London, U.K. These tablets were seized in the UK. Tablets 7A and 8A were supplied by the Garda National Drugs Unit and were seized in the Republic of Ireland.The tablets analysed, along with their physical description are shown in Table 5.10.1. Images of the tablets are shown in Figure 5.10.1.

Tablet Ref. No.	Weight of Tablet	Weight Extracted	Logo	Tablet Colour
#6600	0.3358g	144.19mg	'$'	White, mottled
#6840	0.2733g	143.43mg	'£'	White, mottled
#A6765	0.3087g	114.67mg	'$'	White, mottled
#8A	0.3256g	128.08mg	'$'	White, mottled
#6660	0.3364g	102.74mg	'Fido Dido'	Off White, mottled
#7A	0.3159g	117.98mg	'$'	White , mottled
#6758	0.3257g	152.58mg	'£'	White, mottled
#A6765s*	0.3087g	114.67mg	'$'	White, mottled

Table 5.10.1:Tablets qualitatively analysed by GC-MS.

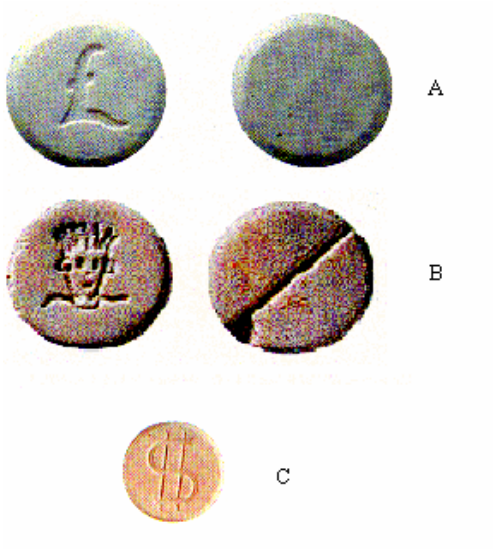


Figure 5.10.I mages of MBDB (8) tablets: (A) Pound logo, (B), Fido Dido logo and (C) Dollar logo.

Preparation of Tablets and standard MBDB (8) for chromatographic analysis

The tablets were powdered using a mortar and pestle. A small amount of powder, circa 1mg, was added to a 10ml test tube. A 1ml volume of water was added and the resulting solution was adjusted to pH 10 with conc. ammonia. This was then extracted into 1ml of petroleum ether. The petroleum ether was evaporated to dryness and reconstituted in a volume of 1ml acetonitrile. A 1 ml aliquot of reference solution 27 was extracted in the same way as 1 mg of powdered tablet.

Chromatographic conditions

The chromatographic conditions are outlined in Table 5.10.2.

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Temperature Program	90°C(1 min) up to 300°C (@ 15°C/min) hold for 10 minutes
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	120°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1 μl
Injection Solvent	ACN

Table 5.10.2: GC-MS conditions used for the qualitative analysis of MBDB (8) in illicitly prepared tablets suspected of containing MBDB (8).

Analysis

Extracted reference solution 28, containing MBDB (**8**), was injected into the chromatographic system.

The tablet extracts were also injected for analysis. The results of the analysis are shown in 6.9.

Section 5.11: Quantitative analysis of MBDB in illicitly manufactured tablets.

Preparation of analytical solutions

Reference solution 27: MBDB•HCl (**8**) synthesised in the Department of Pharmaceutical Chemistry was used to prepare standard solutions. A stock solution of 1 mg/ml MBDB (**8**) was prepared by reconstituting 117 mg of MBDB•HCl in 100 ml of H_2O.

Reference solution 28: A 0.25 mg/ml MBDB (**8**) was prepared by diluting the 1.0 mg/ml standard, solution 27, with sufficient H_2O..

Reference solution 29: A 0.50 mg/ml MBDB (**8**) was prepared by diluting the 1.0 mg/ml standard, solution 27, with sufficient H_2O.

Tablets Analysed

See section 5.10

Preparation of tablets for HPLC analysis

The weight of each tablet, taken for quantitative analyses are shown in Table 5.11.1 (overleaf). The tablets were powdered in a mortar and pestle and weighed out into a separate plastic weighing boats. The contents of the individual weighing boats were quantitatively transferred to separate centrifuge tubes with the aid of 5 ml of acetonitrile. The tubes were then sonicated for twenty minutes before being centrifuged at 3000 rpm for 5 minutes. The supernatant for each tube was then quantitatively transferred to individual 25 ml volumetric flasks and the contents were made up to volume with acetonitrile.

Tablet Reference	Weight of homogenous powder analysed
Dil # 6660	9.8 mg
7A	10.1 mg
Dil # A6765	9.5 mg
Dil #6758	10.8 mg
8A	10.8 mg
Dil # 6840	10.5 mg
Dil # 6600	10.2 mg

Table 5.11.1: Tablets and amounts of tablet quantitatively analysed for MBDB (8) content using HPLC.

Chromatographic conditions

The chromatographic conditions used are outlined in Table 5.11.2.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength
pH 3.0 Phosphate Buffer : ACN : TE	600 : 100 : 1	5 ODS (4.6 mm × 250 mm)	1.5 ml/min	289 nm

Table 5.11.2: Chromatographic conditions for tablets quantitatively analysed for MBDB (8) content using HPLC.

Analysis

A standard curve was prepared by injecting 0.25 mg/ml, 0.50 mg/ml and 0.10 mg/ml MBDB (**8**) standards (reference solutions 29, 28 and 27 respectively. The amount of standard and sample analysed by HPLC was 20µl. Both standards and samples were analysed in duplicate. The results of the analysis can be seen in section 6.10.

Section 5.12: Attempted extraction of Leuckart impurities from MBDB (8) from a spiked sample of an illicitly prepared tablet.

A spiked MBDB (**8**) tablet was extracted (section 5.12.1). The extract was analysed using HPLC-DAD (section 5.12.2) and GC-MS (5.12.3) analysis.

Section 5.12.1 Extraction protocol

Preparation of spiked tablet

A 1 mg amount of each compound **17**, **20** and **21** was added to a 50 mg equivalent of powdered tablet #6758 (see Table 5.10.1, Section 5.10) and the resulting powder was homogenised.

Extraction protocol

An aliquot (10 mg) of homogeneous powdered tablet was weighed accurately. The amount of tablet taken in the case of each tablet is shown in Table 2.10.1. The powder was added to 10 ml of pH 4.0 buffer. The pH was adjusted to pH 4.0 if necessary with 0.2 *M* sodium hydroxide or 0.2 *M* hydrochloric acid. The resulting solution was quantitatively transferred to a 20ml clean dry centrifuge tube and sonicated for 20 after which it was centrifuged at 3000 rpm for 5 minutes. The supernatant was removed and quantitatively transferred to a clean dry 100 ml separating funnel. The contents were then extracted with 10 ml of petroleum ether. The aqueous layer was discarded and evaporated at reduced pressure using a rotary evaporator. The residue in the flask was reconstituted with 600µl acetonitrile. The acetonitrile was then transferred to a V-vial and the acetonitrile was removed at 40°C under a stream of nitrogen. The V-vials containing the residue were stored at -18°C until analysis. Immediately before analysis the residue in each vial was reconstituted in acetonitrile (50µl).

Preparation of impurity recovery solution

As well as having prepared the tablet extract, a tablet solution was prepared which would be used to determine recovery. A 1 mg amount of each compound **17**, **20** and **21** was added to 6 ml of acetonitrile.

Section 5.12.2 HPLC-DAD analysis of spiked tablet extract and impurity recovery solution.

Preparation of analytical solutions

Reference Solution 12: A combined standard of the three impurities, *N*-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared by dissolving 0.15 mg of each in 1 ml of acetonitrile.

Chromatographic conditions

The high pressure liquid chromatographic conditions used in the analysis are outlined Table 5.12.2.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength
pH 3.0 Phosphate Buffer : ACN	66 : 30	5 ODS (4.6 mm × 250 mm)	2.0 ml/min	289 nm

Table 5.12.2.1: HPLC conditions used for the analysis of MBDB (8) tablet extracts.

Analysis

An aliquot (20 μl) of reference solution 12 were injected into the HPLC-DAD, for the purpose of system suitability (retention time, peak tailing, column efficiency). The spiked tablet extract (20μI) (see section 5.12.1) was then analysed by HPLC-DAD. An aliquot (20μI) of the impurity recovery solution was analysed in the same way (see section 5.12.1). The results of this section can be seen in section 6.11.

Section 5.12.3: GC-MS analysis of spiked tablet extract and impurity recovery solution.

Preparation of analytical solutions

Reference Solution 12: A combined standard of the three impurities, *N*-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared by dissolving 0.15mg of each in 1ml of acetonitrile.

Chromatographic conditions

The GC-MS conditions used in the analysis are outlined in Table 5.12.3.1

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Temperature Program	90°C(1 min) up to 300°C (@ 15°C/min) hold for 10 minutes
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	210°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1 μl
Injection Solvent	ACN

Table 5.12.3.1: GC-MS conditions used for the analysis of MBDB (8) tablets.

Analysis

An aliquot (1 μl) of reference solution 12 were injected into the GC-MS, for the purpose of system suitability (retention time, peak tailing and column efficiency. The spiked tablet extract (see section 2.10.1) was then analysed by GC-MS. An aliquot (1μl) of the impurity recovery solution was analysed in the same way. The results of this section can be seen in section 6.11.

Section 5.13: Attempted extraction of Leuckart impurities from MBDB (8) from illicitly manufactured MBDB (8) tablets of an illicitly prepared tablet.

Section 5.13.1: Extraction protocol

Tablets analysed

The tablets analysed and the respective amounts taken in order that 50 mg of MBDB (**8**) was taken are shown in Table 5.13.1.1.

Tablet Name	Weight equivalent to 50mg MBDB (**8**)	Tablet Name	Weight equivalent to 50mg MBDB (**8**)
Dil# 6660	102.74mg	8A	129.05mg
7A	103.98mg	Dil# 6840	143.43mg
Dil#6765	114.45mg	Dil# 6600	116.44mg
Dil# 6758	218.07mg		

Table 5.13.1.1: Table containing weights of tablet required to be taken such that 50mg of MBDB (8) could be extracted

Extraction protocol

An aliquot of homogeneous powdered tablet was weighed accurately in the case of each tablet. The amount of tablet taken in the case of each tablet is shown in Table 5.13.1.1. To the powder was added to 10 ml of pH 4.0 buffer. The pH was adjusted to pH 4.0 if necessary with 0.2 *M* sodium hydroxide or 0.2 *M* hydrochloric acid. The resulting solution was quantitatively transferred to a 20ml clean dry centrifuge tube and sonicated for 20 after which it was centrifuged at 3000 rpm for 5 minutes. The supernatant was removed and quantitatively transferred to a clean dry 100 ml separating funnel. The contents were then extracted with 10 ml of petroleum ether. The aqueous layer was discarded and evaporated at reduced pressure using a rotary evaporator. The residue in the flask was reconstituted with 600 µl acetonitrile. The acetonitrile was then transferred to a V-vial and the acetonitrile was removed at 40°C under a stream of nitrogen. The V-vials containing the residue were stored at -18°C until analysis. Immediately before analysis the residue in each vial was reconstituted in 60 µl.

Section 5.13.2: HPLC-DAD analysis of tablet extracts

Preparation of analytical solutions

Reference Solution 12: A combined standard of the three impurities, *N*-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared by dissolving 0.15 mg of each in 1 ml of acetonitrile.

Chromatographic conditions

The high pressure liquid chromatographic conditions used in the analysis are outlined Table 5.13.2.1.

Constituents	Proportions	Column	Flow Rate	Analytical Wavelength
pH 3.0 Phosphate Buffer : ACN	66 : 30	5 ODS (4.6 mm × 250 mm)	2.0 ml/min	289 nm

Table 5.13.2.1: HPLC conditions used for the analysis of MBDB (8) tablet extracts.

Analysis

An aliquot (20 μl) of reference solution 12 were injected into the HPLC-DAD, for the purpose of system suitability (retention time, peak tailing, column efficiency). The tablet extracts (see Section 5.13.1.1) was then analysed by HPLC-DAD. The results of this section can be seen in section 6.12.

Section 5.13.3: GC-MS analysis of tablet extracts

Preparation of analytical solutions

Reference Solution 12: A combined standard of the three impurities, *N*-formyl MBDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl) pyrimidine (**21**) was prepared by dissolving 0.15mg of each in 1ml of acetonitrile.

Chromatographic conditions

The GC-MS conditions used in the analysis are outlined in Table 5.13.3.1

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	DB-35
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.1 μm
Temperature Program	90°C(1 min) up to 300°C (@ 15°C/min) hold for 10 minutes
Ion Trap Temperature	210°C
Transfer Line Temperature	250°C
Manifold Temperature	210°C
Ionisation Mode	Electron Ionisation
Filament Delay	4 minutes
Injected Volume	1 μl
Injection Solvent	ACN

Table 5.13.3.1: GC-MS conditions used for the analysis of MBDB (8) tablets.

Analysis

An aliquot (1 μl) of reference solution 12 were injected into the GC-MS, for the purpose of system suitability (retention time, peak tailing and column efficiency). The tablet extracts (see section 5.13.1) were then analysed by GC-MS. The results of this section can be seen in section 6.12.

Section 5.14: Derivatisation of *N*-formyl BDB (17) in an attempt to identify peak eluting at 10.2 minutes in GC-MS trace of samples.

Preparation of analytical solutions

Reference solution 30: A 0.10 mg/ml solution of *N*-formyl BDB (**17**) was prepared in acetonitrile.

Derivatising reagent

Methelut (Sigma –Aldrich Chemicals) was used as the methylating agent.

Derivatising procedure, analysis and chromatographic conditions.

An aliquot of 50 µl of Methelut reagent was added to a small vial. An aliquot of 200 µl of reference solution 30 was added to this vial. This solution was mixed and injected into the GC-MS system outlined in Table 5.13.3.1. The remainder of the solution was heated at 80°C for one hour, after which time a 20 µl aliquot was injected into the HPLC system outlined in Table 5.13.2. The results are discussed in section 6.13.

Section 4.1: Impurity profiling

Due to the fact that a large question mark hangs over the therapeutic potential of the ‘’Entactogens’’, the recreational drug-abuser has not been able to rely on illegally diverted pharmaceutically pure forms of these drugs, as may have been the case to an extent with amphetamine and methamphetamine. Instead, he/she, having chosen to use these drugs, will be forced to purchase drugs that have been made exclusively in clandestine laboratories.

Impurity profiling is a technique that has for many years, been used as a means of gathering drug intelligence to help law enforcement agencies combat clandestine drug manufacture. Illegally produced drugs are frequently contaminated as is readily shown via chromatographic analysis (Sinnema, 1981). Contaminants in illicitly manufactured drugs can arise from impure starting materials, side and subsequent reactions, intermediate products and diluents used as cutting agents in the subsequent handling of a drug (Sinnema, 1981). Additives to illicitly prepared tablets may also contain agents responsible for bulking, binding, lubricating and colouring used during tablet manufacture.

Two different approaches can be adopted when impurity profiling. There is on the one hand, what is known as the ‘’signature method’’ where the chromatographic trace is used as a fingerprint. Usually the identity of each peak in the chromatographic trace is not known, but a comparison of two traces collected from the same batch should in theory match and the likelihood of getting an identical chromatographic trace for a different batch should be low. This method has been used with good effect in the profiling of amphetamines (Rashed, 2000; Sten,1998). It has also been used in determining the origin of and/or interrelationship of opiate samples (Neumann, 1984; Desage, 1991; Kaa, 1994 and

Johnston, 1998) and also of cannabis samples (Lehmann, 1995 and de Meijer, 1992). The main drawback of this approach is that two separate samples from the same batch stored separately will likely be affected by many variables e.g. storage conditions, temperature, humidity, exposure to light etc. All of these variables will have a detrimental effect on the integrity of the fingerprint.

The alternative approach takes the fingerprint method a step further by incorporating retention time data for known or likely impurities, data that has been generated by running standards of impurities.

It would be even more desirable if mass spectrometric data collected via the Gas Chromatography-Mass Spectrometer (GC-MS) was available, as this would enable the unequivocal identification of specific impurities. The purity of a particular drug provides information on several levels (King, 1997). The important aspect from an impurity profiling perspective is that certain impurities will only be generated when a compound is made via a particular synthetic route. Hence, they are classed as route specific impurities. The presence of a route specific impurity in a sample will indicate the synthetic route used by the underground chemist and therefore the reagents required. A number of samples, containing these impurities, with very similar impurity profiles may be indicative of a link between

these samples. No information on route specific impurities is gathered by using the fingerprint method.

Details of the synthetic routes employed by the underground chemist are easily obtained through the Internet, underground literature and indeed the literature of the legitimate scientific community (Shulgin, 1991; Shulgin, 1997; Uncle Fester, 1996; Erowid, 2000 and SPDCC, 2000). In general, the single step techniques, which require little knowledge of chemistry, are the techniques most likely to be used in clandestine laboratory environments. An experienced chemist may be able to devise novel syntheses or employ more difficult synthetic routes. However, the easy availability of established techniques should ensure their continued dominance in clandestine laboratory applications. In particular, the variety of reactions successfully used to synthesise amphetamines, its analogues, homologues and derivatives has provided a fertile area for investigation by the clandestine chemist (Dal Cason, 1990). A variety of synthetic reactions can be used to synthesise amphetamines, including reactions such as reductive amination (Haskelberg, 1948), the Leuckart reaction (Moore, 1949), the Ritter reaction (Ritter, 1948), the oxime route (Hey, 1930), the bromopropane route (Anon, 1914) and the nitropropene (Anon., 1953) route. Reductive amination, the bromopropane route and the leuckart reaction can also be used to synthesise methamphetamine. A very significant amount of research has been invested into the impurity profiling of amphetamines and methamphetamine (Van der Ark, 1977; Lambrechts, 1985; Johnson, 1998; Lambrechts, 1984; Lambrects, 1984a; Sinnema, 1981; Van der Ark, 1978; Verweij, 1989; and Barron, 1974). Impurities, route specific and otherwise, have been identified for the synthesis of amphetamine (**3**) and methamphetamine.

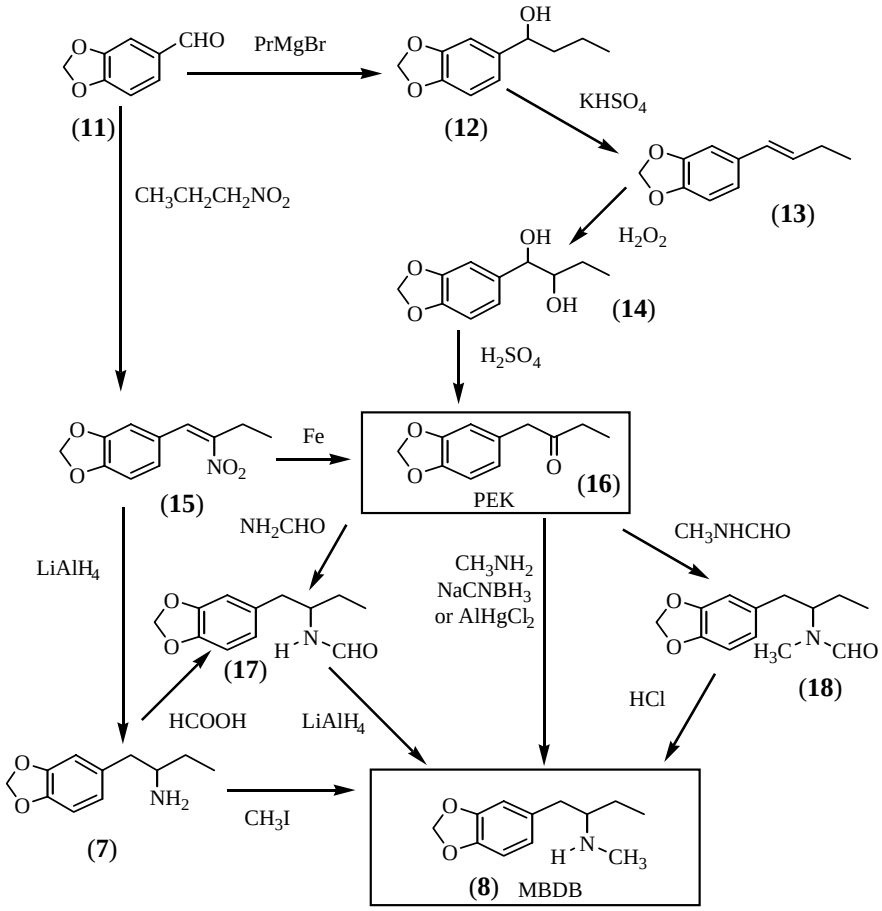
Section 4.2: Impurity profiling MBDB (8) synthesised via the Leuckart-Wallach reaction

In the last chapter a method suitable for the analysis of MDA (**4**), MDMA (**5**), MDEA (**6**) and MBDB (**8**) was established. It is noteworthy that on perusal of the GC-MS chromatogram of the MDMA (**5**) containing extract of a Mitsubishi tablets (Figure 3.6.1a) very small peaks are visible in the trace. There is no doubt that the MDMA (**5**) is of a reasonably high purity, however trace levels of some compounds other than MDMA (**5**) itself are certainly present. Some of these may be impurities or

adulterants, however if they are impurities they may give some clue as to which route of synthesis was used to make the drug, this is investigated further in Chapter III. Due to the fact that the MDA (**4**), MDMA (**5**), are simply amphetamine (**3**) and methamphetamine with a methylenedioxy bridge, the same reactions mentioned above (Ritter, Leuckart etc.) can be used to synthesise the methylenedioxy compounds. The previous work carried out on the impurity profiling of amphetamine (3) and methamphetamine is of great help to the analyst profiling MDA (**4**) and MDMA (**5**). Indeed a significant amount of impurity profiling has been carried out on MDA (**4**) and MDMA (**5**) synthesised via the aforementioned routes. Routes towards the synthesis of MDA (**4**) and MDMA (**5**) which have been impurity profiled include the Leuckart route (Lukaszewski, 1978; Verweij, 1992c; Verweij, 1992a; Renton, 1993 and Bohn, 1993), the nitropropene route (Verweij, 1992a and Verweij, 1992b), the reductive amination route (Verweij, 1990; Verweij, 1992a; Noggle, 1991a; Noggle, 1991b and Clark, 1994) and the bromopropane route (Renton, 1993; Clark, 1994; Noggle, 1991a and Verweij, 1992a). While MBDB (**8**) & MDEA (**6**) have not been profiled to the same extent, the routes of synthesis used for each are in some cases very similar to those used for MDA (**4**) and MDMA (**5**). Hence the impurities are assumed, in some cases, to be analogous to those found in MDA (**4**) and MDMA (**5**). MBDB (**8**) and MDEA (**6**) may however also include impurities, which may differ significantly to those in MDA (**4**) and MDMA (**5**).

The properties and possible risks associated with MBDB (**8**) were recently extensively reviewed as part of a European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) risk assessment (Van Aerts, 2000). Although work has been carried out on the metabolism and immunoassay responses for MBDB (Maurer, 1996; Kintz, 1997 and Kintz, 1997a) and also on the GC-MS analysis of the MBDB (**8**) and its analogues/structural isomers (Clark, 1995 and Noggle, 1991) no impurity profiling has been carried out.

MBDB (**8**) can be prepared via several routes (Scheme 4.1 below). The most popular route of synthesis for amphetamine (**3**) and methamphetamine is undoubtedly the Leuckart reaction, which is a convenient reaction used for the synthesis of amines from ketones and aldehydes (Lukaszewski, 1978). The starting material needed for the synthesis of MBDB (**8**) via the Leuckart reaction is Piperonyl Ethyl ketone (PEK) (**16**).

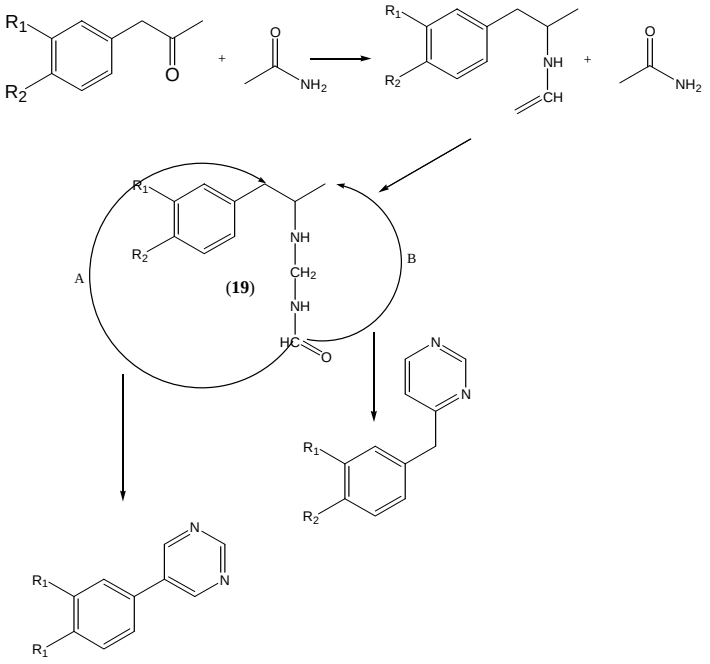


Scheme 4.1: Routes of synthesis for MBDB (leuckart-wallach route is marked with red arrows). PEK (piperonylethylketone), (16) N-formyl MBDB (17) & MBDB (8) are starting material, intermediate and product respectively for the synthesis of MBDB via the leuckart-wallach route of synthesis.

Two routes often used that are often used to synthesise PEK (**16**) are the nitrostyrene route and the glycol route. The nitrostyrene route involves the reaction of piperonal (**11**) and nitropropane to form the nitrostyrene intermediate, 3,4-methylenedioxyphenyl-3-nitro-but-3-ene (**15**), which can be reacted with Fe to form PEK (**16**). Alternatively piperonal (**16**) can be converted to 3,4-methylenedioxybutan-4-ol (**12**) via the Grignard reaction. The tertiary alcohol 3,4-methylenedioxybutan-4-ol (**12**) is then dehydrated to 3,4-methylenedioxyphenylbut-3-ene (**13**) with $KHSO_4$. This methylenedioxyalkene is then oxidised using H_2O_2 to form the 3,4-methylenedioxyphenylbutan-3,4-diol (**14**). This glycol can then be reduced using H_2SO_4 to form PEK (**16**).

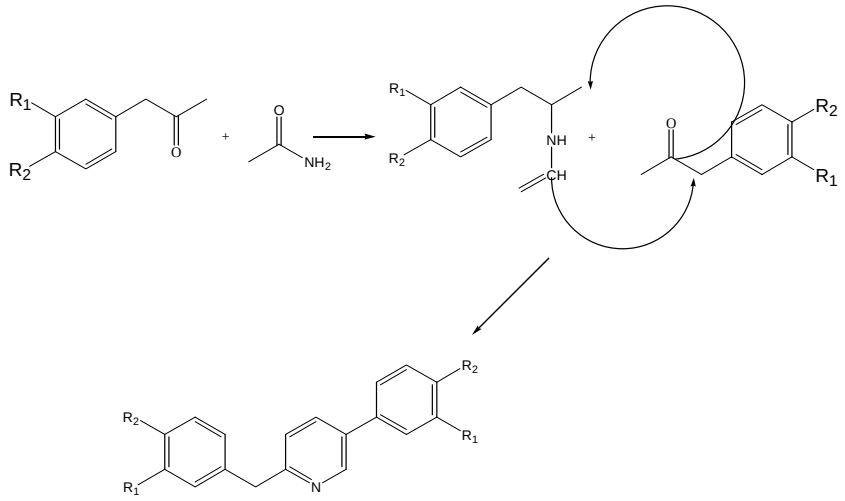
Using PEK (**16**) as the starting material, this reaction involves reacting PEK (**16**) with two molecules of formamide to produce the *N*-formyl derivative (**17**) of an amine, which can then be hydrolysed with HCl to produce BDB (**7**) or methylated with $LiAlH_4$ to form MBDB (**8**). MBDB (**8**) can also be synthesised by taking BDB (**7**) and treating it with formic acid to form the *N*-formyl derivative and treating then with $LiAlH_4$ as before. Alternatively *N*-methyl formamide can be used to form the *N*-methyl, *N*-formyl derivative (**18**), this can then be reduced using HCl to form MBDB (**8**). (see Scheme 4.1, above).

The Leuckart reaction is notorious for producing impurities (Lambrechts, 1984). Among the most common of these impurities in the case of MDA (**4**) and MDMA (**5**), are the *N*-formyl intermediates (**17**) and (**18**) that are usually found in samples as a result of incomplete hydrolysis of the ketone (Lucaszewski, 1978). Other impurities that can arise from the reaction of the ketone (**16**) formamide are usually the result of condensation reactions between the ketone and molecules of formamide (Van der Ark, 1977). A second molecule of formamide can condense to the *N*-formyl-1-methylendioxyphenyl-2-iminopropane (**19**) followed by ring closure (see Scheme 4.2) forming a pyrimidine.

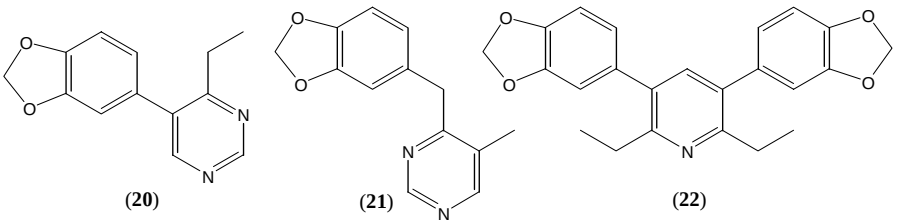


Scheme 4.2 : Proposed condensation of two molecules of formamide and one ketone resulting in the formation of a benzyl pyrimidine (B) and a phenyl pyrimidine (A).

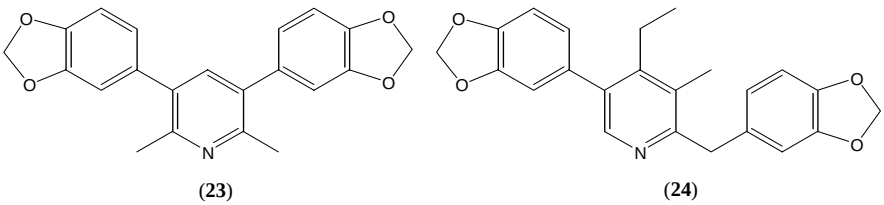
There is also the possibility of two molecules of ketone condensing with one molecule of formamide forming pyridine impurities (see Scheme 4.3).



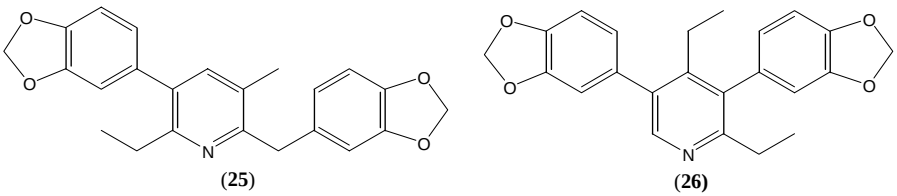
Scheme 4.3 : Proposed condensation of one molecule of formamide and two ketones resulting in the formation of a pyridine.



(**20**) (**21**) (**22**)



(**23**) (**24**)



(**25**) (**26**)

Figure 4.4:Impurities obtained through the Leuckart synthesis of MBDB: ***(20)*** *4-ethyl-5-(3,4-methyelendioxyphenyl)pyrimidine,* ***(21)*** *5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine,* ***(22)*** *2,6-diethyl-3,5-di(3,4-methyelendioxyphenyl)pyridine,* ***(23)*** *2,6-dimethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine,* ***(24)*** *4-ethyl-3-methyl-2-(3,4-methylenedioxybenzyl)-5-(3,4-methylenedioxyphenyl)pyridine,* ***(25)*** *2-ethyl-5-methyl-3-(3,4-methylenedioxyphenyl)-6-(3,4-methylenedioxybenzyl)pyridine &* ***(26)*** *2,4-diethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine*

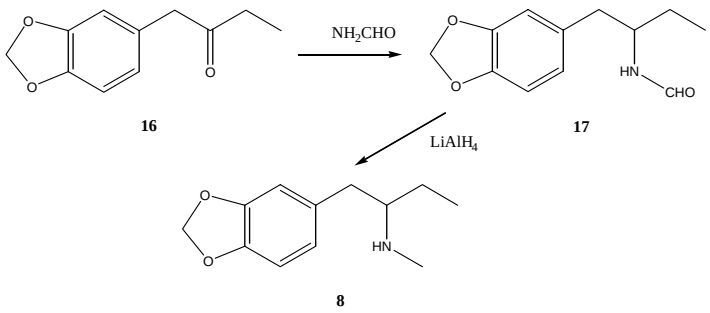
The synthesis of MBDB (**8**) via the Leuckart route was investigated in the Department of Pharmaceutical Chemistry in the School of Pharmacy, Trinity College, Dublin and a number route specific impurities (see Figure 4.2, above) were isolated, purified and then characterised using NMR and MS. It is noteworthy that when MBDB (**8**) is synthesised using PEK (**16**) and *N*-methyl formamide to produce the *N*-methyl, *N*-formyl derivative (**18**) that no pyrimidine or pyridine impurities are produced.

Section 4.3: Objectives

The aim of the work presented here was to establish a selective and specific HPLC separation using a Diode Array Detector (DAD) and a similar GC-MS separation for the analysis of a series of Leuckart route specific impurities/intermediates **17**, **20** and **21**. Using that data generated from the HPLC-DAD and GC-MS spectral libraries for the relevant compounds would be constructed. By developing a suitable solid phase extraction protocol or liquid-liquid extraction, it was hoped that the Leuckart specific impurities/intermediates could be extracted from MBDB•HCl that had been prepared via the Leuckart route in the School of Pharmacy. The best extraction method would be selected on the basis of chromatographic data that would be obtained using the HPLC-DAD and GC-MS methods established. The most suitable extraction method would then be applied to the extraction of illicitly prepared tablets known to contain MBDB (**8**) on the basis of GC-MS and HPLC-DAD analysis. The extracts would be subjected to GC-MS and HPLC-DAD analysis in order to determine whether or not the tablets had been made via the Leuckart reaction investigated.

Section 6.1: Liquid chromatographic separation of seven Leuckart impurities derived from the synthesis of MBDB (8)

As discussed in the introduction the drug, MBDB (8**), referred to as ecstasy, can be synthesised via several routes. One of the routes that can be used is the Leuckart route (see Scheme 6.1.1, below). This route involves the reaction between PEK (**16**) via the *N*-formyl BDB (**17**) intermediate generated from the reaction between one molecule of PEK (**16**) and one molecule of formamide (Moore, 1949) via a *N*-formyl BDB (**17**) intermediate. This reaction also has the potential to generate a number of pyrimidine impurities by the reaction of a second molecule of formamide via an *N*-formyl-1-methylenedioxyphenyl-2-iminopropane intermediate (Van der Ark, 1977). There is also the possibility of two molecules of ketone condensing with one molecule of formamide, forming pyridine impurities.**



16 **17** **8**

Scheme 6.1.1: The synthesis of MBDB (8) via the Leuckart reaction.

A number of these impurities have been isolated from the reaction mixture in the Department of pharmaceutical chemistry in school of pharmacy, Trinity College Dublin, and were subsequently characterised using NMR and MS (Keating, 2001) (see Figure 6.1.1).

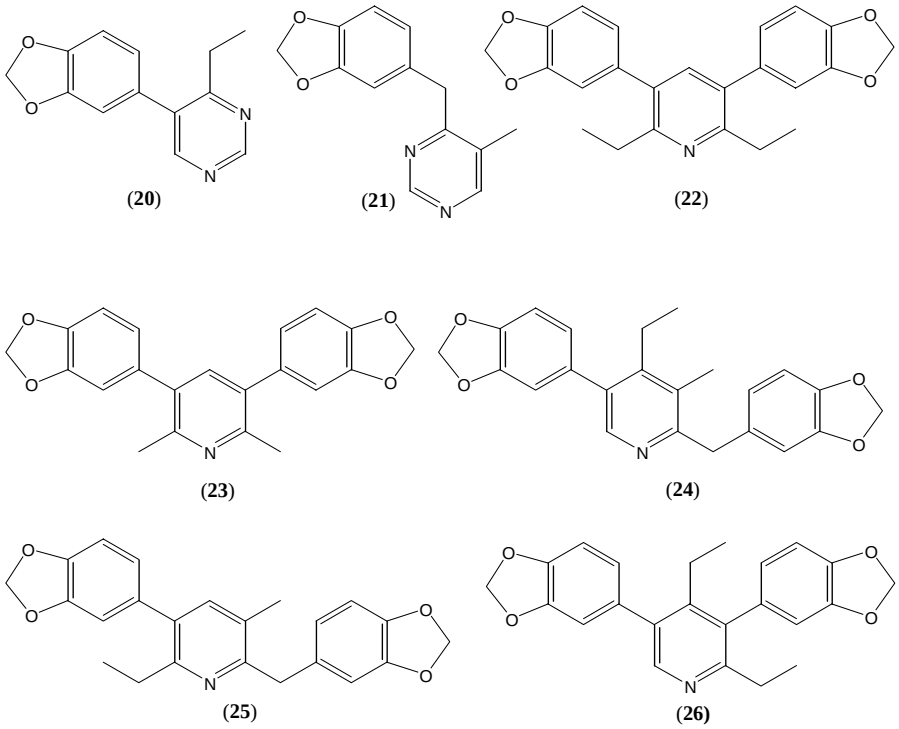


Figure 6.1.1:Impurities obtained through the Leuckart synthesis of MBDB: *(20)* ***4-ethyl-5-(3,4-methyelendioxyphenyl)pyrimidine,*** *(21)* ***5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine,*** *(22)* ***2,6-diethyl-3,5-di(3,4-methyelendioxyphenyl)pyridine,*** *(23)* ***2,6-dimethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine,*** *(24)* ***4-ethyl-3-methyl-2-(3,4-methylenedioxybenzyl)-5-(3,4-methylenedioxyphenyl)pyridine,*** *(25)* ***2-ethyl-5-methyl-3-(3,4-methylenedioxyphenyl)-6-(3,4-methylenedioxybenzyl)pyridine &*** *(26)* ***2,4-diethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine***

The *N*-formyl BDB (17) and any of the pyridine or pyrimidine impurities found in an illicitly prepared tablet would indicate that the tablet was made via the Leuckart reaction as these impurities are route specific. This information would useful in combating clandestine drug manufacture at the law enforcement level.

In order to detect these compounds in an illicitly prepared tablet a suitable means of detecting them is required. The main techniques used for doing this are HPLC and GC-MS. The following section details the result of an attempt to develop a HPLC method to be used to detect one intermediate, *N*-formyl BDB (17**), 2 pyrimidines** 20**,** 21 **and 3 pyridine impurities,** 22**,** 23**,** 25 **and** 26**. Compound** 24**, 4-ethyl-3-methyl-(3,4-methylenedioxybenzyl)-5-(3,4-methylenedioxyphenyl)pyrimidine was excluded as it was isolated at the trace level from the reaction.**

Of the mobile phase systems investigated (see Section 5.3) system 3 was deemed to be the best as it gave the most desirable chromatography in the shortest runtime System 3 consisted of pH 3.0 phosphate buffer: ACN:THF (66:25:13) at a flow rate of 1.5ml/min . A chromatographic trace recorded for the system outlined in section 5.3 involving the

separation of seven leuckart-wallach impurities (17**,** 20**,** 21**,**

22**,** 23**,** 24 **and** 26**) is shown below (Figure 6.1.2).**

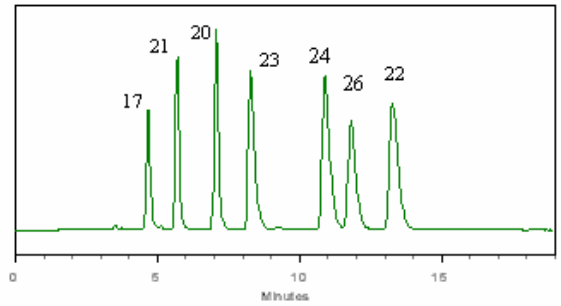


Figure 6.1.2.HPLC trace (289nm) recorded for the separation of seven leuckart-wallach impurities, 17, 20, 21, 22, 23, 25 and 26 derived from the synthesis of MBDB (8) via the leuckart-wallach reaction. For chromatographic conditions see section 5.3.

Chromatographic parameters such as capacity factors, resolution, peak tailing, column efficiency and wavelength maxima were calculated using the CLASS-VP software and are tabulated below (Table 6.1.1). For details of the formulae used see appendix I.

Peak	Peak Identity	t_R (mins)	Width (mins)	Capacity Factor	Resolution	Asymmetry	Theoretical Plates	λ Max >190/ >260
1	17	4.7	1.0	3.7	0.00	1.04	6059	206/285
2	21	5.7	1.23	4.7	4.26	1.2	9850	207/287
3	20	7.1	1.1	6.1	5.51	1.25	11181	205/293
4	23	8.3	1.0	7.27	3.65	1.93	7096	206/284
5	24	10.9	1.03	9.9	6.09	1.66	8457	206/281
6	26	11.8	1.33	10.8	1.85	1.59	8815	205/263
7	22	13.3	1.87	12.27	2.76	1.63	8878	205/271

Table 6.1.1. Table of chromatographic parameters calculated from the chromatographic trace recorded for the separation of seven leuckart-wallach impurities, 17, 20, 21, 22, 23, 25 and 26 derived from the synthesis of MBDB (8) via the leuckart-wallach reaction

The peaks in the mixture, reference solution 8, were identified by comparison with the retention times of the peaks in reference solutions 1-7 which contained compounds **17**, **20**, **21**, **22**, **23**, **25** and **26** respectively. The first peak to elute from the mixture was the most polar compound *N*-formyl BDB (**17**) at circa 4.7minutes. This was followed then by the less polar pyrimidines, 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**) and 4-ethyl-5-(3,4-methyloenedioxy) pyrimidine (**20**) at 5.7minutes and 7.1 minutes respectively. The most polar impurities, the pyridines, 2,6-diethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine (**22**), 2,6-dimethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine (**23**), 2-ethyl-5-ethyl-3-(3,4-methylenedioxybenzyl)pyridine (**25**), and 2,4-diethyl-3,5-di(3,4-methylenedioxyphenyl)pyridine (**26**) eluted at 8.3 minutes, 10.9 minutes, 11.8 minutes and 13.3 minutes respectively. Ideally, in a liquid chromatographic separation one strives for a capacity factor value between 2-10, however values between 1-20 are acceptable (Dolan, 2000). The capacity factors for the seven compounds were between 3.7 and 12.27, which is good. Baseline resolution was achieved in all cases, the lowest resolution value estimated was 1.85, between the sixth (**26**) and the seventh (**22**) peaks. The peak symmetry was found to be within acceptable limits for the first three peaks, however it deteriorated for the latter four peaks 1.93 (**23**), 1.66 (**25**), 1.59 (**26**) and 1.63 (**22**). Ideally, one would accept values of less than 1.2 (Snyder, 1997).

On the basis that the *N*-formyl BDB (**17**) and the pyrimidines (**20** & **21**) were generated in much higher quantities than the pyridines and it was probable that they would be most likely to appear in illicit samples it was decided that the development of an impurity profiling procedure would focus on the route-specific *N*-formyl BDB intermediate (**17**) and the two route specific pyrimidines (**20** & **21**). The pyridines would be excluded, hence their poor peak shape was not a concern. The diode array detector allowed for the estimation of the wavelength which gave the highest absorbance. Although the actual wavelength maxima for the compounds differed slightly, it was felt that 289nm was a suitable wavelength for detecting the *N*-formyl BDB (**17**) and the pyrimidines (**20** & **21**). Calculating the average wavelength for each of these three compounds provided this value.

Section 6.2 HPLC-DAD separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Section 6.2.1 Development of HPLC-DAD separation of three Leuckart impurities derived from the synthesis of MBDB (8)

The twelve HPLC systems described in section 5.4.1 were investigated to determine whether they were suitable for the separation of the *N*-formyl BDB (**17**) and the two route-specific pyrimidines (**20** & **21**) (See Table 5.4.1.1). Various combinations of mobile phases were investigated (see Section 5.4.1) and based on criteria such as run time, peak tailing and resolution, it was decided that system 12 was the most suitable for the separation of the compounds. The other systems, (1-11) were rejected on visual inspection of the chromatograms as they gave poor peak shape or they did not adequately separate the compounds of interest. System 12 involved a pH 3.0 phosphate buffer, acetonitrile and tetrahydrofuran (66:30:5). A typical chromatogram of test solution 8 (containing compounds **17**, **20** and **21)** is shown below (Figure 6.2.1.1). The identity, retention time, width, capacity factor, resolution, peak tailing and column efficiency for each of the peaks was estimated (see Table 6.2.1.1). The formulae used to calculate these parameters are included in appendix I.

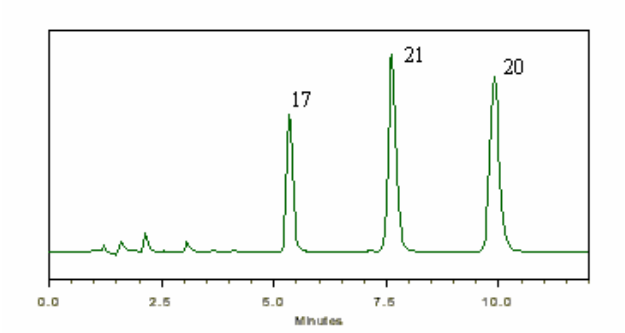


Figure 6.2.1.1 HPLC trace (289nm) recorded for the separation of three leuckart-wallach impurities, 17, 21 and 20 derived from the synthesis of MBDB (8) via the leuckart-wallach reaction, using system 12.

The wavelength maximum for each of the compounds 17**,** 20 **and** 21 **were 285nm, 287nm and 293nm. The most suitable**

wavelength for analysis of all three compounds was found 289nm. Calculating the average wavelength for all three arrived at this value.

Peak	Peak Identity	t_R (mins)	Width (mins)	Capacity Factor	Resolution	Peak Tailing	Theoretical Plates	λ Max >190/ >260
1	17	5.3	0.63	4.33	0.00	1.20	7781.34	204/285
2	21	7.6	0.93	6.63	4.85	1.14	9288.54	203/287
3	20	9..9	0.97	8.90	6.48	1.18	10616.11	203/293

Table 6.2.1.1 Table of chromatographic parameters calculated from the HPLC trace recorded for the separation of three Leuckart impurities, 17, 21 and 20 derived from the synthesis of MBDB (8) (for chromatographic conditions see section 5.4.1).

The order of elution was, as observed for the chromatographic system described in section 6.1. The *N*-formyl BDB (**17**) and the less polar pyrmidines, 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**) and 4-ethyl-5-(3,4-methyloenedioxy) pyrimidine (**20**) eluted at 5.3 minutes, 7.6 minutes and 9.9 minutes respectively. The three peaks were found to be symmetrical with values of 1.2, 1.14 and 1.18 calculated for the peaks of compounds **17**, **20** and **21** respectively. The capacity factors were also within the desired range for the standards, 2-10 (Dolan, 2000), for all three peaks.

Section 6.2.2: Assessment of precision of HPLC-DAD separation of three Leuckart impurities derived from the synthesis of MBDB (8)

The precision of system twelve was assessed by analysing six replicate injection of reference solution 12. Chromatographic parameters such as retention time and peak area were estimated using the formulae in appendix I. The relative standard deviations, R.S.D. (Appendix I) was calculated for the retention times and peak areas of each of the three peaks in the six chromatograms. The results of these calculations are shown in Table 6.4.2.1 below.

Parameter	Peak 1 (**17**)	Peak 2 (**21**)	Peak (**20**)
t_R R.S.D	0.23 %	0 %	0 %
Area R.S.D.	1.76 %	1.39 %	1.57 %

Table 6.4.2.1: Relative standard deviation in retention time and peak area for the three compounds 17, 20 *and* 21.

As can be see there was very little variation between injection based on the R.S.D. values returned, values of less than 2 % are considered to be very good (Riley, 1996).

Section 6.2.3: Assessment of selectivity of HPLC-DAD separation of three Leuckart impurities derived from the synthesis of MBDB (8)

The selectivity of the separation achieved with system 12 was tested by analysing a series of potentially interefering compounds under the chromatographic conditions described in system 12. The compounds, which were tested were; MBDB (**8**), BDB (**7**), MDA (**4**), MDMA (**5**), MDEA (**6**), amphetamine (**3**), ketamine (**27**), 4-methylthioamphetamine (**9**), caffeine (**28**), paracetamol (**29**) and piperonyl ethyl ketone (**16**). None of these compounds were found to interfere with the three compounds of interest (**17**, **20** and **21**) under the conditions of system 12. Most of the compounds eluted within 2.6 minutes. Piperonyl ethyl ketone (**17**), the starting material for the synthesis for MBDB (**8**), was an exception as it eluted much later. An overlaid chromatogram of the three compounds of interest (**17**, **20** and **21**) and a chromatogram of an injection of reference solution 23, (containing a standard solution of piperonyl ethyl ketone (**16**)) (Figure 6.2.3.1), shows that the separation is selective. The resolution between the two closely eluting peaks at circa five minutes was calculated and found to be, 1.57, which is acceptable. It is notable that the chromatogram of reference solution 23 containing PEK (**16**), had more than one peak. This would infer that PEK (**16**) is not a very stable compound and these extra peaks are breakdown products.

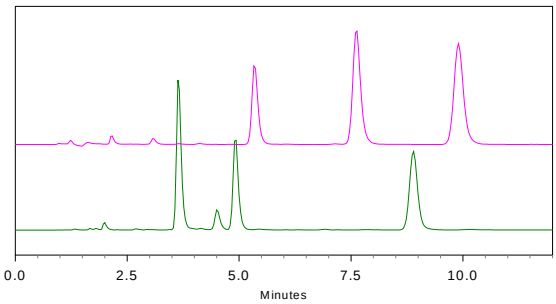


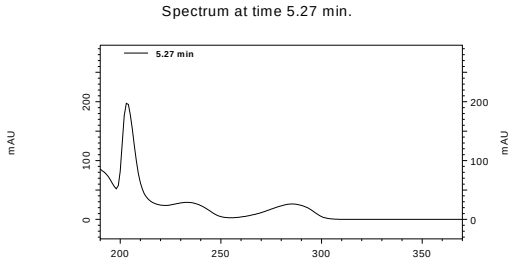
Figure 6.2.3.1.Overlaid chromatogram obtained with the compounds 17 , 20 and 21 (uppermost chromatogram) and a chromatogram obtained with piperonyl ethyl ketone (16) (lower chromatogram).

The liquid chromatographic separation established was considered applicable to the detection of the possible presence of the *N*-formyl BDB (**17**), and the two pyrimidines (**20** and **21**) in illicit tablets, provided that an appropriate sample preparation procedure was established.

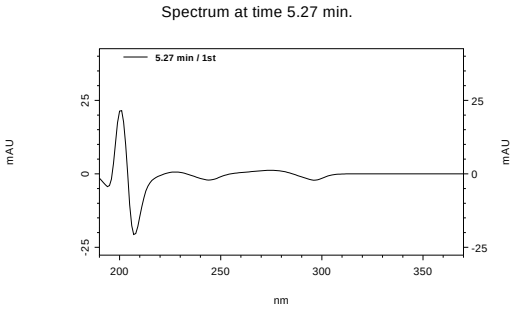
Section 6.3: Construction of UV-Vis spectral library of Leuckart impurities, derived from the synthesis of MBDB (8), and related compounds.

The zero order UV-Vis spectra (zero order spectra are plots of wavelength versus absorbance) collected for the impurities **17**, **20** and **21** were entered into the custom UV-Vis spectral library. Unlike NMR and IR, which are well known for giving highly featured, zero-order, spectra in the liquid phase, UV-Vis spectroscopy does not. Therefore often derivative spectra are calculated as they can be more featured and therefore be more useful from an identification point of view. The CLASS-VP software offers the option to enter derivative spectra into spectral libraries. The 1st and 2nd derivatives of the zero order spectra were also entered into the library. Examples of the zero order, 1st and 2nd derivative spectra for compounds **17**, **20** and **21** are shown below in Figures 6.3.1, 6.3.2 and 6.3.3 respectively. These spectra were collected from a standard injection of reference solution 12 (containing compound **17**, **20** and **21**).

(1a)



(1b)



(1c)

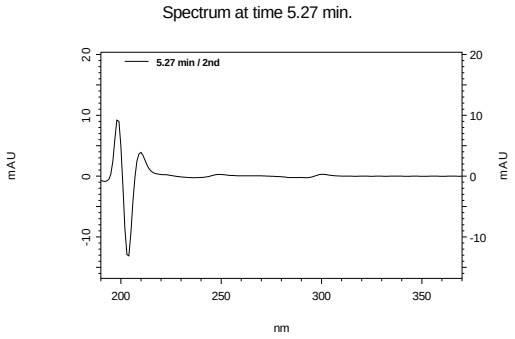
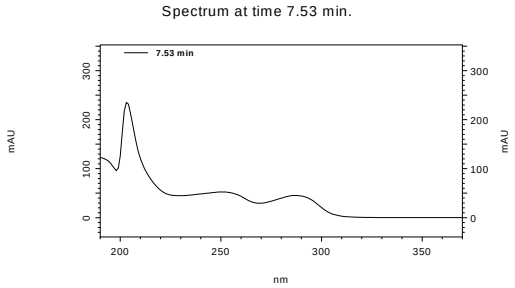
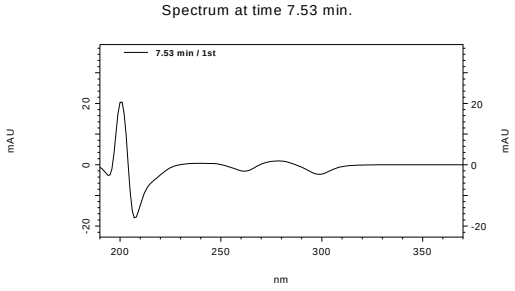


Figure 6.3.1 (1a) UV spectrum of N-formyl BDB (17), (1b) 1nd derivative spectrum of N-formyl BDB (17) and (1c) 2nd derivative spectrum of N-formyl BDB (17).

(2a)



(2b)



(2c)

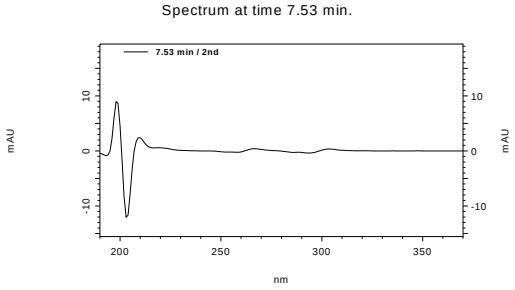
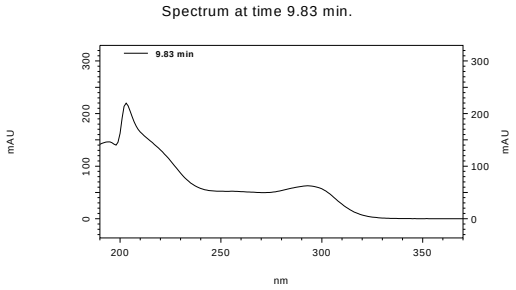
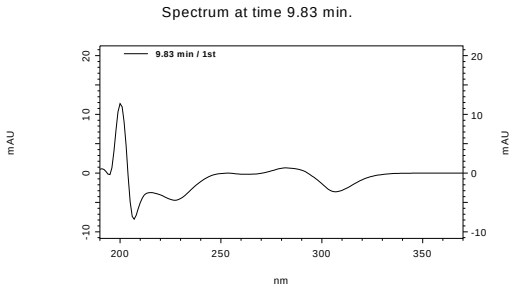


Figure 6.3.2 (2a) zero order UV spectrum of 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (21), (2b) 1st derivative spectrum of 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (21), and (2c) 2nd derivative spectrum of 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (21).

(3a)



(3b)



(3c)

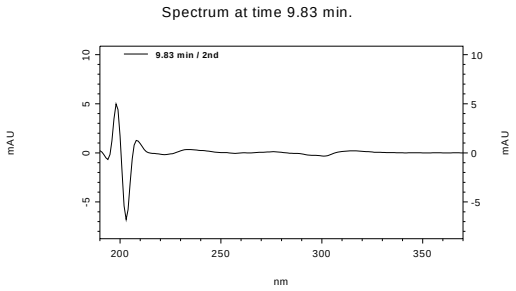


Figure 6.3.3 (1a) zero order UV spectrum of 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (20), (1b) 1nd derivative spectrum of 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (20), and (1c) 2nd derivative spectrum of 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (20.

As can be seen the zero order spectra for the three compounds (17**,** 20 **and** 21**) showed some similarity to one and other (See Figures 6.3.1a, 6.3.2a and 6.3.3a). The *N*-**

formyl BDB (17**) had a spectrum with three maxima at 204nm, 233nm and 285nm respectively (Figure 6.3.1a). The signal at 204nm was highest but as it was close to the solvent cut-off, it was decided that one of the other two maxima would be most useful for analytical work. The 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**) gave a similar spectrum to the *N*-formyl BDB (**17**) (See Figure 6.3.2.a) where the three absorbance maxima which were observed were at 204nm, 251nm and 287nm. Again the third maximum (287nm) was deemed to be most suitable for analytical work. The zero order spectrum for the 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) was found to be different from the other two as the second maximum was not quite as well resolved and hence the only two significant maxima that were observed were at 203nm and 293nm (see Figure 6.3.3a). It was decided that the third maxmum was most suitatble for analytical work as it was again sufficiently far from the solvent cut-off. As mentioned in section 6.2 the analytical wavelength, which would be used for future work,**

would be 289nm as this was the best compromise wavelegth for all three compounds (17**,** 20 **and** 21**).**

One of the drawbacks of diode array detection is that UV spectra for a similar compounds may not differ sufficiently to enable them to be used in unequivocal identification. However in this instance it is fortunate that all three of the target compounds differ sufficiently to enable definite distinction between the compounds of interest spectrally. All of the spectra which were entered into the library were later used in the attempted identification of route specific impurities derived from the synthesis of MBDB (8**) via the Leuckart reaction. Derivative spectra are included here (Figures 6.3.1b, 6.3.1c, 6.3.2b, 6.3.2c, 6.3.3b, 6.3.3c). Derivative spectra are generally more structured than zero order spectra, enabling very tiny differences between the original spectra to be amplified. The second and other even numbered derivative spectra are generally considered to be more useful than first order spectra (Gorog, 1995). In this**

instance the derivative spectra are not that different, probably due to the high spectral bandwidth of the instrument which yields poorly structured 2nd order derivative spectra. The zero order spectra were to be used as the main spectra when searching for the target compounds in chromatograms. The representative UV-Vis spectra of the standards, MBDB (8), BDB (7), MDA (4), MDMA (5), MDEA (6), amphetamine (3), ketamine (27), 4-methylthioamphetamine (9), caffeine (28), paracetamol (29) and piperonyl ethyl ketone (16), were also entered into the library. Spectra were taken over the whole peak so as to allow for concentration differences when searching. An electronic copy of the library is included in appendix II. A copy of the Shimadzu CLASS-VP software is required to use this library (Shimadzu, 1999) .

Section 6.4: GC-MS separation of three Leuckart impurities derived from the synthesis of MBDB (8).

Section 6.4.1 Development of GC-MS separation of three Leuckart impurities derived from the synthesis of MBDB (8)

Although a HPLC-DAD method had been established for the analysis of the Leuckart route specific compounds **17**, **20** and **21**, it was considered worthwhile to attempt to develop a GC-MS separation. It was intended that this method be applicable to the analysis of these impurities in illicitly produced tablets. Using a standard mixture of the three impurities **17**, **20** and **21**, seven temperature programmes were investigated (see Table 5.6.1.2). Of the seven programmes investigated, system 1 was found to be the most suitable. The conditions are described in detail in Section 5.6.1. The temperature programme involved an initial temperature of 90°C (held for 1minute), ramping up to 300°C at a rate of 15°C/min. The oven was held at the final temperature of 300°C for 10 minutes. Compounds **17**, **20** and **21** were separated and gave clear mass spectral fragmentation patterns. A typical reconstructed ion chromatogram (RIC) obtained with a standard containing compounds **17**, **20** and **21** is shown below (Figure 6.4.1.1). A RIC is a plot of the intensity of the ions in the ion trap versus time.

Comment [RM1]: Need chromatogram here.

Figure 6.4.1.1 Reconstructed ion chromatogram trace recorded for the separation of three impurities, 17, 21 and 20 derived from the synthesis of MBDB (8) via the leuckart-wallach reaction, using GC-MS system 1.

Chromatographic parameters such as retention time, peak width, resolution, tailing factor, column efficiency, for the above chromatogram are shown below (see Table 6.4.1.1, for formulae see Appendix I). The primary ions observed from the mass spectra recorded for each peak are also tabulated. All three compounds **17**, **20** and **21** were well separated. The order of elution was determined by comparing the retention time of individual standards containing compounds **17**, **20** and **21** respectively with the chromatographed mixture. The first compound to elute from the column was the 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) at 9.5 minutes. This was followed at 10.0 minutes and 10.2 minutes by the *N*-formyl BDB (**17**) and the 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**) respectively. The peaks were, as expected in capillary GC, extremely symmetrical, with all peaks

showing tailing factor values of 1.0. The resolution between the *N*-formyl BDB (**17**) and the 4-ethyl-5-(3,4-methylenediocyphenyl)pyrimidine (**20**) was found to be 3.88, and the resolution between the *N*-formyl BDB (**17**) and the 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**) was 1.68. As resolution values greater than 1.7 are ideal (Dolan, 2000) this separation was considered acceptable. The efficiency of the column for each of the peaks was good for a 30 metre column. Typically, one would expect values of ca. 90,000 plates (Baugh, 1993). The efficiency of the *N*-formyl BDB (**17**) peak was low, but not so low that the method was not considered useful.

Peak	Peak Identity	t_R (minutes)	Width (minutes)	Resolution	Tailing Factor	Column Efficiency	Primary Ions in mu (abundance)
1	**20**	9.5	0.12	----	1.0	102,400	229mu* (100%), 228 (38%), 168mu (9%) and 115mu (6%)
2	**17**	10.0	0.16	3.88	1.0	63,756	176mu (100%), 222mu (31%), 135mu (32%), 58mu (34%)
3	**21**	10.2	0.12	1.68	1.0	168,428	228mu (100%), 229 (73%), 135mu (30%), 169mu (28%)

*Table 6.4.1.1: Table of chromatographic parameters calculated from the reconstructed ion chromatogram recorded for the separation of three impurities, 17, 21 and 20, derived from the Leuckart synthesis of MBDB (8) using GC-MS system 1 (for chromatographic conditions see section 5.6.1). *mu refers to mass units*

Section 6.4.2: Assessment of precision of GC-MS separation of three Leuckart impurities derived from the synthesis of MBDB (8)

A series of five replicate injections were chromatographed using the conditions outlined in system 1 (see section 5.6.1). The R.S.D. of the retention times of the compound (17, 20 & 21) were less than 0.01% in all cases. The R.S.D. of the peak areas was less than 3% in all cases. The precision of the

method was considered acceptable. Mass spectral data was recorded for each peak and is discussed in section 6.5.

Section 6.4.3: Assessment of selectivity of GC-MS separation of three Leuckart impurities derived from the synthesis of MBDB (8)

The selectivity of the separation achieved with system 1 was tested by analysing a series of potentially interefering compounds under the chromatographic conditions described in programme one (see Section 5.6.1). The compounds, which were tested, were MBDB (**8**), BDB (**7**), MDA (**4**), MDMA (**5**), MDEA (**6**), amphetamine (**3**), ketamine (**27**), 4-methylthioamphetamine (**9**), caffeine (**28**), paracetamol (**29**) and piperonyl ethyl ketone (**16**). None of these compounds were found to interfere with the three compounds of interest (**17**, **20** and **21**) under the conditions of system 1. The PEK (**16**) appeared to breakdown over time as mentioned above (see Section 3.2.3) but was not found to interfere with the compounds of interest.

The method provided a basis for the analysis of route-specific target compounds 17**,** 20 **and** 21 **in MBDB (**8**) tablets, where the MBDB (**8**) had been synthesised via the Leuckart reaction.**

Section 6.5: Construction of Mass spectral library of Leuckart impurities, derived from the synthesis of MBDB (8), and related compounds.

The mass spectra collected for the impurities **17**, **20** and **21**, and potentially interfering compounds were entered into the custom mass spectral library. Mass spectra for compounds **17**, **20** and **21** are shown below in Figure 6.5.1.

(a)

(b)

Figure 6.5.1 Mass spectra for compounds **17** (a) and **20** (b).

(C)

Figure 6.5.1(cont'd): Mass spectra for compound 21 (c).

The last compound to elute was the 5-methyl-5-(3,4-methylenedioxybenzyl)pyrimidine (21**). It is clear from the electron ionisation mass spectral fragmentation pattern (see Figure 6.5.1.c) that the molecule does not undergo significant fragmentation. Given that the molecular mass of the compound was calculated to be 228 mass units (mu), the molecular ion was at this mass in the spectrum, as expected. The molecular ion was the base peak in the mass spectrum compound** 21**. There was a large M+1 peak (73%), which is likely due to proton addition. Proton addition to the molecular ion in electron ionisation is not that common, but can occur and seems to have occurred in this instance (Todd, 1995). There was also a significant ion at 169mu, which is difficult to interpret, but would seem to be derived from fragmentation of both the pyrimidine and the**

methylenedioxy ring. Although there are other fragment peaks, they are all generally less than 10% of the abundance of the base peak. For confirmational analysis in GC-MS one strives for a fragmented spectra, as it is not impossible that many compounds of the mass 228mu would have poor mass spectral fragmentation patterns. The possibility of derivatisation in order to obtain a more fragmented and therefore unique spectrum was considered. However, it was felt that as none of the potentially interfering compounds eluted at similar retention times, hence this spectrum would suffice for the analysis of the target compounds. Derivatisation also adds an additional analytical step, which can lead to errors. In addition, in the case of the pyrimidines there is no suitable functionality that could be easily exploited in order to form a derivative. A proposed fragmentation pattern for 5-methyl-5-(3,4-methylenedioxybenzyl)pyrimidine (21**) is shown below (Figure 6.5.2).**

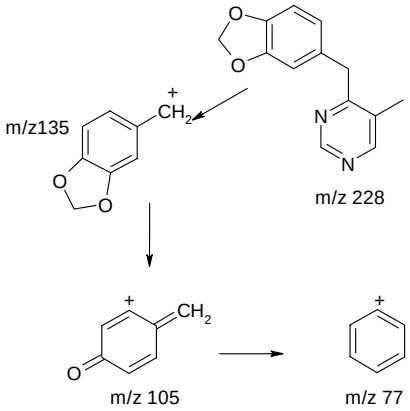


Figure .5.2: Proposed fragmentation pattern for 5-methyl-5-(3,4-methylenedioxybenzyl)pyrimidine (21).

The other pyrimidine, 4-ethyl-5-(3,4-methylendioxybenzyl) (20**) eluted first and unlike compound** 21 **it gave a poor fragmentation pattern (see Figure 6.5.2.b), when compared to compound** 21**.Again, the large M+1 peak was observed, but this time it was the base peak, indicating significant proton addition. The fragments previously observed with compound** 21 **at 115mu and 169mu were again observed in the mass spectrum of compound** 20**, but were at a very low abundance. It is proposed that these were due to the fragmentation of the pyrimidine and the methylenedioxy ring.**

The *N*-formyl BDB (17**) gave a good fragmentation pattern. The main ions observed were the molecular ion at 222mu, and further ion fragments at 176mu, 161mu, 135mu and 58mu. The proposed pathway of fragmentation is shown below (see Figure 6.5.2).**

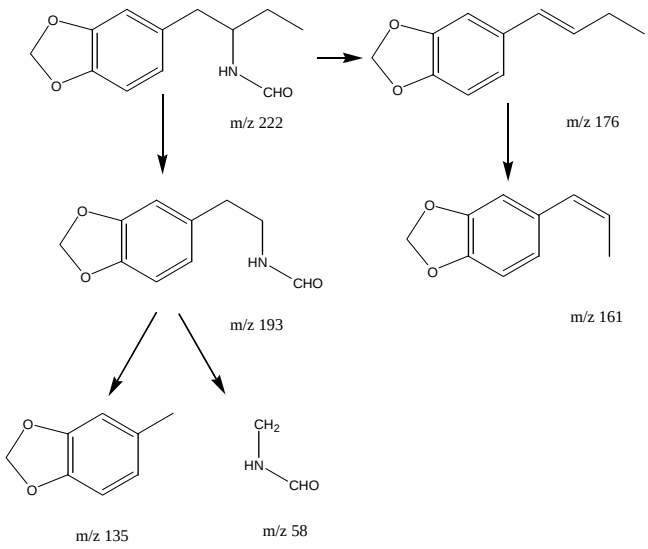


Figure 6.5.2 proposed fragmentation pattern for N-formyl BDB (17).

The mass spectra of compounds 17**,** 20 **and** 21 **were entered in to the custom library once suitable representative mass spectra had been found. Spectra which were considered to be representative of the compounds were entered into the**

mass spectral library. The representative Mass spectra of the standards, MBDB (8**), BDB (**7**), MDA (**4**), MDMA (**5**), MDEA (**6**), ketamine (**27**), 4-methylthioamphetamine (**9**), caffeine (**28**), paracetamol (**29**) and piperonyl ethyl ketone (**16**), were also entered into the library. Amphetamine (**3**) was excluded as it seemed to be eluting during the 4 minute filament delay at the start of the chromatographic run. An electronic copy of this library is included in Appendix II. The Varian sotware is required when using this library (Varian, 1989-1998).**

It was decided that instead of using the reconstructed ion chromatogram (RIC) mode, which plots all the ion intensities recorded while a compound elutes from the column into the mass spectrometer, Selected Ion Monitoring, SIM, would be used. SIM is a technique employed in mass spectrometry that allows the selection of a selected ions for recording. The ions are usually the base peak or some other ion that gives a high abundance in the

compounds being analysed. This SIM technique reduces the background signal and allows for much better selectivity. The SIM mode was used for all subsequent analyses and the ions that were chosen for the compounds 17**,** 20 **and** 21 **were 176mu, 229mu and 228mu respectively. These ions were chosen as they were the most intense (see Figure 6.5.1 (a), (b) and (c)) ions observed for the mass spectra of the respective analytes.**

Section 6.6: Solid Phase Extraction of Leuckart impurities from MBDB (8)

In attempting to develop a method for the extraction of the target compounds 17**,** 20 **and** 21 **from MBDB (**8**) synthesised via the Leuckart reaction the starting point was a method described by Lambrechts *et al.* (Lambrechts, 1985). They employed bonded phase silica sorbents in an attempt to develop a rapid sampling protocol for HPLC analysis of impurities in illicit amphetamine (**3**). A method was developed which allowed the detection of *N*-**

formylamphetamine, 4-methyl-5-phenylpyrimidine and *N*,*N*-di(β-phenylisopropyl)formamide in a seizure of illicit amphetamine by using a protocol involving a 100mg C8 bond elut® solid phase extraction cartridge and the following protocol.

Two column volumes HPLC grade MeOH

↓

Two column volumes of HPLC grade H_2O

↓

1 ml of 50mg/ml amphetamine in pH7.0 buffer

↓

Two column volumes of HPLC grade H_2O

↓

Column was dried under vacuum

↓

Analytes eluted with 3×100µl of HPLC grade Acetonitrile where each Acetonitrile aliquot was allowed to remain on the phase for 2 minutes.

↓

The resulting solution was then analysed by HPLC

The compounds of most significance in the resultant chromatogram were collected at the waste tube of the detector and submitted for GC-MS analysis, which allowed for identification of the aforementioned analytes.

The approach adopted in the present study differed in that the HPLC and GC-MS analyses had been established and validated for the qualitative analysis of three target compounds (sections 6.2-6.5). Then, using 100mg C8 and C18 solid phase extraction cartridges and the Varian Sorbent Extraction Technology Handbook (Blevins, 1993) a suitable procedure was developed for extraction of the target compounds from MBDB (8**) synthesised via the Leuckart reaction.**

Section 6.6.1: Development of a suitable system for the elution of interfering components from a matrix containing Leuckart impurities, derived from the synthesis of MBDB (8), from C8 and C18 solid phase extraction cartridges.

Using the HPLC chromatographic system outlined in section 5.8.1 an attempt was made to determine the most suitable elution solvent. The best elution solvent is the one, which elutes the interfering compounds, but allows retention of the target compounds (Blevins, 1993). Complete elution of

MBDB (8) itself and interfering compounds from both C8 and C18 phases was the aim of the experiment, with retention of compounds 17, 20 or 21. The ability of the elution systems investigated (systems 1-4 table 5.8.1.1) was assessed based on the peak area returned for MBDB (8) on injection into the HPLC system at 289nm. Peak areas were graphed for both C8 and C18 solid phase extraction cartridges and are shown below (Figure 6.6.1.1).

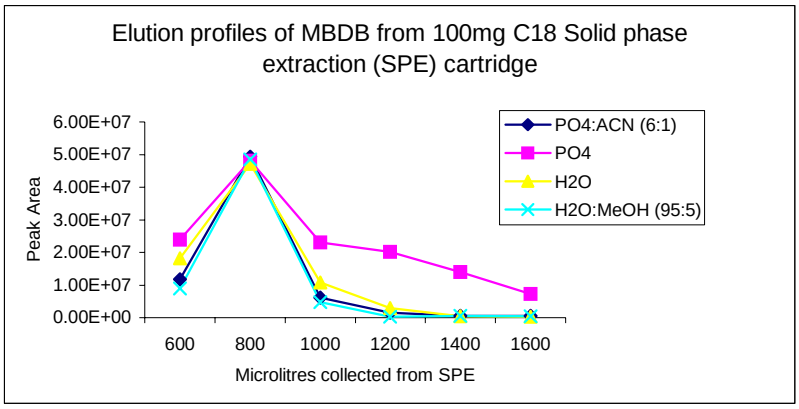


Figure 6.6.1.1: Graph of elution profiles of MBDB (8), from a 100mg C18 solid phase extraction cartridge, using different elution solvents.

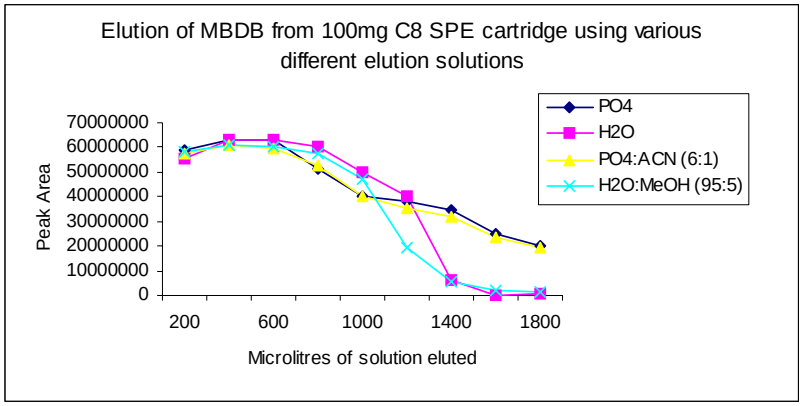


Figure 6.6.2: Graph of elution profiles of MBDB (8), from 100mg C8 solid phase extraction cartridge, using different elution solvents.

In the case of the C18 cartridge 3 solvent systems namely, H_2O, pH 3 phosphate:ACN (6:1) and H_2O:MeOH (95:5) performed similarly, but since further work indicated the organic modifier might have the effect of disturbing the target analytes, H_2O was chosen as the elution solvent. In the case of the C8 cartridge both H_2O and H_2O:MeOH (95:5) performed best. H_2O was again chosen as the most suitable solvent for the elution of MBDB (**8**) for C8. The MBDB•HCl would appear to be water-soluble and to have no affinity for the non-polar stationary phase of the extraction cartridges in the presence of H_2O. The target compounds **20** and **21** are not as water soluble, however the *N*-formyl BDB (**17**) is slightly more water-soluble (Keating, 1999).

Section 6.6.2: Assessment of the retention of impurities when subjected to MBDB (8) elution protocol.

Using H_2O, the ability of the C8 and C18 cartridges to retain *N*-formyl BDB (**17**), 4-ethyl-5-(3,4-methylenedioxyphenyl)pyrimidine (**20**) and 5-methyl-4-(3,4-methylenedioxybenzyl)pyrimidine (**21**), was assessed. By chromatographically analysing the eluate from the experiment described in section 5.8.2 it was evident, that the MBDB (**8**) elution protocol that involved H_2O, discussed above did not appear to be eluting the target compounds **17**, **20** and **21**. Featureless chromatograms were observed for these extracts.

Section 6.6.3: Development of a suitable system for the elution of Leuckart impurities, derived from the synthesis of MBDB (8), from C8 and C18 solid phase extraction cartridges

The next step involved the selection of the most suitable solvent, for the elution of the designated impurities from the two stationary phases. The best solvent is the one, which completely elutes the previously retained analytes in the smallest volume (Blevins, 1993). Acetonitrile and tetrahydrofuran were tested as potential, impurity elution solvents. Several fractions of each were collected from C8 and C18, SPE cartridges and chromatographed. The peak areas from the chromatograms for all three compounds versus microlitres of respective solvent (THF and ACN). The plots of how each compound eluted from its respective phase in either THF or ACN are shown below (see Figure 6.6.3.1, 6.6.3.2, 6.6.3.3 and 6.6.3.4).

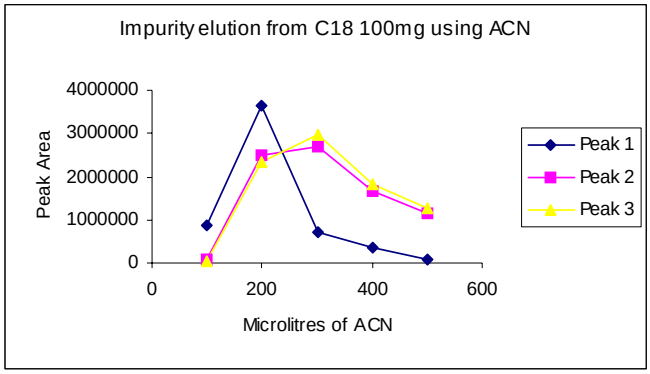


Figure 6.6.3.1: Graph of elution profiles of compounds 17, 20 and 21, from 100mg C18 solid phase extraction cartridge using ACN. Where peak 1 is compound 17, peak 2 is compound 21 and peak 3 is compound 20.

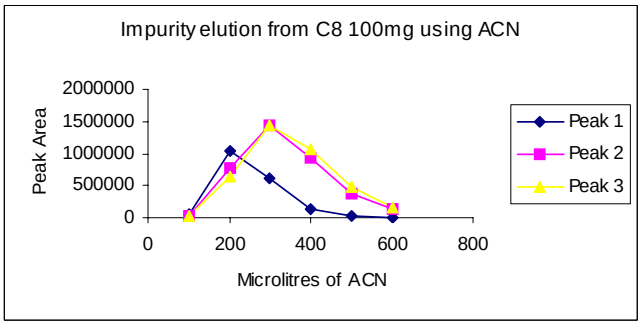


Figure 6.6.3.2: Graph of elution profiles of compounds 17, 20 and 21, from 100mg C8 solid phase extraction cartridge using ACN. Where peak 1 is compound 17, peak 2 is compound 21 and peak 3 is compound 20.

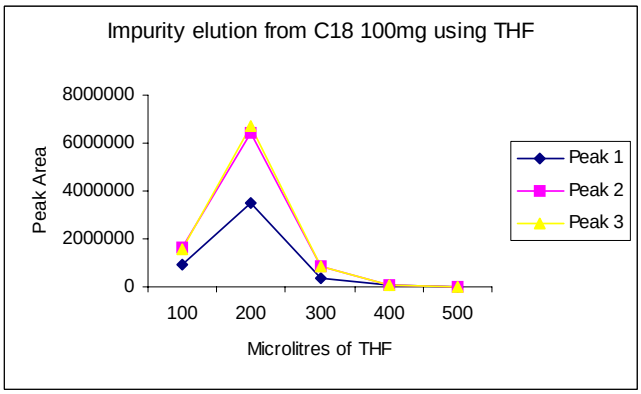


Figure 6.6.3.3: Graph of elution profiles of compounds 17, 20 and 21, from 100mg C18 solid phase extraction cartridge using THF. Where peak 1 is compound 17, peak 2 is compound 21 and peak 3 is compound 20.

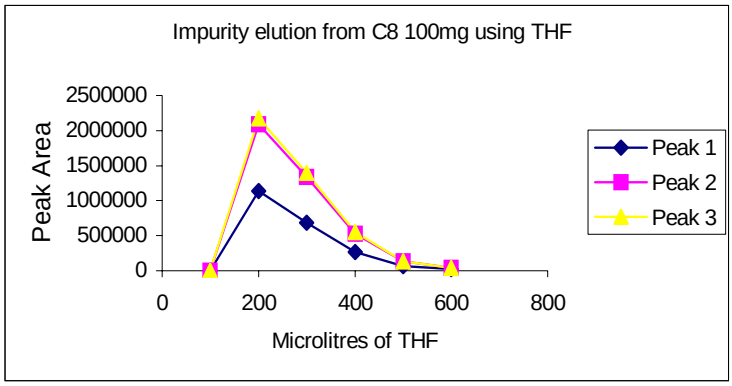


Figure 6.6.3.4: Graph of elution profiles of compounds 17, 20 and 21, from 100mg C8 solid phase extraction cartridge using THF. Where peak 1 is compound 17, peak 2 is compound 21 and peak 3 is compound 20.

Based on the results above graphs it was evident that ACN was not the best solvent as it was still giving peak signals after 500 µl of solvent had been passed through the cartridge. The THF had eluted all three compounds successfully after 500 µl as can be seen form the above graphs and hence THF was chosen as the most suitable elution solvent for both the C8 and C18 solid phase extraction cartridges. As the main objective of the procedure was to enrich the impurities as much as possible, an added advantage of using THF is that it was easily evaporated at room temperature, under a stream of nitrogen gas. Once evaporated the residue could be reconstituted in the desired amount of HPLC grade ACN. It was felt that 60 µl of ACN was a suitable volume as it could then be used for two HPLC injections and two GC-MS injections. One could use a lower volume if the levels of impurity were extremely low.

The protocol that was employed on the basis of the above results for extracting compounds 17**,** 20 **and** 21**, synthesised via the Leuckart reaction, is outlined in section 5.8, the results of which follow.**

Section 6.6.4: Extraction of Leuckart impurities from a sample of MBDB (8) prepared via the Leuckart reaction.

Using water to elute MBDB (8) and THF to then elute the target compounds 17, 20 and 21 the solid phase extraction, protocol was applied to MBDB (8) synthesised by the Leuckart reaction, in the Department of Pharmaceutical Chemistry, T.C.D. The extracts were subjected to HPLC analysis and GC-MS analysis as described in Section 5.8.4, in an attempt to confirm the Leuckart route as the route of synthesis. Typical HPLC chromatograms for extraction of 50mg of MBDB (8), extracted using 100mg C8 and C18 are shown below (Figure 6.6.4.1 and 6.6.4.2 respectively).

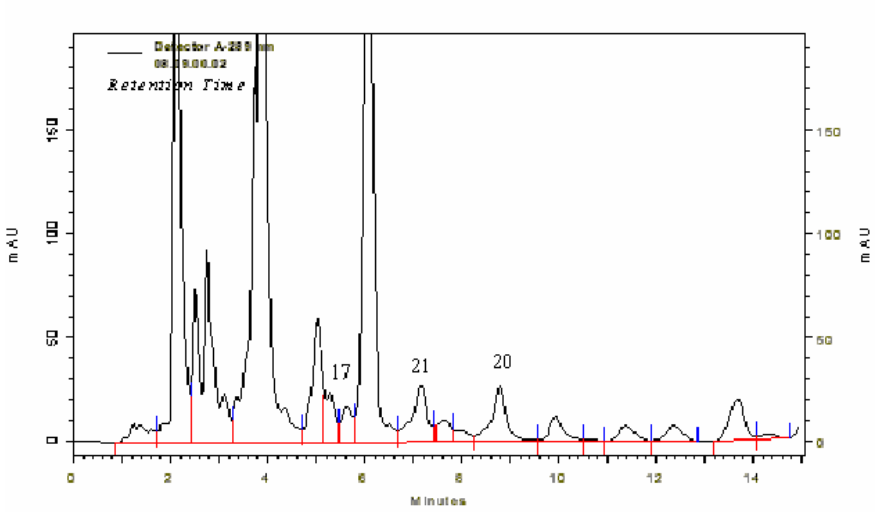


Figure 6.6.4.1 HPLC (289nm) of 50mg MBDB•HCl solid phase extracted with a C18 cartridge using H_2O to elute MBDB (8) and THF to elute analytes.

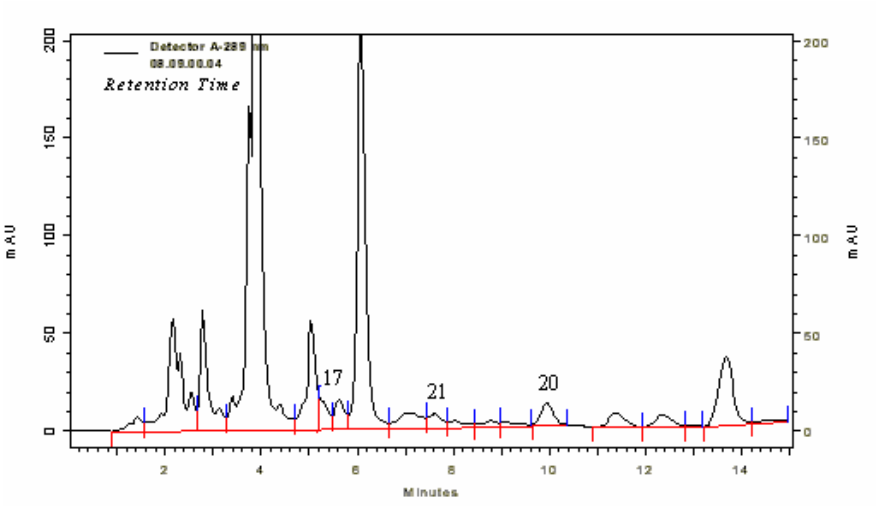


Figure 6.6.4.2 HPLC (289nm) of 50mg MBDB•HCl solid phase extracted with a C8 cartridge using H_2O to elute MBDB (8) and THF to elute analytes.

Using the UV-Vis library previously described in section 6.3, the above chromatograms were compared to the UV-Vis spectra in the custom UV-Vis spectral library. Compounds 17, 21 **and** 20 **were found to be eluting at at 5.3 minutes, 7.6 minutes and 9.9minutes respectively. The spectra of the components in each of the traces eluting at 5.3 minutes, 7.6 minutes and 9.9 minutes, in each chromatogram were found to match those of the *N*-formyl BDB (**17**) , 5-methyl-(3,4-MDbenzyl)pyrimidine (**21**) and 4-ethyl-5-(3,4-MDP)pyrimidine (**20**) respectively.**

The CLASS-VP software for the HPLC enables background correction. This is simply carried out by first setting a background spectrum, which is generally selected at some point in the chromatogram where there is no peak eluting or at the base of the peak of interest. Once the background spectrum is set it can be subtracted from the analyte spectrum. This process improves the quality of the analyte spectrum and should allow for more accurate identification. The signal was low in the case of all three components, however background correction of the spectra yielded reasonable spectra. The match quality value was calculated by the software and are tabulated below for both chromatograms (see Table 6.6.4.1). A value as close to 1.0 as possible is considered to be very good (Shimadzu, 1997) and a value greater than 0.99 was returned each case, however it is worth noting that there is a high background especially around the *N*-formyl BDB (17**), hence the possibility of isomeric/structurally similar compounds in the mixture could not be ruled out.**

Compound	Spectral Match Quality
17	**0.995**
20	**0.998**
21	**0.992**

Table 6.6.4.1: Spectral match quality for compounds 17, 21 and 20.

GC-MS chromatograms were also obtained for the impurities from the MBDB (8**) and are shown below (see Figure 6.6.4.3 for C18 and Figure 6.6.4.4 for C8). Both pyrimidines were present in the C8 and C18 extracts when compared to the spectra in the library esablished in section 6.5. The 4-ethyl-5-(3,4-MDP)pyrimidine (**20**) eluted at 9.5minutes and 5-methyl-4-(3,4-MDbenzyl)pyrimidine (**21**) at 10.2 minutes. The library was searched for these compounds and gave poor results. The chromatograms were visually inspected and it was confirmed that the compounds aforementioned were indeed present. There was no evidence of the *N*-formyl BDB (**17**) however there was a peak eluting at 10.3 minutes which would appear to be *N*-formyl MBDB (**18**). The identity of this was confirmed using the technique (described in section 5.14) of methylating a sample of *N*-formyl BDB (**17**) to form *N*-formyl MBDB (**18**), the results of which can be found in section 6.13. The occurrence of the *N*-formyl MBDB (**18**) may be due to an impurity in the formamide used in the synthesis (i.e. an *N*-methlyformamide impurity). The *N*-formyl MBDB (**18**) may have been too water soluble to be retained on either phase hence the absence from the trace. The *N*-formyl MBDB (**18**), being slightly more polar than the desmethyl analogue (**17**) may have been retained hence, the *N*-formyl MBDB (18) may have been the large peak eluting at 6.1 minutes the HPLC chromatograms (Figures 6.6.4.2 & 6.6.4.2), this is investigated further in section 6.3.**

Figure 6.6.4.3 GC-MS chromatogram of 50mg MBDB•HCl solid phase extracted with a C18 cartridge using H_2O to elute MBDB (8) and THF to elute analytes. The trace shows the extracted ions 228mu, 229mu and 176mu.

Figure 6.6.4.4 GC-MS chromatogram of 50mg MBDB•HCl solid phase extracted with a C8 cartridge using H_2O to elute MBDB (8) and THF to elute analytes. The trace shows the extracted ions 228mu, 229mu and 176mu.

The result of the above experiment lead to the conclusion that it was possible to detect Leuckart specific impurities generated via the synthesis of MBDB (8**) using the solid phase extraction protocols developed using both C8 and C18 cartridges. The procedure developed was done so by extracting the impurities from pure MBDB•HCl, which had been synthesised to a very high standard and rigourously purified in the Department of Pharmaceutical Chemistry in the School of Pharmacy (see Appendix III). The next step was to carry out an extraction of the impurities using liquid-liquid extraction at different of pH values to see if any improvement on the solid phase extraction protocol could be established.**

Section 6.7: Development of liquid-liquid extraction for the isolation and enrichment of Leuckart impurities from MBDB (8)

Although a solid phase extraction protocol, capable of confirming that a batch of MBDB (8**) was synthesised via the Leuckart route was established, it was considered worthwhile to investigate the possibility of using liquid-liquid extraction as an alternative method. The optimum liquid-liquid extraction pH was chosen, by extracting samples of MBDB•HCl at different values of pH (3, 4, 4.9, 6 and 7) into petroleum ether. The chromatographic systems used to monitor the**

success of the various extractions were the GC-MS and HPLC systems outlined in section 5.9. The most suitable pH was chosen on the basis that it gave a higher recovery of impurities from the same batch of MBDB (8). It was considered that pH 4 was the most suitable. Typical chromatograms (HPLC and GC-MS) obtained after MBDB•HCl was extracted at pH 4 extractions, are shown below (Figure 6.7.1 and Figure 6.7.2 respectively).

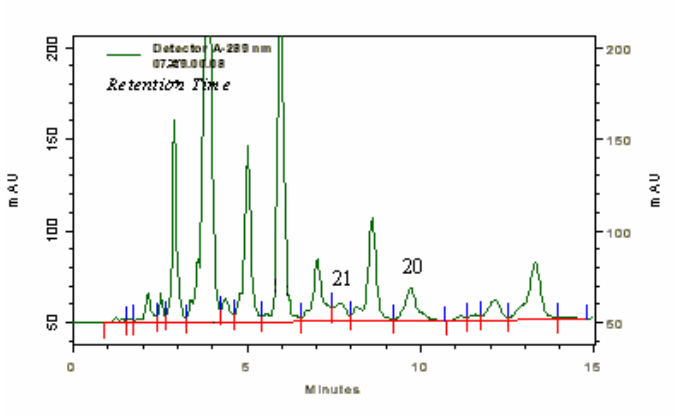


Figure 6.7.1 HPLC chromatogram (289nm) of 50mg MBDB•HCl (8) liquid-liquid extracted at pH 4 with petroleum ether.

Figure 6.7.2 SIM GC-MS chromatogram of 50mg MBDB•HCl **(8)** *liquid-liquid extracted at pH 4 with Petroleum Ether. Trace was constructed using ions 228, 229 and 176.*

The peak areas for each of the compounds (17**,** 20 **and** 21**) were calculated and plotted against extraction pH for the GC-MS and for the HPLC. These plots are shown below (Figure 6.7.3 and Figure 6.7.4 respectively). As can be seen from the Figure 6.7.4 (GC-MS) the best pH for the extraction of all three compounds was pH 4. Although pH 5 appeared to be better than pH 4 for extracting the *N*-formyl MBDB (**18**), it was obvious from chromatograms that there is a lot of compounds being extracted at this higher pH. It may have been possible that the pKa of MBDB (**8**) was being approached and less of the MBDB (**8**) was ionised, such that the unionised amine was partitioning into the organic phase. However the pKa was determined experimentally for MBDB (**8**) (see appendix IV) and was found to be 10.25, hence it is unlikely that MBDB (**8**) is what is being carried over between pH 5 and pH 7. It is more plausible that neutral impurities**

were interfering at this stage. These impurities were not of interest at this stage as there are no reports in the literature about route specific neutral impurities, hence they were considered as an interference. The HPLC data also indicated that pH 4 was the optimum (see Figure 6.7.3.), however the *N*-formyl BDB (17**) peak appeared not to match the library spectrum sufficiently well. It appeared that there was an interfering compound eluting. Because of this the *N*-formyl BDB (**17**) was excluded from figure 6.7.3. This observation undermined the selectivity of the HPLC assay. This was not a problem with respect to the GC-MS assay, which serves to highlight the desirability of different methodologies. It is however, worth noting that it was possible to identify the pyrimidine peaks for compounds 20 and 21 easily from the UV spectra acquired using the HPLC-DAD method developed in section 6.2.**

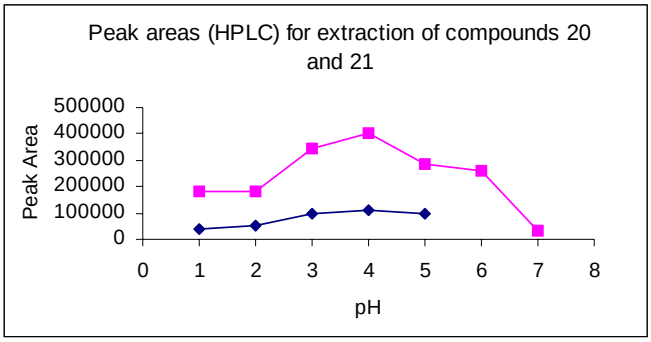


Figure 6.7.3 Graph of HPLC (289nm) trace peak areas for compounds 17, 20 and 21 versus extraction pH for 50 mg of MBDB•HCl into petroleum ether. Compound 20 in marked in pink and compound 21 is plotted in navy.

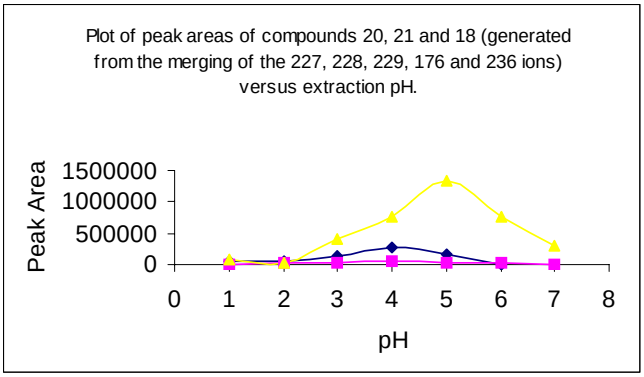


Figure 6.7.4 Graph of GC-MS trace peak areas (for ion 228mu, 229mu and 176mu) for compounds 17, 20 and 21 versus extraction pH for 50 mg of MBDB•HCl into petroleum ether. Compound 18 is plotted in yellow (suspected N-formyl MBDB (17)), compound 20 is plotted in navy and compound 21 is plotted in pink

Section 6.8: Comparison of Solid Phase Extraction and Liquid-Liquid Extraction.

Having investigated both solid phase extraction and the pH extraction method, the best extraction protocol based on the peak areas obtained via GC-MS analysis for extractions of the same sample of MBDB (8**), was the pH 4 extraction procedure. Although the *N*-formyl MBDB (**18**) was not initially a target compound it was felt that, it was so significant that it could not be excluded. This procedure would be adopted for the analysis of the tablets. A bar chart constructed from the GC-MS peak areas of the pyrimidines** 20 **and** 21 **was constructed for the above liquid –liquid extractions and the solid phase extractions and is shown below (Figure 6.7.5). This chart demonstrates quite clearly that the method of choice is the pH 4 extraction, when the pyrimidines are concerned. This pH is also suitable for the extraction of the *N*-formyl MBDB (**18**) as shown in Figure 6.8.1**

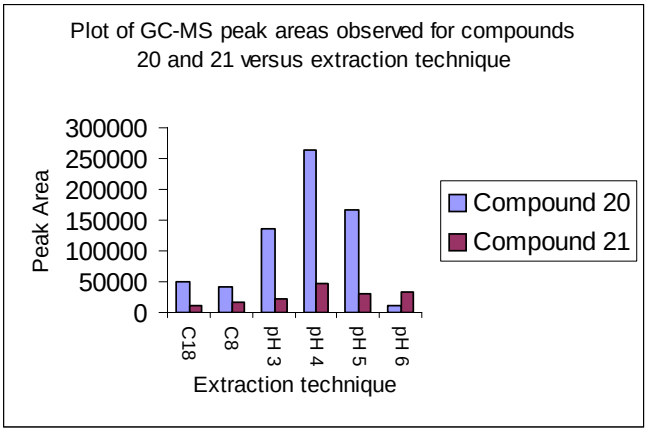


Figure 6.8.1:Bar chart constructed from the GC-MS peak areas of the pyrimidines 20 and 21 versus the extraction technique employed.

Section 6.9: Qualitative analysis of illicitly prepared MBDB (8)

Although the HPLC-DAD analysis (see Section 6.10) had been performed on the tablets, it was felt that it would be worthwhile to get mass spectral data for the tablets, given the interference with the *N*-formyl MBDB (18**) noted earlier. Using the GC-MS procedure outlined in section 5.10 the identity of the main active ingredient in the illicitly prepared tablets was checked against standard MBDB (**8**). All of the tablets were found to contain MBDB (8) as the most significant peak eluting at the same as the standard, 7.4 minutes, where the mass spectra were comparable to the mass spectrum of MBDB (**8**). The fit algorithm in the GC-MS Saturn software returned values in excess of 970 which is considered acceptable when presented along with retention time data (Varian, 1989-1998). The results are tabulated below (Table 6.9.1).**

Tablets Analysed	Retention Time	Ions (in order of intensity)
Standard MBDB	7.4 minutes	208mu, 72 mu, 135 mu, 177 mu.
Dil #6660	7.4 minutes	208mu, 72 mu, 135 mu, 177 mu.
7A	7.4 minutes	208mu, 72 mu, 135 mu, 177 mu.
Dil #A6765	7.4 minutes	208mu, 72 mu, 135 mu, 177 mu.
Dil #6758	7.4 minutes	208mu, 72 mu, 135 mu, 177 mu.
8A	7.4 minutes	208mu, 72 mu, 135 mu, 177 mu.
Dil #6840	7.4 minutes	208mu, 72 mu, 135 mu, 177 mu.
Dil #6600	7.4 minutes	208mu, 72 mu, 135 mu, 177 mu.

Table 6.9.1: Retention time and primary ion data for the qualitative analysis of MBDB (8) in illicitly prepared tablets and standard MBDB (8).

Section 6.10: Quantitative analysis of MBDB (8) in illicitly manufactured tablets

In order treat each tablet similarly, the concentration of MBDB (8**) in each of the tablets had to be determined, such that a 50mg MBDB (**8**) equivalent of each tablet could be subjected to extraction into petroleum ether at pH 4. All of**

the tablets were analysed using the HPLC method outlined in section 5.11. MBDB (8**) was found to elute at 12.3 minutes in the above system.**

Each of the tablets was treated as detailed in Section 5.11 before injection into the HPLC system. All of the tablets contained MBDB (8**) as the only significant peak in the chromatogram. The UV-Vis spectra for MBDB (**8**) in the library were compared with the suspected MBDB (**8**) peak, eluting at 12.30 minutes in each of the samples. Each of the tablets main peaks eluted at the same time as MBDB (**8**) and matched the MBDB (**8**) library entry. The fit was always greater 0.99, which is considered good. The areas of the peaks for the MBDB (**8**) standards (see Section 5.11) were calculated and a standard curve was constructed (Figure 6.10.1). The R^2 value was 0.999 and the equation of the line was y=10572x+63672 as calculated with the aid of Microsoft Excel (Microsoft®, 1997).**

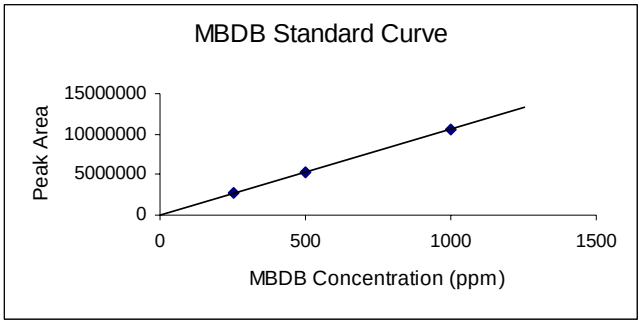


Figure 6.10.1: MBDB (8) Standard Curve

The peak areas were calculated and the concentration values were interpolated from the standard curve for each of the samples analysed. The amount of MBDB (8) per tablet was calculated using the following equation:

$$\text{MBDB mg} = \frac{(\text{Interpolated concentration mg})}{(\text{weight of tablet taken})} \times \text{weight of tablet mg}$$

The results are tabulated below (Table 6.10.1).

Tablet Reference	Weight of homogenous powder analysed	Tablet Weight	Peak Area	Interpolated Concentration	Concentration of MBDB (**8**)
Dil # 6660	9.8 mg	336.4 mg	5130192	0.48 mg/ml	163.72 mg
7A	10.1 mg	278.41 mg	4592539	0.43 mg/ml	133.88 mg
Dil # A6765	9.5 mg	308.70 mg	4446056	0.41 mg/ml	134.84 mg
Dil #6758	10.8 mg	465.49 mg	3803090	0.35 mg/ml	106.73 mg
8A	10.8 mg	328.07 mg	4520714	0.42 mg/ml	127.11 mg

Dil # 6840	10.5 mg	273.30 mg	3936944	0.40 mg/ml	95.27 mg
Dil # 6600	10.2 mg	335.8 mg	4708358	0.44 mg/ml	144.19 mg

Table 6.10.1: Quantitative data for the determination of MBDB (8) in illicitly prepared tablets.

As can be seen from table 6.10.1 there was a significant difference in the concentration of MBDB (8**) in the tablets analysed. The concentration of MBDB (**8**) ranged from 95.27mg 163.72mg. The average MBDB (**8**) content was 129.39mg, with a standard deviation of 21.15mg. Doses ranging from 150-210mg seem to be normal for MBDB (**8**) (Shulgin, 1991), but significant variation has been recorded. Rothe and co-workers claim that on average MBDB (**8**) tablets contained 97mg (Rothe, 1997). The risk assessment published by the EMCDDA (Van Aerts, 2000) noted that the mean amount of MBDB (**8**) in tablets was 100mg, whereas Kintz reports that the concentrations are in the region of 76-228mg (Kintz, 1997). The amount of each tablet (equivalent to 50mg) required for each of the tablets was calculated (Table 6.10.2).**

Tablet Name	Weight equivalent to 50mg MBDB	Tablet Name	Weight equivalent to 50mg MBDB
Dil# 6660	102.74mg	8A	129.05mg
7A	103.98mg	Dil# 6840	143.43mg
Dil#6765	114.45mg	Dil# 6600	116.44mg
Dil# 6758	218.07mg		

Table 6.10.2: Table containing weights of tablet required to be taken such that 50mg of MBDB (8) could be extracted

Section 6.11: Attempted extraction of Leuckart impurities from MBDB (8) from a spiked sample of an illicitly prepared tablet.

A 50mg equivalent of MBDB (8**) from typical tablet, A6765, was spiked with approximately 2% each of compounds** 17**,** 20 **and** 21**. The tablet was extracted as outlined in Section (5.12.1). The resultant HPLC chromatogram for this experiment is shown below (Figure 6.11.1). As can be seen, compounds** 20 **and** 21 **were well extracted but the recovery of compound** 17 **was poor. The SIM GC-MS trace of the sample is also shown (Figure 6.11.2). Compounds** 20 **and** 21 **are clearly extracted, however compound** 17**,** ***N*****-formyl BDB, is not extracted with the same efficiency as the other two**

compounds. So the limitation of the method was that it was not effective at extracting *N*-formyl BDB (17).

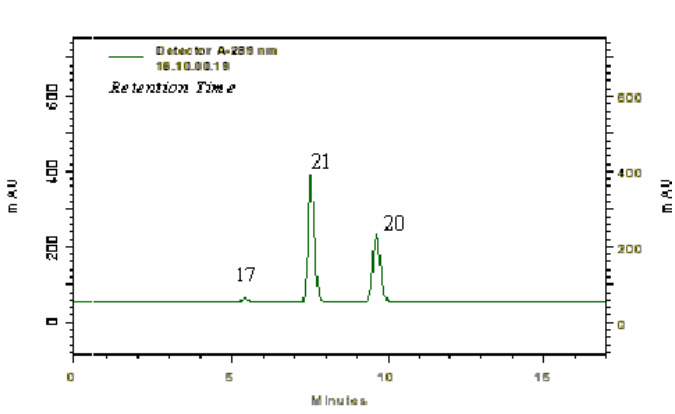


Figure 6.11.1 HPLC trace (289nm) for spiked sample A6765s.

Figure 6.11.2 SIM GC-MS trace of ions 176mu, 228mu and 229mu for spiked sample A6765.

Although it had been previously established that the pH 4 liquid –liquid extraction method was the best of the methods investigated (see Section 6.8) the actual recovery was not calculated. The recovery was assessed by comparing the peak areas returned for GC-MS and HPLC-DAD for the standard peaks fund in the spiked tablet after extraction to the peak areas of the impurity recovery solution analysed in section 5.13. The impurity recovery solution was prepared such that, if the impurities in the spiked tablet were exhaustively extracted then the solution to the following equation should be 100%;

$$\text{Recovery} = \frac{\text{Peak Area Observed for Impurity Recovery Solution}}{\text{Peak Area Observed for Spiked Tablet Extract Solution}} \times \frac{100}{1}$$

Table 16.11.1 shows the respective peak area values observed for the tablet extract solution and the impurity recovery solution as well as the solutions to the above equation for the respective method (HPLC-DAD and GC-MS).

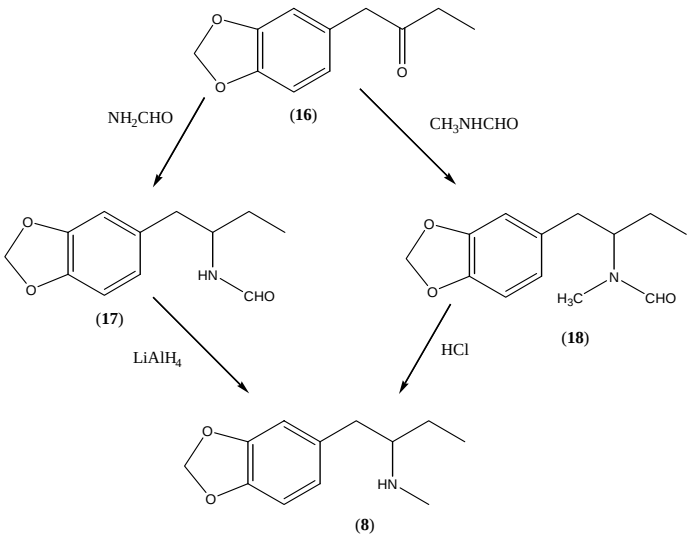
Compound Number	Impurity Recovery Solution Peak Area (Arb. Units)	Spiked Tablet Extract Solution Peak Area (Arb. Units)	Recovery
17 (HPLC-DAD)	908	197836	0.4%
17 (GC-MS)	7532	1425941	0.5%
20 (HPLC-DAD)	143489	213488	67%
20 (GC-MS)	825169	1304528	63%
21 (HPLC-DAD)	183255	200561	91%
21 (GC-MS)	1378970	1565740	88%

Table 16.11.1: Respective peak area values observed for the tablet extract solution and the impurity recovery solution as well as the solutions to the above equation for the respective method (HPLC-DAD and GC-MS).

As already mentioned the recovery of the *N*-formyl BDB was very poor, the recovery of pyrimidines was much better (see Table 6.11.1). The recovery of compound 21 was particularly good. There was little difference between the recovery estimates between the two method of analysis (GC-MS and HPLC-DAD (see Table 6.11.1).

Section 6.12: Attempted extraction of Leuckart impurities from MBDB (8) from illicitly manufactured MBDB (8) tablets.

All of the tablets were extracted as described in section 5.12 and from the chromatograms, none were found to contain any of compounds **17**, **20** or **21**, which would infer that the MBDB (8) was not synthesised via the route investigated. Samples, #6840, #8A and 7A did however appear to contain compound **18**, *N*-formyl MBDB, eluting at circa 10.2 minutes, on the GC-MS evidence, at a low level. *N*-formyl MDA and *N*-formyl MDMA have been detected in samples of MDA (**4**) and MDMA (**5**) as specific intermediate products of the Leuckart synthesis (Verweij, 1992c). It follows therefore that detection of *N*-formyl BDB (**17**) and *N*-formyl MBDB (**18**) in a sample of MBDB (**8**) would be also be proof that the Leuckart synthesis was involved. A variation of the Leuckart route that could be exploited in the synthesis of MBDB (**8**) is via the *N*-formyl MBDB (**18**) (see Scheme 6.12.1, below).



*Scheme 6.12.1: Leuckart routes from PEK **(16)** to MBDB **(8)**.*

It is noteworthy that this latter route does not yield pyridine or pyrimidine impurities (Keating 1999). The results would appear to indicate that none of the tablets were prepared via the Leuckart route of synthesis investigated here, inferring that they were likely to be synthesised via another route. However the presence of what may be *N*-formyl MBDB (**18**) (see section 6.13) could indicate that the tablets may have been prepared by the Leuckart route, via the *N*-formyl MBDB (**18**) intermediate. Alternatively, the MBDB (**8**) may have been synthesised via the other Leuckart route where N-methyl formamide is present as an impurity in the formamide used as a reagent (intermediate via compound **17**, scheme 6.12.1). It is likely that this is where compound **18** arose from in the MBDB (**8**) prepared in the School of Pharmacy (see section 6.6 and 6.7). The spiking experiment rules out the possibility that this compound is formed during sample preparation and/or GC-MS analysis. The *N*-formyl MBDB (**18**) was not present in spiked street tablet sample (#A6765s) which had been spiked with compounds **17**, **20** and **21**. If compound **17** were the source of compound **18** one would have expected it to arise during this experiment, but this was not the case.

Section 6.13 Derivatisation of *N*-formyl BDB (18) in an attempt to identify peak eluting at 10.2 minutes in GC-MS trace of samples.

The compound eluting at circa 10.3 minutes was positively identified as *N*-formyl MBDB (18**). A sample of *N*-formyl (**17**), which had been prepared in the Department of Pharmaceutical Chemistry, was derivatised to form the *N*-formyl MBDB (**18**). The GC-MS trace for the Methelut-derivatised *N*-formyl BDB (**17**) is shown below (Figure 6.13.1) and the corresponding mass spectrum for the peak is**

also shown (Figure 6.13.2). This mass spectrum is identical to the mass spectrum of the unknown compound found to be eluting at 10.2 minutes in section 6.6.4.

Figure 6.13.1: GC-MS trace for N-methylated derivative of N-formyl BDB (17).

Figure 6.13.2 Mass spectrum for compound eluting at 10.2 minutes from GC-MS system employed in section 5.14.

A dilution of the solution containing the reagents, starting material and reaction products was injected into the HPLC system outlined in section 2.2, system 12. Figure 6.13.3 shows the chromatogram obtained. The peak eluting at 5.3 minutes was compound **17**, hence the peak eluting at 6.1 minutes must be that of the *N*-formyl MBDB (**18**).

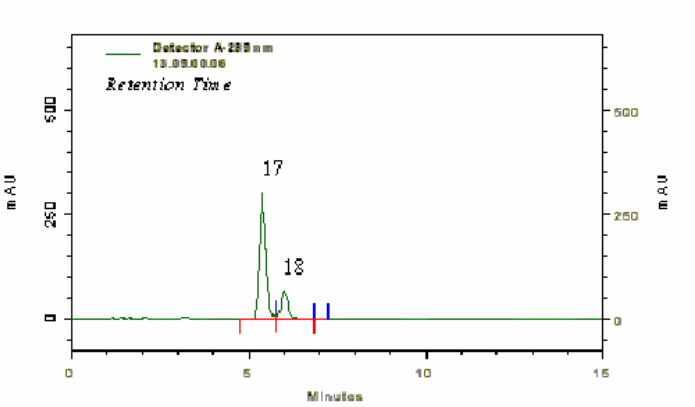


Figure 6.13.3: HPLC trace (289nm) of derivatised N-formyl BDB (17).

On the basis of this HPLC data, the large peak eluting at circa 6.1 minutes in the HPLC chromatograms of the C8 and C18 extracts in section 6.6.4 is most likely *N*-formyl MBDB (18**). The spectrum of the standard *N*-formyl MBDB (**18**) was compared to the spectra of the large peaks in Figures 6.6.4.1 and 6.6.4.2 and it was discovered that the**

spectra of the standard and sample were a good match (>0.90). It is noteworthy that the GC-MS procedure is preferred as the unequivocal identification of *N*-formyl MBDB (18**) was achieved.**

Section 6.14:Conclusion

HPLC-DAD and GC-MS procedures were established for the identification of Leuckart route specific impurities. An extraction protocol was then established and applied to a series of samples. The results indicated that it was likely that four of the tablets analysed were synthesised via the Leuckart route as the intermediate *N*-formyl MBDB (18**) was detected. This compound was also detected in the MBDB (**8**) synthesised in the School of Pharmacy, which had been used synthesised via the Leuckart Route and may have arisen due to impure starting materials via the reaction used.**

Section 7: Introduction

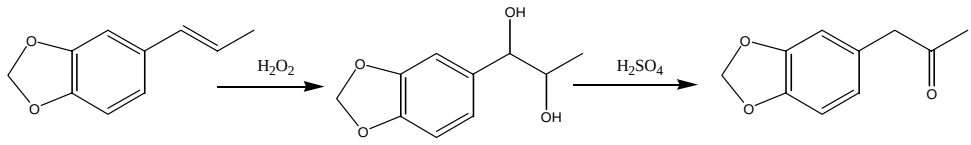
As mentioned before there are many routes available for the synthesis of phenylalkylamine (ecstasy) type compounds. The main routes used in the synthesis of MDA (**4**), MDMA (**5**), MDEA (**6**), MBDB (**8**) and 4-MTA (**9**) are outlined below.

Section 7.1: Synthesis of Propanamines, MDA (4), MDMA (5) and MDEA (6).

The synthesis of MDA (**4**), MDMA (**5**) and MDEA (**6**) among others has been excellently reviewed by Dal Cason *et al*. (1990). The most important aspects of that particular paper with respect to the work presented here are outlined below.

Synthesis of PMK

Two of the main starting materials for the synthesis of MDA (**4**) are Piperonal (**11**) and Piperonalmethylketone (PMK, **33**). PMK can be prepared by converting Isosafrole (**34**) into Isosafrole glycol (**32**) and then by oxidising the alcohols to form the ketone (See scheme 7.1). Alternatively it can be synthesised via the nitropropene route (see below). Isosafrole (**34**) can be synthesised from safrole (**40**), which can be acquired from Sassafras oil by distillation (French, 1995).

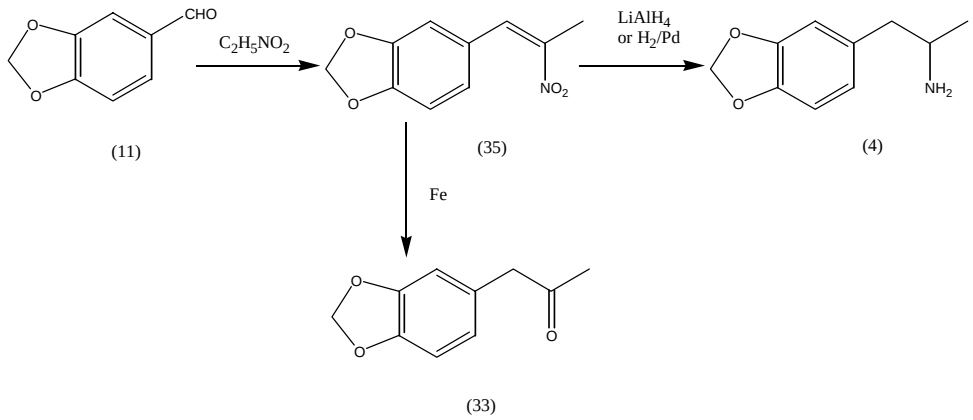


(34) (32) (33)

Scheme 7.1: Reaction scheme for the synthesis of PMK (33) from Isosafrole (34).

Nitropropene Route

This method involves the condensation reaction between piperonal (**11**) and nitroethane to form the intermediate piperonyl nitropropene (**35**), which can be treated with $LiAlH_4$ or H_2/Pd to form MDA (**4**), (Scheme 7.2). The nitropropene can be used to synthesise PMK (**33**) using Iron as a catalyst. Then using any one of a variety of reactions, which are discussed below, the appropriate methylenedioxyphenyl ring-substituted amphetamine can be manufactured.



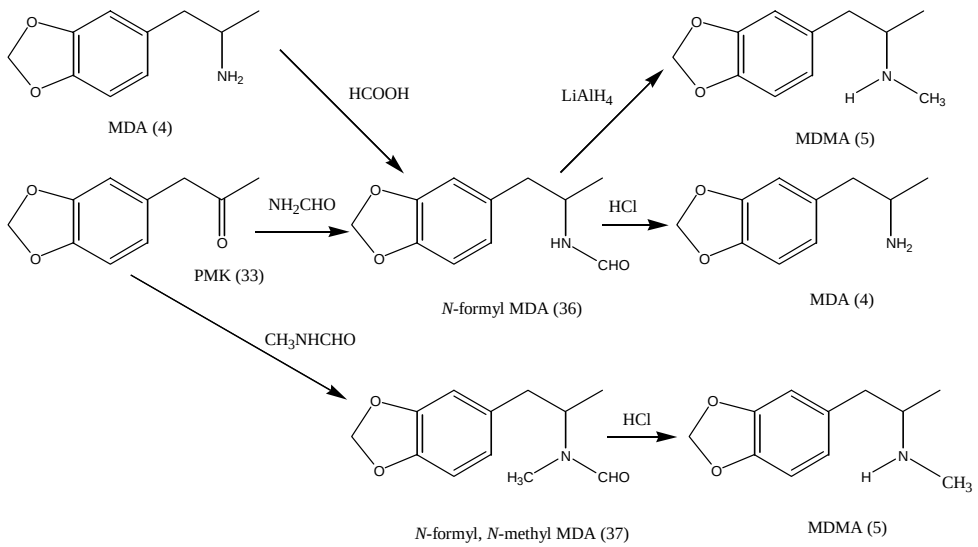
(11) (35) (4)

(33)

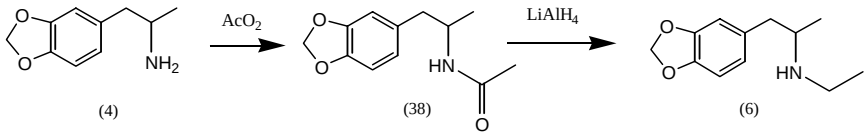
Scheme 7.2:Reaction scheme for the synthesis of PMK (33) and MDA (4) via the nitropropene route (35).

The Leuckart-Wallach Reaction

Using PMK (**33**) as the starting material this reaction involves the reaction between PMK (**33**) and two molecules of formamide to produce the *N*-formyl derivative (**36**) of the amine, which can then be hydrolysed with HCl to produce MDA (**4**) or methylated with $LiAlH_4$. MDMA (**5**) can also be synthesised by taking MDA (**4**) and treating it with formic acid to form the *N*-formyl derivative (**36**) and treating then with $LiAlH_4$ as before. Alternatively *N*-methyl formamide can be used to form the *N*-methyl, *N*-formyl derivative (**37**), this can then be reduced using HCl to form MDMA (**5**). (see Scheme 7.3). MDEA (**6**) can also be synthesised in this manner by treating MDA (**4**) with acetic anhydride to form the *N*-acetyl derivative of MDA (**38**). This *N*-acetyl intermediate (**38**) is then treated with $LiAlH_4$ to form MDEA (**6**) (Scheme 7.3a)



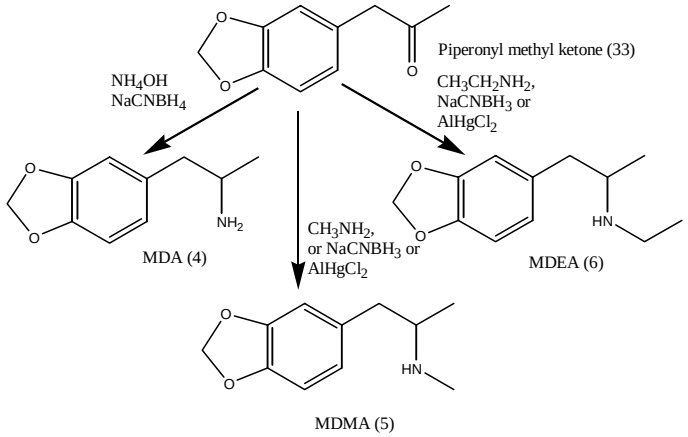
Scheme 7.3:Reaction scheme of the synthesis of MDA (4) and MDMA (5) via the Leuckart route.



Scheme 7.3a:Reaction scheme for the synthesis of MDEA (6) via the Leuckart route.

<u>Reductive Amination</u>

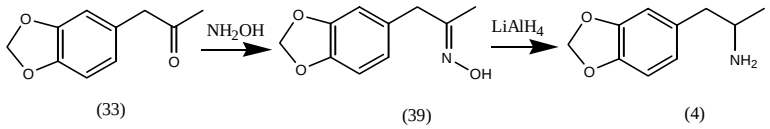
Reductive amination involves the reaction between PMK (**33**) and methylamine at reduced pressure and slightly elevated temperatures (ca. 80°C). The ketone (**33**) reacts with the amine to form the intermediate imine as the intermediate product, which is then subsequently reduced to the corresponding amine. MDMA (**5**) can also be manufactured in this way using methylamine and either $NaCNBH_3$ or $AlHgCl_2$. MDEA (**6**) can also be synthesised by this particular route. MDEA (**6**) can also be synthesised in this way but instead of using methylamine as is the case with MDMA (5) one would use ethylamine. (See Scheme 7.4).



Scheme 7.4:Synthesis of MDA (4), MDMA (5) and MDEA (6) via the reductive amination route.

The Oxime route

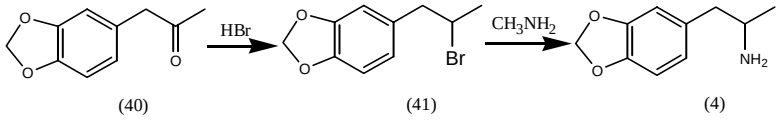
This reaction involves the formation of the oxime from the reaction between PMK (**33**) and a hydroxylamine molecule, which yields MDA (**4**) on hydrogenation with $LiAlH_4$ (see scheme 7.5).



Scheme 7.5:Reaction scheme for the synthesis of MDA (4) via the oxime route.

Bromopropane Route

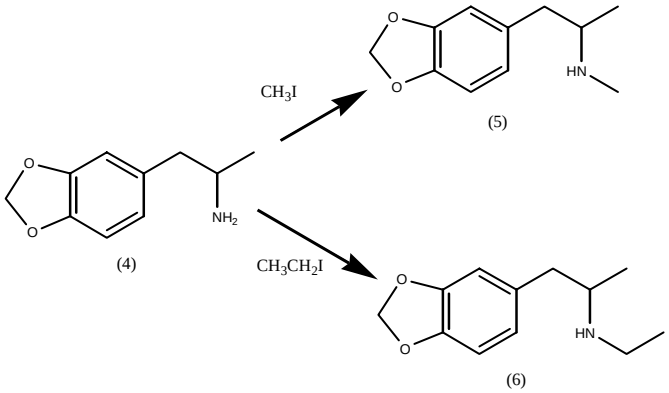
MDMA (**5**) can be synthesised directly from safrole via the bromopropane route (Merck, 1914). This involves the bromination of safrole (**40**) using HBr. The methylenedioxyphenyl-2-bromopropane intermediate can then be treated with methylamine to form MDMA (**5**) (see Scheme 7.6).



Scheme 7.6:Reaction scheme for the synthesis of MDA (4) via the bromopropane route.

Alkylation using Alkyl Iodides

MDMA (**5**) (Braun, 1980) or MDEA (**6**) can be formed by reacting MDA (**4**) with methyl or ethyl iodide reagent (see scheme 7.7).



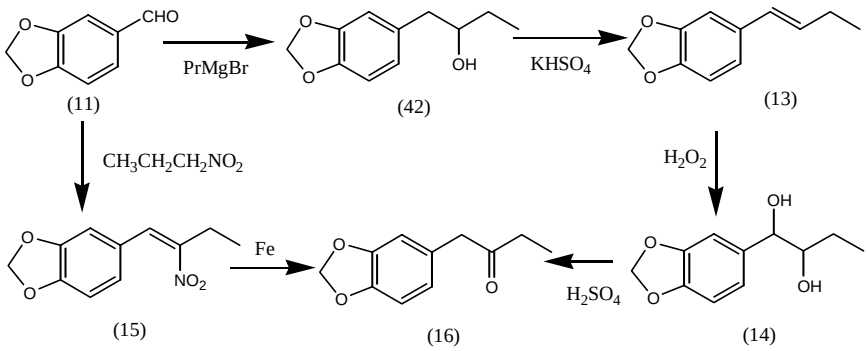
Scheme 7.7:Reaction scheme for the synthesis of MDEA (6) and MDMA (5) from MDA (4) via Alkylation

Section 7.2: Synthesis of Butanamines, BDB (7) & MBDB (8).

There is very little information available on the synthesis of MBDB (**8**) in the literature, however the EMCDDA report on the risk assessment of MBDB (**8**) (EMCDDA, 1999) suggests that reductive amination and nitropropene routes of synthesis may be popular in clandestine laboratories. However it is important to note that BDB (**7**) and MBDB (**8**) differ from MDA (**4**) and MDMA (**5**) by only one extra carbon atom on the alkyl side chains respectively, while the amine function remains at the two position in all cases. Hence it follows that the reactions for the synthesis of MDA (**4**) and MDMA (**5**) would directly apply to the synthesis of BDB (**7**) and MBDB (**8**). Butane type precursors are therefore required for BDB (**7**) and MBDB (**8**) instead of the propane type precursors needed for MDA (**4**) and MDMA (**5**). The possible reactions for the synthesis of BDB (**7**) and MBDB (**8**) are outlined below.

Synthesis of PEK (16)

As with MDA (**4**) and MDMA (**5**) one of the main starting precursors for BDB (**7**) and MBDB (**8**) the ketone possessing an extra carbon in the side-chain. This is called piperonyl ethyl ketone or PEK (**16**). This can be synthesised in much the same way as PMK (**33**) starting with piperonal. The nitrobutene derivative can also be formed from piperonal (**11**) which again under Iron catalytic conditions can be converted to PEK (**16**) (see scheme 7.8).

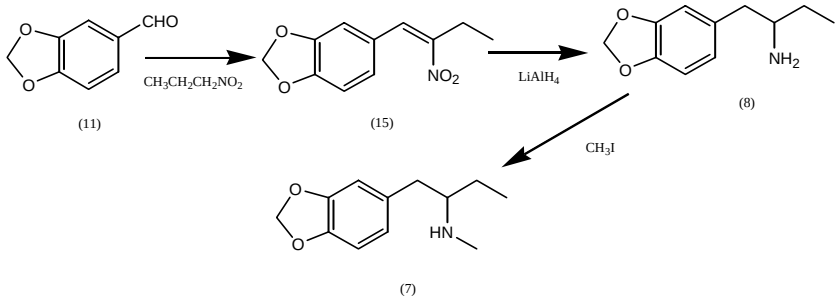


Scheme 7.8:Reaction scheme for the preparation of PEK (16).

Once PEK (**16**) is prepared one can easily synthesise MBDB (**8**) via the Leuckart or Reductive Amination routes (see below).

Nitrobutene Route to MBDB (8)

Using nitropropane and piperonal one can synthesise the nitrobutene derivative (**15**), which under reducing conditions forms the methylenedioxyphenyl-2-butylamine or (BDB) (**7**) which can be alkylated using methyl Iodide (see scheme 7.9).



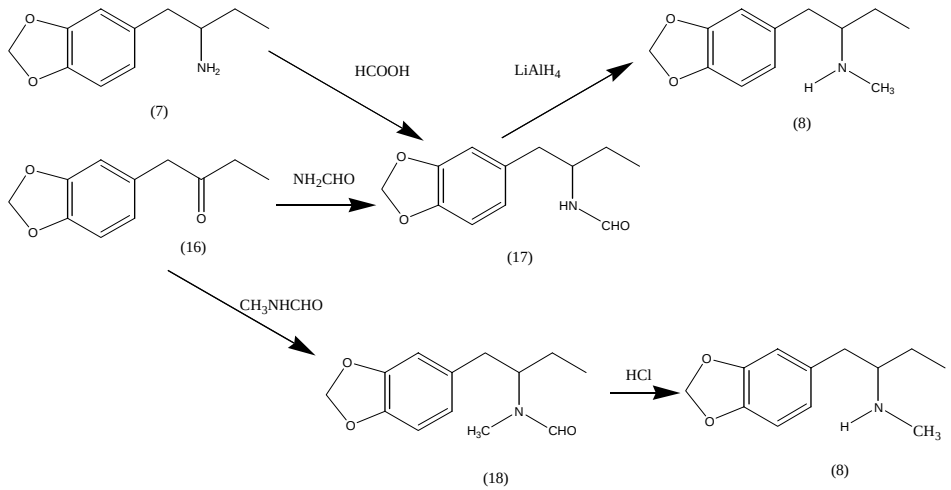
Scheme 7.9:Reaction scheme for the synthesis of MBDB (8) from Nitrobutene followed by Alkylation.

Reductive Amination to MBDB (8)

This is straightforward as with the propanamines where methylamine and $NaCNBH_3$ or $AlHgCl_2$ are used to aminate PEK (**16**), forming BDB (**7**) or MBDB (**8**).

Leuckart-Wallach Reaction to MBDB (8)

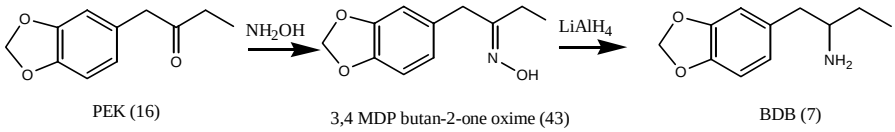
Using BDB (**7**) from the nitrobutene route or PEK (**16**) one can form the *N*-formyl derivative (**17**) and then reduce to MBDB (**8**) with $LiAlH_4$. One can also form the *N*-methyl, *N*-formyl-derivative (**18**) and then use a less aggressive reducing reagent (HCl) to form MBDB (**8**) (see scheme 7.10).



Scheme 7.10:Reaction scheme for the synthesis of MBDB (8) via the Leuckart-Wallach Route

The Oxime route

This reaction involves the formation of the oxime (**43**) from the reaction between the PEK (**16**) and a hydroxylamine molecule, which yields BDB (**7**) on hydrogenation with $LiAlH_4$ (see scheme 7.11). BDB (**7**) can be converted to MBDB (**8**) via alkylation with CH_3I.



Scheme 7.11:Oxime Route

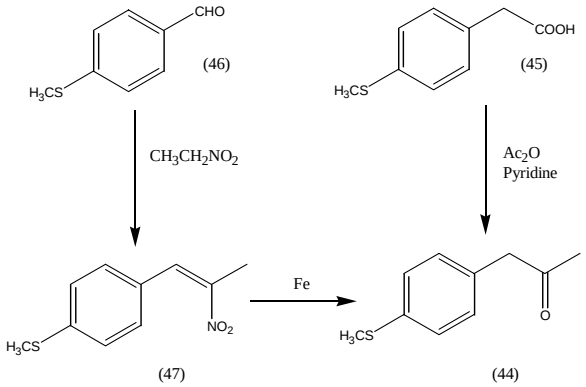
Section 7.3: Synthesis of 4-MTA (9).

As with the butanamines there is very little information available on the reactions that could be used in the synthesis of 4-MTA (**9**). The nitropropene route is the only route discussed in the literature (Holland, 1963; Poortman, 1998 & 1999), however 4-MTA (**9**) could be made by any of the reactions

used to synthesise MDA (**4**). The only difference this time is that the precursors used will not be 3,4-methylenedioxylated, but instead will be methylthioylated at the 4 position of the benzene ring.

Synthesis of 4-Methylthiophenylpropan-2-one

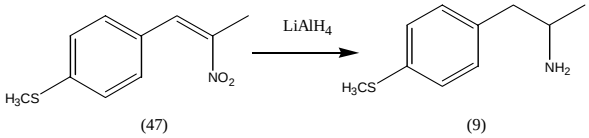
One of the main starting materials for the synthesis of 4-MTA (**9**) would be 4-methylthiophenylpropan-2-one (MPP) (**44**). MPP (**44**) can be prepared by converting 4-methylthiophenylacetic acid (**45**) to the ketone (**44**) (See scheme 7.12). MPP (**44**) can also be synthesised via the nitropropene route starting with 4-methylthiobenzaldehyde (**46**) via a 4-methylthiophenylnitrpropene (**47**) intermediate (see Scheme 7.12).



Scheme 7.12: Reaction scheme for the synthesis of MPP (44) from 4-methylthiophenylacetic acid (45) and also via the nitropropene route

Nitropropene Route

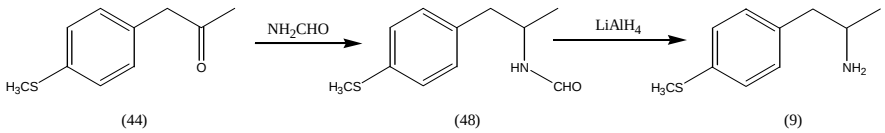
The nitropropene intermediate (**47**) mentioned above can be reduced directly to form 4-MTA (**9**) (see Scheme 7.13).



Scheme 7.13: Reaction scheme for the synthesis of 4-MTA (9) via the Nitropropene route.

The Leuckart-Wallach Reaction

This involves the reaction between MPP (**44**) and two molecules of formamide to produce the *N*-formyl 4-MTA (**48**), which can then be hydrolysed with HCl to produce 4-MTA (**9**) (see Scheme 7.14).

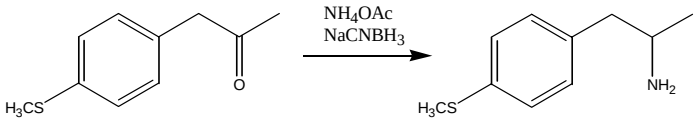


(44) (48) (9)

Scheme 7.14:Reaction scheme for the synthesis of 4-MTA (9) via the Leuckart-Wallach route

Reductive Amination

This involves the reaction between MPP (**44**) and $NaCNBH_3$ and ammonium acetate (see Scheme 7.15), to form 4-MTA (**9**) in one step.

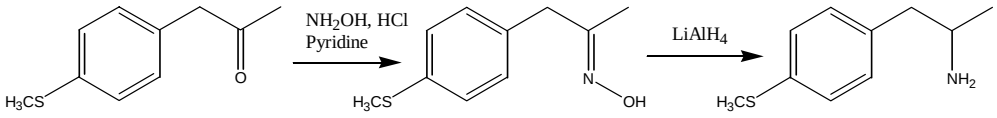


(44) (9)

Scheme 7.15:Reaction scheme for the synthesis of 4-MTA (9) via the reductive amination route

The Oxime route

This reaction involves the formation of the oxime from the reaction between the MPP (**44**) and a hydroxylamine molecule forming an oxime intermediate (**49**), which yields 4-MTA (**9**) on hydrogenation with $LiAlH_4$ (see scheme 7.16).



(44) (49) (9)

Scheme 7.16: Reaction scheme for the synthesis of 4-MTA via the oxime route

7.4: Objectives

Chapter II showed the benefits of using mass spectrometry as a means of determining the route of synthesis. Identification of the route was simplified by using standard reference material isolated from MBDB (**8**) derived from the Leuckart reaction. If a mass spectral library of compounds known to be route specific impurities or intermediates were established for MDA (**4**), MDEA (**5**), MDMA (**6**) MBDB (**8**) and 4-MTA (**9**) it may be possible to use it to identify the routes of synthesis of these compounds in a forensic setting. The reactions outlined above have been studied in the Department of Pharmaceutical Chemistry, School of Pharmacy, Trinity College Dublin. Stable impurities,

intermediates and products (e.g. MDA (**4**), MDMA (**5**) etc.), were isolated in the Dept. of Pharmaceutical Chemistry. These compounds were made available to the Dept. of Pharmacognosy for the purpose of establishing a mass spectral library that could be used in the determination of the type of reactions used to synthesise ecstasy-type drugs.

Section 8.1: Instrumentation & Solvents

GC-MS

The GC-MS system consisted of a Hewlett Packard 5973 MSD coupled to a 6890 GC system. Samples were introduced into the system with the aid of 6890 Injector Autosampler. A Hewlett Packard HP-5MS column (30m x 0.25mm x 0.25μm film thickness) was used throughout the experiments detailed below.

Miscellaneous Instruments

A fisons bench-top whirlimixer was used to agitate samples.

Solvents

All solvents used were HPLC grade unless otherwise stated.

Section 8.2: GC-MS analysis of standard impurities, intermediates and products derived from the synthesis of Ecstasy.

Origin of impurities, intermediates & products.

All of the compounds chosen for inclusion in the mass spectral database were either synthesised or isolated from intermediates or products by JJ Keating, in the Department of Pharmaceutical Chemistry at the School of Pharmacy, Trinity College Dublin. The exact structures were elucidated with the aid of H^1 and C^{13} NMR. The details of synthesis, isolation and structural elucidation can be found in the Ph.D. Thesis of JJ Keating (2001).

Preparation of analytical solutions of impurities, intermediates and products.

All solutions were prepared by dissolving approximately 0.01mg, of material to analysed, in 100μl of acetonitrile. The solutions were vortex mixed in a small vials before injection into the chromatographic system.

Reference solution 1: Piperonal (**11**)

Reference solution 2: Isosafrole (**34**)

Reference solution 3: 3,4-MDP-butane-1,2-diol (**14**)

Reference solution 4: Piperonyl methyl ketone (**33**)

Reference solution 5: N-formyl MDA (**36**)

Reference solution 6: 1-(3,4-MDP)-2-nitro-1-propene (**35**)

Reference solution 7: 1-(3,4-methylenedioxyphenyl)-propan-2-oxime (**39**)

Reference solution 8: 2-methyl-3-(3,4-MDP)-aziridine (**50**)

Reference solution 9: MDA (**4**)

Reference solution 10: 2-(3,4-methylenedioxybenzyl)-aziridine (**51**)

Reference solution 11: 4-methyl-5-(3,4-MDP)-pyrimidine (**52**)

Reference solution 12: 4-(3,4-Mdbenzyl)-pyrimidine (**53**)

Reference solution 13: 2,6-dimethyl-3,5-di(3,4-MDP)-pyridine (**23**)

Reference solution 14: 2,4-dimethyl-3,5-di(3,4-MDP)-pyridine (**54**)

Reference solution 15: 6-methyl-5-(3,4-MDP)-2-(3,4-MDbenzyl)-pyridine (**55**)

Reference solution 16: 2,4-dimethyl-3-(3,4-MDP)-6-(3,4-Mdbenzyl)-pyridine (**56**)

Reference solution 17: 4-methyl-5-(3,4-MDP)-2-(3,4-Mdbenzyl)-pyridine (**57**)

Reference solution 18: Safrole (**40**)

Reference solution 19: 3,4-MDP-butane-1,2-diol (**14**)

Reference solution 20: 3,4-MDP-2-bromopropane (**41**)

Reference solution 21: N-formyl, *N*-methyl MDA (**37**)

Reference solution 22: MDMA (**5**)

Reference solution 23: N-acetyl MDA (**38**)

Reference solution 24: MDEA (**6**)

Reference solution 25: 3,4-MDP-(2-nitro-but-1-ene) (**15**)

Reference solution 26: Piperonyl ethyl ketone (**16**)

Reference solution 27: 3,4-methylenedioxyphenyl-(butan-2-one oxime) (**43**)

Reference solution 28: N-formyl BDB (**17**)

Reference solution 29: N-formyl, *N*-methyl BDB (**18**)

Reference solution 30: 4-ethyl-5-(3,4-MDP)-pyrimidine (**20**)

Reference solution 31: 2,6-diethyl-3,5-di-(3,4-MDP)-pyridine (**22**)

Reference solution 32: 5-methyl-4-(3,4-MDbenzyl)-pyrimidine (**21**)

Reference solution 33: 2,6-dimethyl-3,5-di-(3,4-MDP)-pyridine (**23**)

Reference solution 34: 4-ethyl-3-methyl-2-(3,4-Mdbenzyl))-5-(3,4-MDP)-pyridine (**24**)

Reference solution 35: 2-ethyl-5-methyl-3-(3,4-MDP)-6-(3,4-Mdbenzyl)-pyridine (**25**)

Reference solution 36: 2,4-diethyl-3,4-di-(3,4-MDP)-pyridine (**26**)

Reference solution 37: 3,4-MDP-3-ethyl aziridine (**58**)

Reference solution 38: BDB (**7**)

Reference solution 39: MBDB (**8**)

Reference solution 40: 4-methylthio-benzaldehyde (**46**)

Reference solution 41: 1-methylthio-4-(2-nitro-propenyl)-benzene (**47**)

Reference solution 42: 1-(4-methylthio-phenyl)-propan-2-one (**44**)

Reference solution 43 N-formyl 4-MTA (**48**)

Reference solution 44 1-(4-methylthio-phenyl)-propan-2-oxime (**49**)

Reference solution 45 2-ethyl-3-(4-methylthio-phenyl)-aziridine (**59**)

Reference solution 46 4-(4-methylthio-benzyl)-5-(4-methylthio-phenyl)-pyrimdine (**60**)

Reference solution 47 2-(4-methylthio-benzyl)-3-(3-methylthio-phenyl)-aziridine (**61**)

Reference solution 48 4-methyl-5-(4-methylthio-phenyl)-pyrimidine (**62**)

Reference solution 49: 4-(4-methylthio-benzyl)-pyrimidine (**63**)

Reference solution 50: 2,6-dimethyl-3,5-bis-(4-methylthio-phenyl)-pyridine (**64**)

Reference solution 51: 2,4-dimethyl-3,5-bis-(4-methylthio-phenyl)-pyridine (**65**)

Reference solution 52: 2-methyl-6-(4-methylthiobenzyl)-3-(4-methylthio-phenyl)-pyridine (**66**)

Reference solution 53: 2,4-dimethyl-6-(4-methylthio-benzyl)-3-(4-methylthio-phenyl)-pyridine (**67**)

Reference solution 54: 4-methyl-2-(4-methylthio-benzyl)-5-(4-methylthio-phenyl)-pyridine (**68**)

Reference solution 55: 1-methyl-4-methyl thio-benzene (**69**)

Reference solution 56: N,N-bis-[1-methyl-2-(4-methylthio-phenyl)-ethyl]-formamide (**70**)

Reference solution 57: 4-MTA (**9**)

Reference solution 58: 3,4-MDP-propane-1,2-diol (**71**)

Chromatographic Conditions

The GC-MS conditions used in the analysis are outlined in Table 8.2.1

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	HP-5MS
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.25 μm
Transfer Line Temperature	280°C
Ionisation Mode	Electron Ionisation
Injection Mode	Splitless
Initial Temperature (Hold Time)	90°C (1 minute)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	280°C (6 minutes)
Total Run Time	19.7 minutes
Detected Mass Range	40 – 600 m/z

Table 8.2.1: Chromatographic conditions used for the analysis of impurities, intermediates and products derived from the synthesis of ecstasy.

Analysis

Each of the reference solution extracts were chromatographed (in duplicate) using the above chromatographic system. The results of this section are discussed in section 9.

8.3: Construction of Mass Spectral Library.

The Hewlett-Packard Chemstation software (Hewlett Packard, 1986-1996) that controlled the mass spectral detector allowed for the construction of mass spectral libraries. Compounds were entered into the custom spectral library from chromatographic data generated in section 8.1. Library creation using Chemstation is fully explained in the software help tool. The help tool in the HP software was used in order to create and modify the library entitled Mslib.l. An electronic copy of this library, called Mslib.l,

can be found on the supplementary disk at the back of this thesis (see Appendix II). This electronic copy requires Chemstation software or some other compatible software in order to function. The results of this section are shown in section 9.1.

Section 8.4: GC-MS analysis of ecstasy of licit origin

Origin of Ecstasy

Samples of MDMA (**5**), MDA (**4**), 4-MTA (**9**), BDB (**7**) and MDEA (**6**) were prepared in the Department of Pharmaceutical Chemistry by JJ Keating. The route used in the synthesis of each is shown in Table 8.4.1.

Compound	Route of Synthesis
MDMA.HCl	Leuckart Route
4-MTA.HCl	Reductive Amination Route
BDB.HCl	Oxime Route
MDEA.HCl	Reductive Amination Route
MDA .HCl	Leuckart Route
MDA.HCl	Oxime Route
MDA (crude reaction product)	Oxime route

Table 8.4.1: Licitly produced ecstasy analysed by GC-MS.

Preparation of ecstasy prior to GC-MS analysis

An aliquot of the powder (10mg) was added to water (5ml) that had been made alkaline by the addition of conc. ammonia (0.5 ml). This solution was extracted with 5ml of petroleum ether. The ether layer was transferred to a clean dry test tube, where it was evaporated under a stream of nitrogen at 40°C. The residue was reconstituted in 10ml of acetonitrile. The resulting solution was injected into the chromatographic system.

Chromatographic Conditions

The GC-MS conditions used in the analysis are outlined in Table 8.4.2.

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	HP-5MS
Column Internal Diameter	0.25 mm

Column Length	30 M
Column Film Thickness	0.25 μm
Transfer Line Temperature	280°C
Ionisation Mode	Electron Ionisation
Injected Volume	1μl
Injection Mode	Splitless
Initial Temperature (Hold Time)	90°C (1 minute)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	280°C (6 minutes)
Total Run Time	19.7 minutes
Detected Mass Range	40 – 600 m/z

Table 8.4.2: Chromatographic conditions used for the analysis of licitly produced ecstasy.

Analysis

Each of the extracts were chromatographed (in duplicate) using the above chromatographic system. The results of this section are discussed in section 9.2.

Section 8.5: GC-MS analysis of ecstasy of illicit origin

Origin of Ecstasy

Samples of ecstasy tablets were supplied by the Garda National Drugs unit. Physical descriptions of the tablets are included below in Table 8.5.1.

Tablet Number	Appearance	No. in Batch	Average Weight
1	Yellow, Round, Bi-convex	6	0.2532g
2	White, Round, Bi-convex	6	0.2974g
3	White, Round, Flat, Half scored on one side, other side bearing bird logo	6	0.3321g
4	White, Round, Flat, Half scored on one side, other side bearing bird logo	6	0.2987g
5	Salmon, Round, Bi-convex, Half-scored on one side, other side bearing a Mitsubishi logo.	100	0.3050g

Table 8.5.1: Illicitly produced tablets analysed by GC-MS.

Preparation of Tablets prior to GC-MS analysis

Tablet were powdered and homogenised in a mortar and pestle. An aliquot of the powder (30mg) was added to water (5ml) that had been made alkaline by the addition of conc. ammonia (0.5 ml). This solution was extracted with 5ml of petroleum ether. The ether layer was transferred to a clean dry test tube, where it was evaporated under a stream of nitrogen at 40°C. The residue was reconstituted in 3ml of acetonitrile. A portion (1µl) resulting solution was injected into the chromatographic system.

Chromatographic Conditions

The GC-MS conditions used in the analysis are outlined in Table 8.5.2.

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	HP-5MS
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.25 µm
Transfer Line Temperature	280°C
Ionisation Mode	Electron Ionisation
Injected Volume	1µl
Injection Mode	Splitless
Initial Temperature (Hold Time)	90°C (1 minute)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	280°C (6 minutes)
Total Run Time	19.7 minutes
Detected Mass Range	40 – 600 m/z

Table 8.5.2: Chromatographic conditions used for the analysis of illicitly produced ecstasy tablets.

Analysis

Each of the extracts were chromatographed (in duplicate) using the above chromatographic system. The results of this section are discussed in section 9.3.

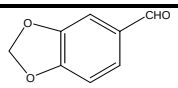
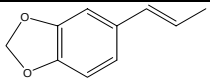
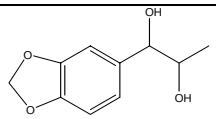
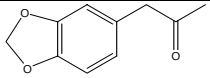
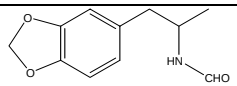
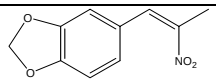
Section 9.1: GC-MS analysis of standard impurities, intermediates and products derived from the synthesis of Ecstasy.

The reactions outlined in the introduction were studied in the Department of Pharmaceutical Chemistry, School of Pharmacy, Trinity College Dublin for synthesis of MDA (**4**), MDMA (**5**), MDEA (**6**), MBDB (**8**) and 4-MTA (**9**). Stable impurities and intermediates and products, which were isolated in the Dept. Pharmaceutical Chemistry, were made available to the Department of Pharmacognosy for the purpose of establishing a mass spectral library.

By analysing selected compounds by GC-MS a sample mass spectrum from the chromatogram could be stored in a specially constructed library. This library could then be used when trying to identify compounds in illicitly prepared ecstasy tablets or reaction mixtures from clandestine labs etc. The type of information that it was hoped would be gleaned would be the identification of the route of synthesis by the identification of certain route specific impurities/intermediates. This kind of information would be of use to law enforcement authorities.

The idea of establishing a mass spectral database is not a new one in the case of the propanamines (especially MDA (**4**) and MDMA (**5**)) (Bohn, 1993; Verweij, 1992a). However there has been no attempt made to establish such a library in the case of MDEA (**6**), MBDB (**8**) and 4-MTA (**9**).

The structure and name for the impurities and/or intermediates included in the library for MDA (4), MDMA (**5**), MDEA (**6**), MBDB (**8**) and 4-MTA (**9**) are shown in Tables 9.1.1, 9.1.2, 9.1.3, 9.4 and 9.1.5 respectively. The route from which the impurity/intermediate was obtained from and whether that impurity is route specific or not is also indicated in the following tables.

Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
Piperonal [5.85 minutes], (**11**)		Precursor for Piperonal methyl ketone (PMK) (**33**) and for 1-(3,4-MDP)-2-nitro-1-propene (**35**).	149 (100), 150 (95), 63 (35), 121 (32), 65 (21), 91 (12).
Isosafrole [6.27 minutes], (**34**)		Precursor for PMK (**33**).	162 (100), 104 (44), 103 (41), 131 (40), 77 (31), 51 (24).
3,4-MDP-propane-2,3-diol [7.12 minutes], (**71**)		Intermediate between Isosafrole (**34**) and PMK (**33**).	65 (42), 93 (74), 123 (21), 151 (100), 196 (24).
Piperonyl methyl ketone [7.08 minutes], (**33**)		Intermediate between 1-(3,4-MDP)-2-nitro-1-propene (**35**) and MDA (**4**) (nitropropene route). Precursor to N-formyl MDA (**36**) (Leuckart route). Starting material for MDA (**4**) via one step reductive amination route. Precursor to oxime route.	135 (100), 77 (27), 178 (25), 51 (21).
***N*-formyl MDA** [10.34 minutes], (**36**)		Leuckart route specific intermediate between PMK (**33**) and MDA (**4**).	162 (100), 135 (75), 77 (40), 72 (40). 207 (12).
1-(3,4-MDP)-2-nitro-1-propene [9.91 minutes], (**35**)		Intermediate between piperonal and MDA (**4**). Also precursor to PMK (**33**).	103 (100), 77 (62), 160 (73), 175 (15), 207 (7), 149 (17), 63 (28), 150 (18), 131 (16)

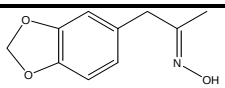
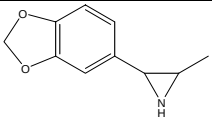
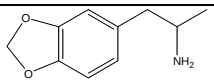
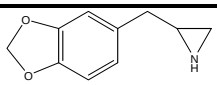
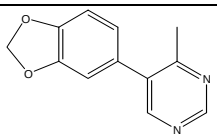
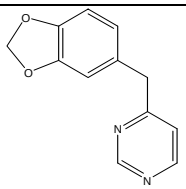
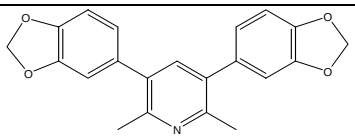
1-(3,4-methylenedioxyphenyl)-propan-2-oxime *[8.60 minutes], **(39)***		Oxime route specific intermediate between PMK (**33**) and MDA (**4**).	42 (34), 51 (44), 77 (45), 105 (16), 118 (115), 135 (100), 136 (43), 146 (23), 160 (30), 177 (19), 193 (49).
2-methyl-3-(3,4-MDP)-aziridine [7.65 minutes], (**50**)		Oxime route specific impurity.	42 (84), 51 (30), 77 (31), 78 (37), 106 (32), 135 (92), 136 (100), 177 (49).
MDA [7.11 minutes], (**4**)		Product amine sold as ecstasy. Can easily be converted to MDMA (**5**).	44 (100), 77 (8), 135 (8), 136 (19).

Table 9.1.1: Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of MDA (4) (continued overleaf).

Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
2-(3,4-methylenedioxybenzyl)-aziridine [8.14 minutes], (**51**)		Oxime route specific impurity.	51 (14), 7 (16), 89 (12), 121 (14), 135 (100), 147 (28), 148 (22), 177 (50).
4-methyl-5-(3,4-MDP)-pyrimidine [10.09 minutes], (**52**)		Leuckart route specific impurity derived from reaction between PMK (**33**) and *N*-formyl MDA (**36**).	51 (24), 77 (30), 135 (6), 155 (31), 156 (19), 185 (10), 213 (59), 214 (100), 215 (26).
4-(3,4-Mdbenzyl)-pyrimidine [9.86 minutes], (**53**)		Leuckart route specific impurity derived from reaction between PMK (**33**) and *N*-formyl MDA (**36**).	62 (16), 76 (10), 88 (13), 102 (14), 145 (12), 146 (13), 155 (21), 156 (13), 183 (7), 213 (50), 214 (100), 215 (25).
2,6-dimethyl-3,5-di(3,4-MDP)-pyridine [18.24 minutes], (**23**)		Leuckart route specific impurity derived from reaction between PMK (**33**) and *N*-formyl MDA (**36**).	288 (11), 346 (27), 347 (100), 348 (26).

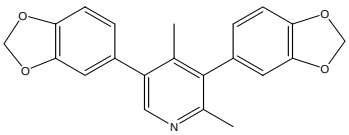
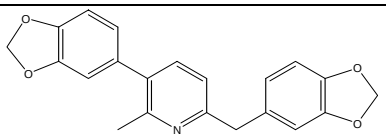
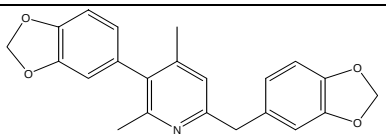
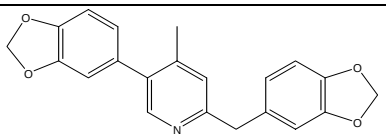
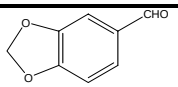
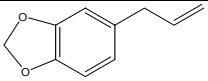
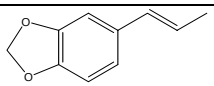
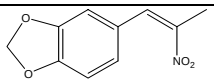
2,4-dimethyl-3,5-di(3,4-MDP)-pyridine [18.72 minutes], (**54**)		Leuckart route specific impurity derived from reaction between PMK (**33**) and *N*-formyl MDA (**36**).	288 (10), 346 (14), 347 (100), 348 (26).
6-methyl-5-(3,4-MDP)-2-(3,4-MDbenzyl)-pyridine [17.94 minutes], (**55**)		Leuckart route specific impurity derived from reaction between PMK (**33**) and *N*-formyl MDA (**36**).	77 (13), 135 (10), 15 (8), 288 (10), 318 (13), 346 (52), 347 (100), 348 (24).
2,4-dimethyl-3-(3,4-MDP)-6-(3,4-Mdbenzyl)-pyridine [18.03 minutes], (**56**)		Leuckart route specific impurity derived from reaction between PMK (**33**) and *N*-formyl MDA (**36**).	77 (11), 135 (8), 165 (36), 302 (9), 332 (15), 346 (19), 347 (11), 360 (65), 361 (100), 362 (27).
4-methyl-5-(3,4-MDP)-2-(3,4-Mdbenzyl)-pyridine [19.04 minutes], (**57**)		Leuckart route specific impurity derived from reaction between PMK (**33**) and *N*-formyl MDA (**36**).	77 (13), 158 (158), 288 (17), 318 (10), 346 (13), 347 (100), 348 (23).

Table 9.1.1 (continued): Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of MDA (4).

Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
Piperonal [5.85 minutes], (**11**)		Precursor for Piperonal methyl ketone (PMK) (**33**) and for 1-(3,4-MDP)-2-nitro-1-propene (**35**)	149 (100), 150 (95), 63 (35), 121 (32), 65 (21), 91 (12).
Safrole [5.42 minutes], (**40**)		Precurser for 3,4-methylenedioxy-bromo-2-propane (**41**).	50 (15), 51 (40), 63 (16), 77 (43), 103 (41), 104 (54), 105 (14), 131 (45), 132 (16), 135 (34), 161 (29), 162 (100), 161 (13).
Isosafrole [6.27 minutes], (**34**)		Precursor for PMK (**33**)	162 (100), 104 (44), 103 (41), 131 (40), 77 (31), 51 (24).
1-(3,4-MDP)-2-nitro-1-propene		Intermediate between piperonal (**11**) and MDA	103 (100), 77 (62), 160 (73), 175 (15),

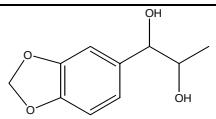
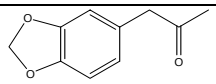
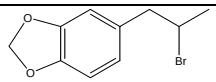
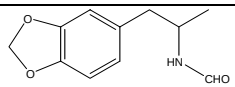
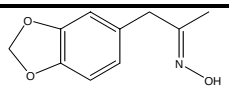
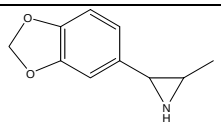
[9.91 minutes], (**35**)		(**4**). Also precursor to PMK (**33**).	207 (7), 149 (17), 63 (28), 150 (18), 131 (16)
3,4-MDP-propane-2,3-diol [7.12 minutes], (**71**)		Intermediate between Isosafrole (**11**) and PMK (**33**).	65 (42), 93 (74), 123 (21), 151 (100), 196 (24).
Piperonyl methyl ketone [7.08 minutes], (**33**)		Intermediate between 1-(3,4-MDP)-2-nitro-1-propene (**35**) and MDA (**4**) (nitropropene route). Precursor to N-formyl MDA (**36**). Starting material for MDMA (**5**) via one step reductive amination route. Precursor to oxime route.	135 (100), 77 (27), 178 (25), 51 (21).
3,4-MDP-2-bromopropane [8.09 minutes], (**41**)		Intermediate between Safrole (40) and MDMA (5) in the bromopropane route.	51 (16), 77 (21), 105 (15), 135 (100), 163 (19), 242 (19), 244 (21)
***N*-formyl MDA** [10.34 minutes], (**36**)		Leuckart route specific intermediate between PMK (**33**) and MDA (**4**).	162 (100), 135 (75), 77 (40), 72 (40). 207 (12).

Table 9.1.2: Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of MDMA (5) (continued overleaf).

Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
1-(3,4-methylenedioxyphenyl)-propan-2-oxime [8.60 minutes], (**39**)		Oxime route specific intermediate between PMK (**33**) and MDA (**4**).	42 (34), 51 (44), 77 (45), 105 (16), 118 (115), 135 (100), 136 (43), 146 (23), 160 (30), 177 (19), 193 (49).
2-methyl-3-(3,4-MDP)-aziridine [7.65 minutes], (**50**)		Oxime route specific impurity.	42 (84), 51 (30), 77 (31), 78 (37), 106 (32), 135 (92), 136 (100), 177 (49).

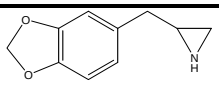
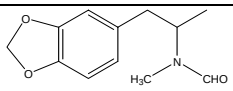
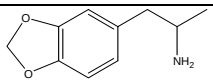
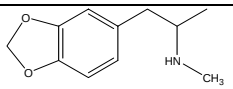
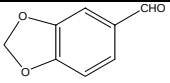
2-(3,4-methylenedioxybenzyl)-aziridine [8.14 minutes], (**51**)		Oxime route specific impurity.	51 (14), 7 (16), 89 (12), 121 (14), 135 (100), 147 (28), 148 (22), 177 (50).
***N*-formyl, *N*-methyl MDA** [10.52 minutes] (**37**)		Leuckart route specific intermediate between PMK (**33**) and MDMA (**5**).	51 (33), 72 (41), 77 (41), 105 (11), 135 (75), 136 (20), 162 (100), 207 (9).
MDA [7.11 minutes], (**4**)		Product amine sold as ecstasy, which can easily be reduced to form MDMA (**5**).	44 (100), 77 (8), 135 (8), 136 (19).
MDMA [7.53 minutes], (**5**)		Product amine sold as ecstasy.	51 (5), 58 (100), 77 (6), 135 (5).

Table 9.1.2 *(continued)*: Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of MDMA (5).

Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
Piperonal [5.85 minutes], (**11**)		Precursor for Piperonal methyl ketone (PMK) (**33**) and for 1-(3,4-MDP)-2-nitro-1-propene (**35**).	149 (100), 150 (95), 63 (35), 121 (32), 65 (21), 91 (12).

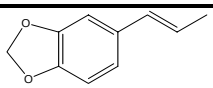
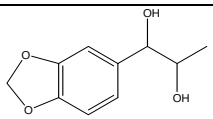
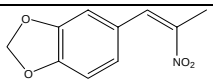
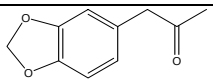
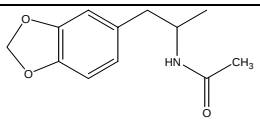
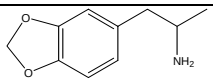
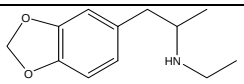
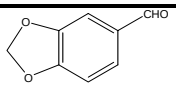
Isosafrole [6.27 minutes], (**34**)		Precursor for PMK (**33**).	162 (100), 104 (44), 103 (41), 131 (40), 77 (31), 51 (24).
3,4-MDP-propane-2,3-diol [7.12 minutes], (**71**)		Intermediate between Isosafrole (**34**) and PMK (**33**).	65 (42), 93 (74), 123 (21), 151 (100), 196 (24).
1-(3,4-MDP)-2-nitro-1-propene [9.91 minutes], (**35**)		Intermediate between piperonal (**11**) and PMK (**33**).	103 (100), 77 (62), 160 (73), 175 (15), 207 (7), 149 (17), 63 (28), 150 (18), 131 (16).
Piperonyl methyl ketone [7.08 minutes], (**33**)		Intermediate between 1-(3,4-MDP)-2-nitro-1-propene (**35**) and MDA (**4**) (nitropropene route).	135 (100), 77 (27), 178 (25), 51 (21).
***N*-acetyl MDA** [10.10 minutes], (**38**)		Intermediate between MDA (**4**) and MDEA (**6**).	51 (21), 77 (26), 86 (17), 135 (33), 162 (100), 16 (14), 221 (6).
MDA [7.11 minutes], (**4**)		Intermediate between PMK (**33**) and N-acetyl MDA (**38**)	44 (100), 77 (8), 135 (8), 136 (19).
MDEA [7.94 minutes], (**6**)		Product amine sold as ecstasy	51 (10), 72 (100), 77 (13), 105 (4), 135 (12).

Table 9.1.3: Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of MDEA (6).

Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
Piperonal [5.85 minutes], (**11**)		Precursor for Piperonal ethyl ketone (PMK) (**16**)	149 (100), 150 (95), 63 (35), 121 (32), 65

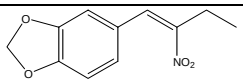
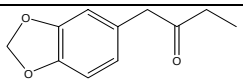
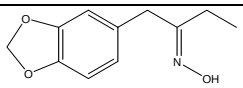
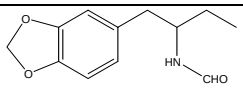
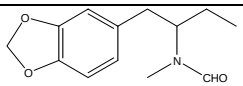
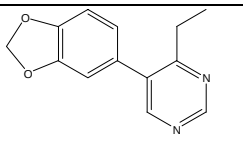
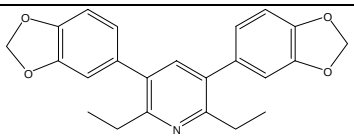
		and for 1-(3,4-MDP)-2-nitro-1-butene (**15**)	(21), 91 (12).
3,4-MDP-(2-nitro-but-1-ene) [10.21 minutes], (**15**)		Intermediate between piperonal (**11**) and BDB (**7**) or between piperonal (**11**) and PEK (**16**).	51 (27), 63 (35), 91 (43), 102 (32), 115 (100), 116 (51), 117 (65),145 (32), 150 (16), 159 (25), 174 (54), 221 (52).
Piperonyl ethyl ketone [7.95 minutes], (**16**)		Precursor for *N*-formyl BDB (**17**) and *N*-formyl, *N*-methyl BDB (**18**) via the Leuckart reaction. Precursor for MBDB (**8**) via reductive amination route. Precursor for oxime route to BDB (**7**).	51 (21), 57 (21), 77 (25), 105 (6), 135 (100), 192 (22).
3,4-methylene dioxyphenyl-(butan-2-one oxime) [9.66 minutes], (**43**)		Intermediate between PEK (**16**) and BDB (**7**).	51 (36), 63 (39), 77 (30), 91 (55), 102 (32), 115 (100), 116 (47), 117 (66), 135 (17), 145 (29), 159 (13), 174 (72), 221 (50).
***N*-formyl BDB** [10.67 minutes], (**17**)		Intermediate between PEK (**16**) and MBDB (**8**).	58 (60), 86 (65), 135 (62), 161 (12), 176 (100), 221 (9).
***N*-formyl, *N*-methyl BDB** [10.99 minutes], (**18**)		Intermediate between PEK (**16**) and MBDB (**8**).	72 (67), 77 (18), 88 (11), 100 (100), 135 (20), 176 (60), 235 (2)
4-ethyl-5-(3,4-MDP)-pyrimidine [10.30 minutes], (**20**)		Leuckart route specific impurity derived from reaction between PEK (**16**) and *N*-formyl BDB (**17**).	62 (26), 84 (17), 115 (30), 145 (17), 169 (35), 197 (17), 212 (11), 227 (99), 228 (100), 229 (27).
2,6-diethyl-3,5-di-(3,4-MDP)-pyridine [18.18 minutes], (**22**)		Leuckart route specific impurity derived from reaction between PEK (**16**) and *N*-formyl BDB (**17**).	135 (8), 346 (10), 374 (100), 375 (57).

Table 9.1.3: Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of MBDB (8) (continued overleaf).

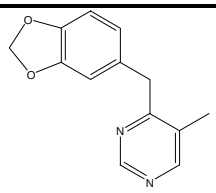
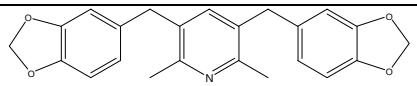
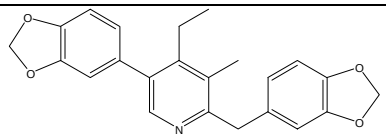
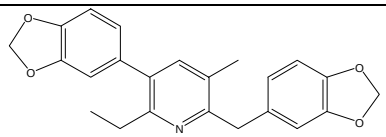
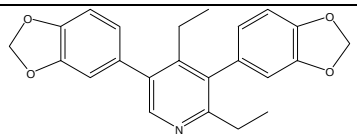
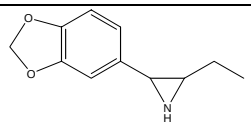
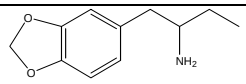
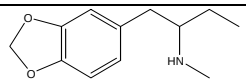
Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
5-methyl-4-(3,4-MDbenzyl)-pyrimidine [10.89 minutes], (**21**)		Leuckart route specific impurity derived from reaction between PEK (**16**) and *N*-formyl BDB (**17**).	51 (24), 77 (27), 135 (47), 169 (28), 227 (83), 228 (100), 229 (21).
2,6-dimethyl-3,5-di-(3,4-MDP)-pyridine [18.72 minutes], (**23**)		Leuckart route specific impurity derived from reaction between PEK (**16**) and *N*-formyl BDB (**17**).	77 (20), 135 (15), 207 (13), 360 (11), 374 (54), 375 (100), 376 (27).
4-ethyl-3-methyl-2-(3,4-Mdbenzyl))-5-(3,4-MDP)-pyridine [20.90 minutes], (**24**)		Leuckart route specific impurity derived from reaction between PEK (**16**) and *N*-formyl BDB (**17**).	44 (28), 73 (26), 135 (14), 207 (38), 253 (12), 281 (15), 374 (100), 375 (46).
2-ethyl-5-methyl-3-(3,4-MDP)-6-(3,4-Mdbenzyl)-pyridine [19.78 minutes], (**25**)		Leuckart route specific impurity derived from reaction between PEK (**16**) and *N*-formyl BDB (**17**).	77 (12), 135 (12), 172 (9), 252 (15), 316 (8), 346 (14), 360 (15), 374 (92), 375 (100), 376 (32).
2,4-diethyl-3,5-di-(3,4-MDP)-pyridine [18.37 minutes], (**26**)		Leuckart route specific impurity derived from reaction between PEK (**16**) and *N*-formyl BDB (**17**).	135 (8), 346 (10), 374 (100), 375 (58), 376 (13).
3,4-MDP-3-ethyl aziridine [8.30 minutes], (**58**)		Aziridine impurity derived from the the synthesis of BDB (**7**) from 3,4-methylene dioxy phenyl-(butan-2-one oxime) (**43**)	54 (27), 65 (20), 77 (29), 91 (32), 119 (45), 135 (47), 149 (42), 162 (13), 176 (100), 190 (58), 191 (70).
BDB [8.00 minutes], (**7**)		BDB (**7**) (product amine which can be easily converted to MBDB (**8**)	58 (100), 72 (11), 135 (12), 136 (20).
MBDB [8.23 minutes], (**8**)		MBDB (**8**) product amine sold as ecstasy.	51 (19), 72 (100), 77 (23), 135 (19).

Table 9.1.3 (continued): Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of MBDB (8).

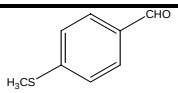
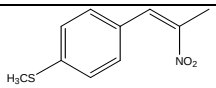
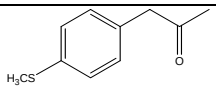
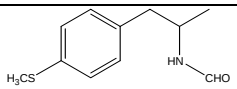
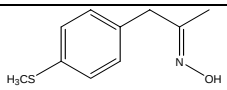
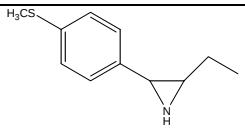
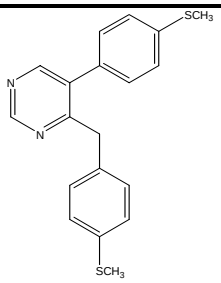
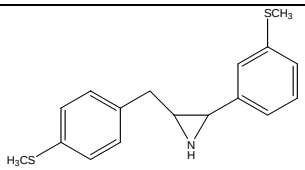
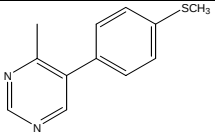
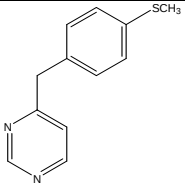
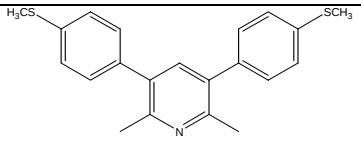
Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z (% abundance)**
4-methylthio-benzaldehyde [7.15 minutes], (**46**)		Precursor for the formation of the1-methylthio-4-(2-nitro-propenyl)-benzene (**47**).	50 (18), 51 (18), 54 (11), 69 (16), 77 (13), 108 (14), 109 (16), 123 (14), 151 (88), 152 (100), 153 (15).
1-methylthio-4-(2-nitro-propenyl)-benzene [10.86 minutes], (**47**)		Intermediate between 4-methylthio-benzaldehyde (**46**) and 1-(4-methylthio-phenyl)-propan-2-one (**44**).	63 (15), 77 (15), 89 (19), 103 (10), 115 (100), 116 (64), 147 (32), 162 (69), 209 (43).
1-(4-methylthio-phenyl)-propan-2-one [8.16 minutes], (**44**)		Precursor to 4-MTA (**9**) via reductive amination. Precursor for *N*-formyl 4-MTA (**48**) (Leuckart route). Also precursor for 1-(4-methylthio-phenyl)-propan-2-oxime (**49**) (Oxime route).	63 (12), 78 (15), 89 (11), 121 (25), 122 (33), 137 (100), 180 (24).
***N*-formyl 4-MTA** [10.64 minutes], (**48**)		Intermediate between 1-(4-methylthio-phenyl)-propan-2-one (**44**) and 4-MTA (**9**). Leuckart route specific impurity.	444 (56), 72 (46), 91 (11), 122 (20), 137 (55), 164 (100), 209 (10).
1-(4-methylthio-phenyl)-propan-2-oxime [9.57 minutes], (**49**)		Intermediate between 1-(4-methylthio-phenyl)-propan-2-one (**44**) and 4-MTA (**9**). Route specific impurity of the oxime route.	63 (18), 78 (21), 89 (21), 121 (39), 12 2(51), 131 (37), 137 (100), 162 (23), 195 (46).
2-ethyl-3-(4-methylthio-phenyl)-aziridine [9.40 minutes], (**59**)		Route specific aziridine impurity derived from the oxime route	51 (17), 63 (19), 69 (14), 77 (23), 91 (23), 104 (20), 109 (15), 117 (29), 122 (12), 130 (20), 135 (32), 136 (39), 137 (41), 146 (16), 150 (41), 151 (53), 164 (8), 178 (100), 192 (37), 193 (52)

Table 9.1.4: Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of 4-MTA (9) (continued overleaf).

Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
4-(4-methylthio-benzyl)-5-(4-methylthio-phenyl)-pyrimdine [19.19 minutes], (**60**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	121 (13), 122 (19), 137 (32), 244 (9), 290 (14), 323 (13), 337 (55), 338 (100).
2-(4-methylthio-benzyl)-3-(3-methylthio-phenyl)-aziridine [16.90 minutes], (**61**)		Route specific aziridine impurity derived from the oxime route	55 (18), 137 (23), 89 (24), 91 (25), 121 (35), 122 (76), 123 (22), 137 (100), 148 (16), 164 (9), 207 (3), 281 (66), 301 (17).
4-methyl-5-(4-methylthio-phenyl)-pyrimidine [10.55 minutes], (**62**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	121 (16), 122 (24), 137 (47), 168 (56), 169 (28), 201 (40), 215 (39), 216 (100), 217 (31), 218 (12).
4-(4-methylthio-benzyl)-pyrimidine [10.66 minutes], (**63**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	122 (18), 137 (29), 244 (30), , 290 (14), 323 (12), 337 (55), 338 (100), 339 (30), 340 (12).
2,6-dimethyl-3,5-bis-(4-methylthio-phenyl)-pyridine [20.35 minutes], (**64**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	175 (10), 288 (7) 303 (7), 336 (9), 351 (100), 352 (30), 353 (13).

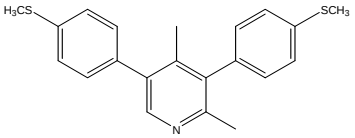
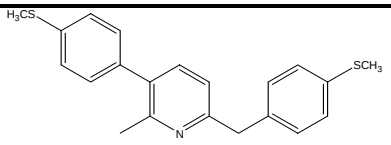
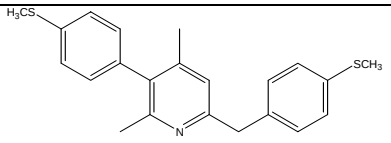
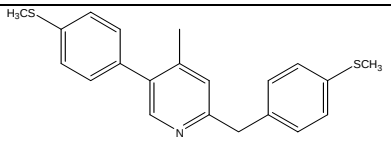
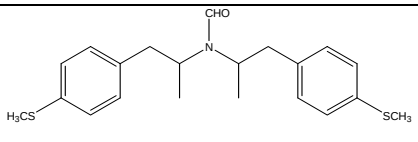
2,4-dimethyl-3,5-bis-(4-methylthio-phenyl)-pyridine [20.38 minutes], (**65**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	175 (9), 257 (6), 288 (7), 289 (8), 303 (6), 336 (8), 351 (100), 352 (31), 353 (15).

Table 9.1.4 (continued): Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of 4-MTA (9) (continued overleaf).

Name [Retention Time, minutes]	**Structure**	**Route**	**Mass Spectral fragmentation m/z** (% abundance)
2-methyl-6-(4-methylthiobenzyl)-3-(4-methylthio-phenyl)-pyridine [20.54 minutes] (**66**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	122 (9), 137 (11), 152 (10), 254 (6), 289 (9), 303 (13), 321 (7), 336 (44), 350 (58), 351 (100), 352 (31), 364 (24), 365 (37).
2,4-dimethyl-6-(4-methylthio-benzyl)-3-(4-methylthio-phenyl)-pyridine [2.47], (**67**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	137 (10), 159 (18), 303 (17), 317 (7), 335 (12), 350 (59), 364 (65), 365 (100), 366 (31), 367 (13).
4-methyl-2-(4-methylthio-benzyl)-5-(4-methylthio-phenyl)-pyridine [21.46 minutes], (**68**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	127 (9), 152 (15), 289 (11), 303 (10), 321 (7), 336 (31), 350 (100), 351 (92), 352 (31), 353 (11), 354 (2).
N,N-bis-[1-methyl-2-(4-methylthio-phenyl)-ethyl]-formamide [21.01 minutes], (**70**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	70 (11), 91 (7), 117 (25), 137 (32), 164 (100), 165 (91), 166 (19), 236 (38), 373 (1).

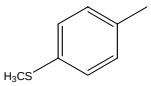
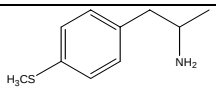
1-methyl-4-methylthio-benzene [4.46 minutes], (**69**)		Leuckart route specific impurity in the synthesis of 4-MTA (**9**) via *N*-formyl 4-MTA (**48**).	45 (17), 91 (58), 123 (29), 138 (100).
4-MTA [7.78 minutes], (**9**)		Product amine sold as ecstasy.	44 (100), 78 (12), 91 (15), 122 (21), 137 (21), 138 (65).

Table 9.1.4 (continued): Names, structures, synthetic route information and mass spectral data for selected intermediates, impurities and product for the synthesis of 4-MTA.

Section 9.2: GC-MS analysis of ecstasy of licit origin

A total of seven samples of various ecstasy type drugs which had been prepared in the School of Pharmacy via known routes were analysed for the presence for any of the impurities entered into the mass spectral library.

The first sample to be analysed was a sample of MDMA (**5**) that had been prepared via the Leuckart route of synthesis. MDA (**4**) and MDEA (**6**) were detected as impurities. There is also a spectrum that on visual inspection shows similarities to *N*-formyl, *N*-methyl MDA (**37**) at 10.5 minutes (the same retention time as the *N*-formyl, *N*-methyl MDA (**37**) standard. The presence of this component indicates that the MDMA (**5**) was indeed synthesised via the Leuckart route.

The second sample to be analysed was 4-MTA (**9**). This had been prepared via the reductive amination route and as expected there were no impurities observed

The third sample analysed was BDB (**7**), which had been prepared via the Leuckart route. This extract was found to contain the 3,4-methylenedioxyphenyl-(butan-2-one oxime) (**43**) eluting at 9.3 minutes (Quality 93%) which may have been due to the synthesis of PEK (**16**) and also the *N*-formyl BDB (**17**), eluting at 10.7 minutes which was due to the Leuckart reaction (see Figure 9.2.1). Both the retention time and the mass spectrum for the unknown matched those of the standard well (mass spectral match

quality, 93 %). See Figure 9.2.2 for the selected ion monitoring of the 176 ion which is the base peak of the mass spectrum of *N*-formyl BDB (**17**).

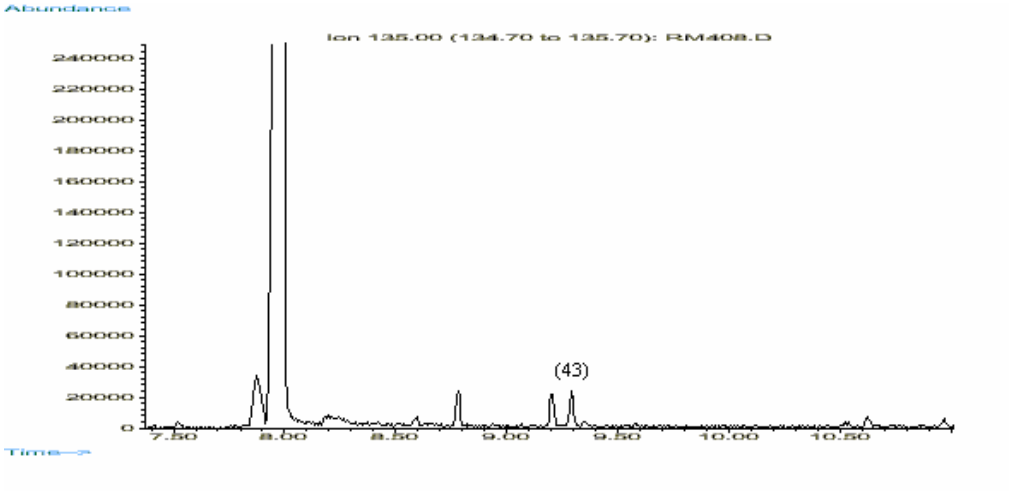


Figure 9.2.1: Selected Ion Monitoring Chromatogram of the 135 ion for the analysis of BDB made via the Leuckart route. The 3,4-methylenedioxyphenyl-(butan-2-one oxime) (43) is annotated above its peak..

The oxime is most likely an intermediate resulting from the synthesis of PEK (**16**) via the oxime route, while the *N*-formyl BDB (**17**) impurity is the intermediate between PEK (**16**) and MBDB (**8**) for the Leuckart reaction.

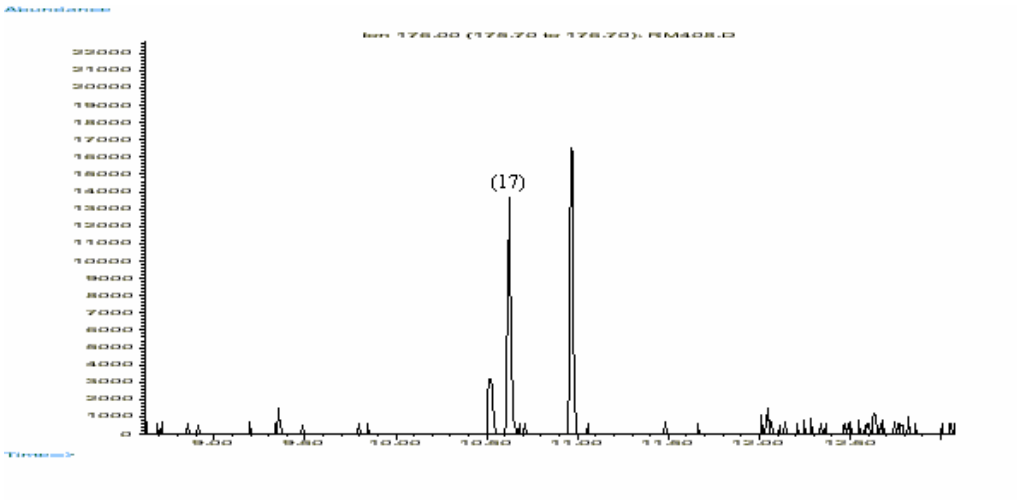


Figure 9.2.2: Selected Ion Monitoring Chromatogram of the 176 ion for the analysis of BDB made via the Leuckart route. The compound N-formyl BDB (17) is annotated above its peak.

The next sample analysed was MDEA (**6**) synthesised via the reductive amination route. There were no obvious impurities present other than, low levels of MDA (**4**) and MDMA (**5**) (less than 0.2% based on peak area measurements), which was as expected for the reductive amination route.

The fifth sample analysed was MDA (**4**) which had been prepared via the Leuckart reaction, MDMA (**5**) and MDEA (**6**) were present as minor impurities, however the presence of *N*-formyl MDA (**36**) noted at 10.0 minutes indicating that the sample had been prepared via the Leuckart reaction (see Figure 9.2.3). The mass spectrum of the unknown and the standard *N*-formyl MDA (**36**) had a match quality of 81%, which was considered good. Hence, the MDA (**4**) was proven to have been manufactured via the Leuckart route.

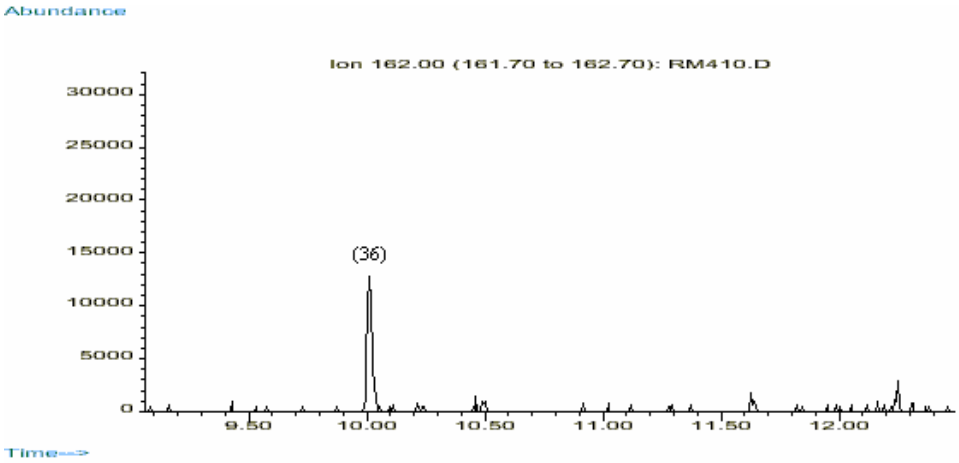


Figure 9.2.3: Selected Ion Monitoring Chromatogram of the 162 ion for the analysis of MDA (4) made via the Leuckart route. The compound N-formyl MDA (36) is annotated above its peak.

The sixth sample analysed was MDA (**4**) prepared via the oxime route. Although containing MDA (**4**), there were no impurities observed. This may have been due to the fact that the procedure used to clean up the product was particularly rigorous (vacuum distillation, flash column chromatography) and leaving the level of impurity at an undetectable level. One would expect to have detected the route specific aziridine impurities (**50**) and (**51**), however these compounds were not detected.

The final sample, which was analysed, was a sample of MDA (**4**), which had been prepared via the oxime route. The sample analysed in this case was the crude reaction product prior to clean up. Although the chromatogram contains many peaks (see Figure 9.2.4), the aziridine impurities (**50**) and (**51**) which are route specific for the oxime route to MDA (**4**) were easily identified with the aid of the mass spectral library. The retention times matched those of the standard (7.6 minutes and 8.1 minutes for compounds (**50**) and (**51**) respectively) and the mass spectral match quality in both cases was greater than 95%. This highlights the effect that sample clean-up has on the impurity profile of drugs.

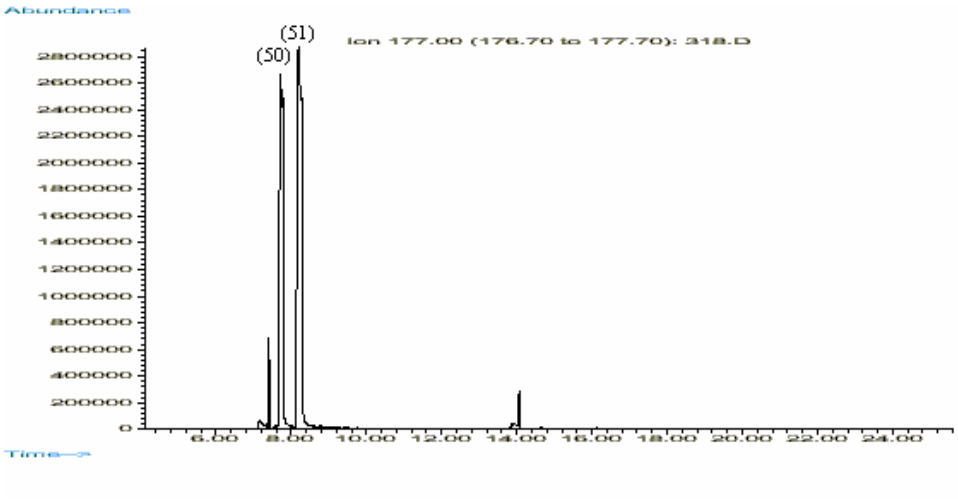


Figure 9.2.4: Selected Ion Monitoring Chromatogram of the 177 ion for the analysis of crude MDA (4) made via the Oxime route. The aziridines (50) and (51) are annotated above their respective weeks.

Section 9.3: GC-MS analysis of Ecstasy of illicit origin

The tablets discussed in section 8.5 were extracted and analysed under the conditions described. The GC-MS chromatograms obtained were searched for the presence of any compounds that had been entered into the mass spectral library, Mslib.l. It was found that some of the tablets did indeed contain impurities that indicated the route of synthesis.

The first tablet analysed was the yellow bi-convex tablet. The main peak in the chromatogram was found to be MDMA (**5**) eluting at 7.58 minutes under the conditions described (See Figure 9.3.1). There was also small peak eluting at 7.11 minutes which was identified as MDA (**4**) with the aid of the library. This was not considered unusual as this type of impurity may be as a result of any number of reasons, e.g. adulteration, poor laboratory hygiene between batches etc. The most likely source of MDA (**4**) would be incomplete reaction in the synthesis of MDMA (**5**) or side reaction due to low level impurities in the starting materials resulting in the formation of MDA (**4**).

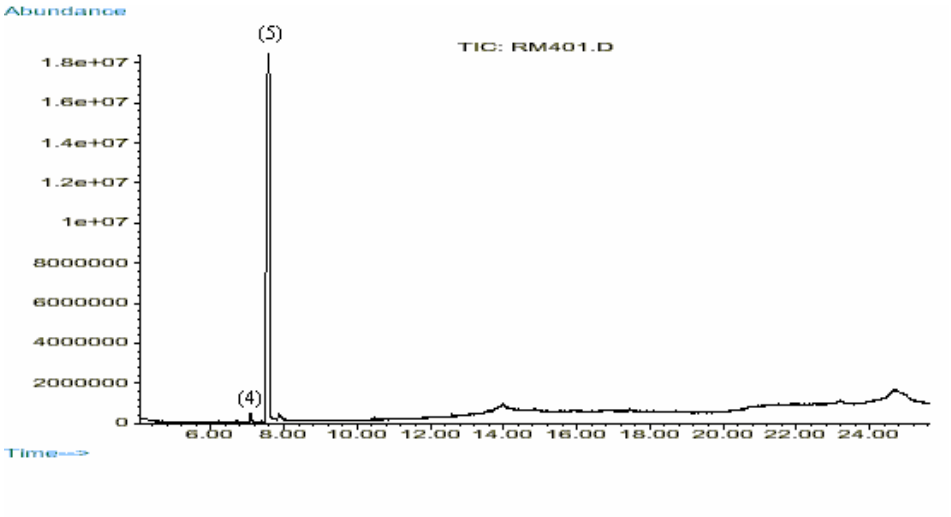


Figure 9.3.1:Reconstructed Ion Chromatogram for the extract of Tablet 1. MDA (4) and MDMA (5) are annotated on their respective peaks.

Of more interest was the presence of compound eluting at 10.5 minutes (the same retention time as the standard *N*-formyl, *N*-methyl MDA (**37**)), (see Figure 9.3.2). The peak area was found to be 0.2 % of the peak area of the main MDMA (**5**) peak. When the library was used to search for the identity of this compound it was found that the quality of the match between this unknown compound and *N*-formyl, *N*-methyl MDA (**37**) was 97 %, which is excellent. The compound was therefore considered most likely to be *N*-formyl, *N*-methyl MDA (**37**).

The consequence of this discovery is apparent if one peruses Table 9.1.2, where it is stated that *N*-formyl, *N*-methyl MDA (**37**) is the intermediate route specific product between PMK (**33**) and MDMA (**5**) in the synthesis of MDMA (5) via the Leuckart reaction. Hence it is plausible to state that this particular tablet was most likely synthesised using the Leuckart reaction via the *N*-formyl, *N*-methyl-MDA (**37**), intermediate.

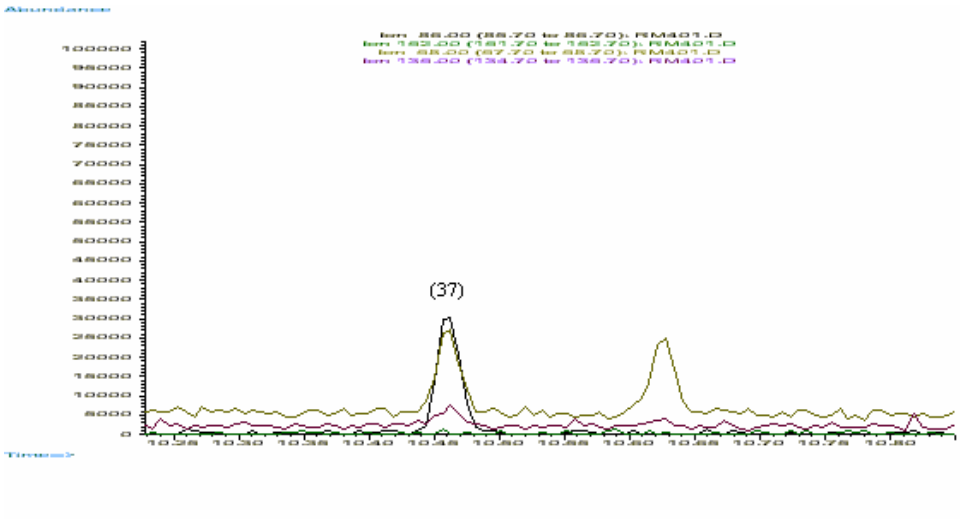


Figure 9.3.2: Selected ion monitering chromtaogram for ions 86, 58, 153 and 162 for the extract of Tablet 1.

The second tablet analysed contained mainly MDA (**4**) (see Figure 9.3.3). There was also a small MDMA (**5**) peak noticeable at 7.6 minutes. The only other peak that could be identified with the aid of the mass spectral library was a small peak eluting at 10.0 minutes (the same retention time as *N*-formyl MDA (**36**)) (see Figure 9.3.4). The mass spectrum of the unknown compound was compared against the spectra in the library and was found to match the library spectrum for *N*-formyl MDA (**36**) to a quality level of 91%, which was considered very good. The information presented in Table 9.1.1 states that the presence of this particular impurity indicates that the MDA (**4**) was synthesised using the Leuckart reaction. Hence this illicit preparation contained MDA (**4**) manufactured using the Leuckart reaction.

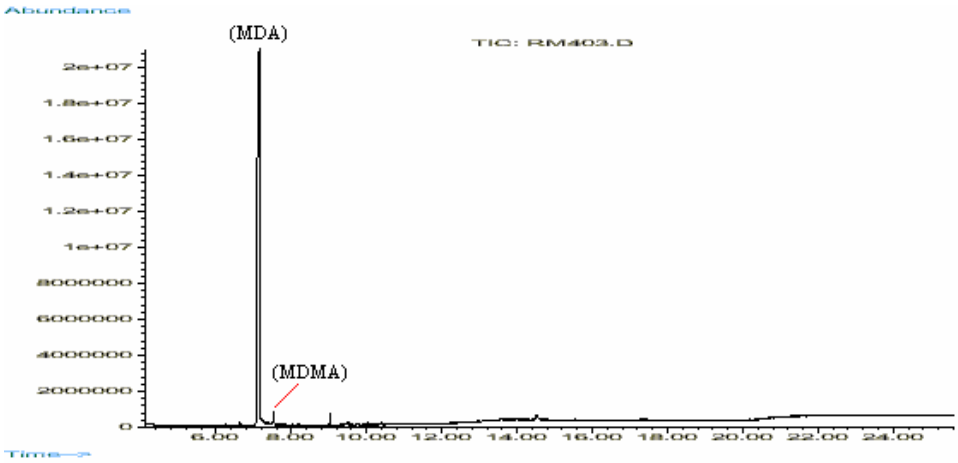


Figure 9.3.3:Reconstructed Ion Chromatogram for the extract of Tablet 3. MDA (4) and MDMA (5) are annotated at their respective peaks.

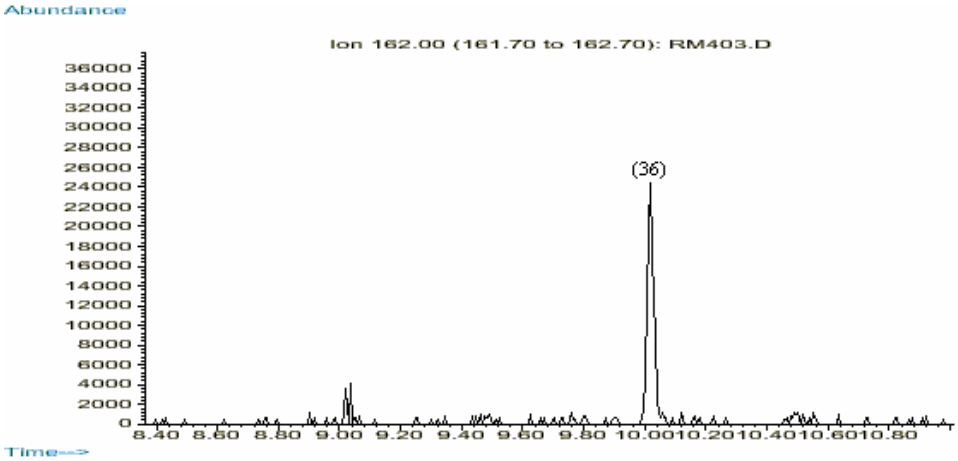


Figure 9.3.4: Selected ion monitering chromatogram for ion162 (specific for N-formyl MDA) for the extract of Tablet 3. (N-formyl MDA (36) is annotated.

Tablet 3 was a white tablet bearing a bird logo (see Figure 9.3.5). Analysis of the extract of tablet 3 revealed a similar pattern to that described for the extract of tablet 1. MDMA (**5**) was the main component eluting at 7.5 minutes and there was small pea eluting at 7.11 minutes (less than 2% the area of the main peak), which was identified as MDA (**4**). There was an MDEA (**6**) peak found eluting at 8.00 minutes, which compares well with the retention time for standard MDEA (**6**). There was also

the presence of the peak at 10.5 minutes which was successfully identified as *N*-formyl, *N*-methyl MDA (**37**). It was hard to identify as it was co-eluting with some other compound, however a match for the *N*-formyl, *N*-methyl MDA (**37**) library entry of 59 % was recorded, which is considered acceptable when the retention times for standard and unknown were compared (10.5 minutes versus 10.5 minutes, respectively) (Kavanagh, 2001). The area of the peak was particularly small; less than 0.2% the area of the primary peak. This MDMA (**5**) also seemed to be made via the Leuckart route, but could not be linked to the previous batch as it contained additional components (e.g. MDEA (**6**)), which were not evident in tablet 1.

Figure 9.3.5: Image of tablet 3 and 4.

The fourth tablet was the same as tablet 3 in as much as that the tablets were both white, of the same dimensions and bearing a similar bird logo. The main peak was MDMA (**5**), there was a trace of both MDEA (**6**) and MDA (**4**) and the presence, at a low level, of *N*-formyl, *N*-methyl MDA (**37**) could be confirmed with the aid of the library (spectral match quality 70%, retention time 10.5 minutes). The two tablets were certainly supplied to the School of Pharmacy as part of the same seizure so it is plausible that the two tablets were part of the same manufactured batch. On visual inspection the chromatograms had very similar chromatographic profiles, however the peak areas of the compounds MDA (**4**), MDEA (**6**) and *N*-formyl, *N*-methyl MDA (**37**) were estimated with the aid of the software's integration tool and divided by the area of the MDMA (**5**) peak. In the case of both tablets, the values estimated were plotted on a chart (see Figure 9.3.6). As can be seen from the chart the impurities seem to occur at the same level, with a similar ratio to each other, adding credence to the theory that they may be from the same batch. It is worth noting however, that it would be desirable to have more peaks to compare to each other or a software tool such as Spectrogram (Doolin, 2000) which would allow a qualitative mathematical comparison of the two separate chromatograms. This product was designed and manufactured by VG Gas Analysis Systems (Cheshire, U.K) but is not available for purchase at the time of writing.

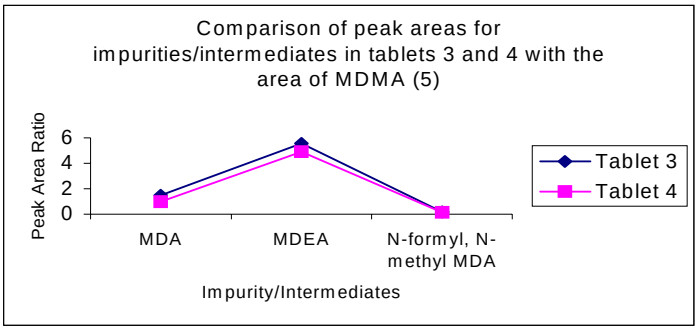


Figure 9.3.6: Chart showing peak area ratios for impurities in tablet 3 and 4 compared to the area of MDMA (5) the main constituent.

The fifth and final tablet to be analysed was a tablet bearing a Mitsubishi logo (see Figure 9.3.7). This tablet contained mainly MDMA (**5**) but it also contained a peak eluting at the same time as PMK (**33**) standard, 7.0 minutes (see Figure 9.3.8). Figure 9.3.8 shows the SIM chromatogram for the 135 ion, which is a common ion, found in compounds sharing the methylenedioxyphenyl moiety. The spectral match quality between the standard and the unknown was 74 %, which was considered good. The absence of Leuckart route impurities may indicate that the tablet was not made via this route. The presence of PMK (**33**) would rule out the Bromopropane route, as PMK (**33**) is not an intermediate of this route. The same applies to alkylation, which proceeds via a nitropropene intermediate (**35**) to MDA (**4**) and then alkylation to MDMA (**5**), without the need for PMK (**33**). The other possibility is a one step route from PMK to MDMA (**5**), via the reductive amination route. This route is known to be a favoured route of synthesis for the manufacture of MDMA (**5**) (King, 1999). This route is not known to yield any route specific impurities at a detectable level (Keating, 2001). Therefore it is possible that the route of synthesis used was the reductive amination route, but this cannot be proven as this conjecture is based predominantly on the absence of certain impurities as opposed to the presence of known route specific impurities.

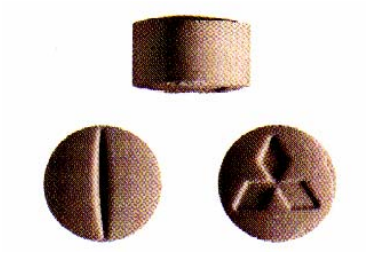


Figure 9.3.7:Image of Mitsubishis tablets analysed.

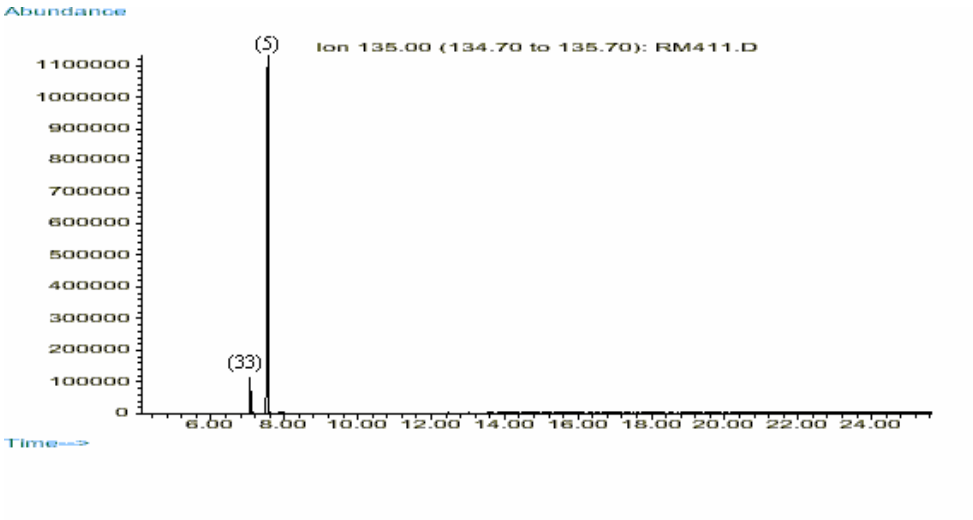


Figure 9.3.8: Selected ion monitering for ion135 for the extract of Tablet 5. The compounds MDMA (5) and PMK (33) are annotated at their respective peaks.

Section 9.4: Conclusion

The mass spectral library was easily set up once the relevant compounds were available. The library proved a useful tool in the analysis of ecstasy of illicit and licit origin, in terms of its ability to aid the determination of route of synthesis. In some cases, especially when the drug was licitly prepared identification proved difficult e.g. MDA (**4**) via the oxime route. This is primarily due to the likelihood that the samples were rigorously cleaned. Such clean up is less likely to be employed in the case of the illicit samples, but not out of the question. It is also noteworthy that the intermediates were easier to detect than most of the impurities, which had been isolated and included in the library e.g. The pyridine and pyrimidine impurities of the Leuckart routes. It may be that, by taking and extracting larger quantities of drug, detection of these impurities would be achievable.

10.1: Introduction

The metabolism of MDA (**4**), MDMA (**5**), MDEA (**6**), BDB (**7**), MBDB (**8**) and 4-MTA (**9**) is of interest on several levels as the presence/absence indicates whether a drug has been abused or not. In the emergency medicine setting it is important to be able to determine the type of compound that an admission may have ingested in order to administer suitable treatment. In accidental deaths associated with drug abuse it is important for coroners to be able to ascertain the types of drugs that the victim had ingested, which may or may not have lead to the patients death. Workplace drug testing has also become a common feature in society and therefore samples of blood and urine are often analysed for drug content prior to a hiring decision being made. Metabolism studies are also used in an attempt to determine the toxicity of certain drugs, as the parent drug itself may not be dangerous in its initial form, however it may be rendered so on metabolism. Since metabolites are claimed to be responsible for the hypothermic, the neurotoxic and/or hepatotoxic effects (Hiramatsu, 1990 & Carvalho, 1996), of amphetamine type drugs, detailed knowledge of the metabolism is necessary.

In general urine and blood are analysed for parent drugs and metabolites, with immunoassay being used as a screening procedure. Commercial amphetamine immunoassays e.g. radioimmunoassay (Cody, 1990; Kintz, 1997a), enzyme immunoassays (Kunsman, 1990; Kintz, 1997a & Howard-Taylor, 1999) or fluorescence polarisation immunoassays (Kunsman, 1990; Kintz, 1997a & Hensslin, 1996) have been used for screening methylenedioxyamphetamines. The poor selectivity of immunoassay techniques has been associated with false positive results where compounds similar to amphetamine such as phentermine, the appetite suppressant, and ephedrine, the bronchodilator and decongestant, have been found to yield erroneously positive amphetamine results (Howard-Taylor, 1999). It is noteworthy that five enzyme immunoassay systems, recently tested for accuracy all gave spurious results, however the manufacturers of all five systems stated that any positive results should be confirmed by GC-MS (Howard-Taylor, 1999). Confirmation and quantification methods using GC-MS, HPLC, CE and TLC in detection of amphetamine type designer drugs in urine and blood were recently reviewed (Kraemer, 1998). The determination of

'Ecstasy' components in alternative biological specimens (saliva, sweat and hair) has also been reviewed (Kintz, 1999).

In recent years there has been a significant amount of research carried out on the metabolism of Ecstasy-type compounds (Maurer, 2000; Garret, 1991; Helmlin, 1996; Kintz, 1999; Kintz, 1997; Sadeghipour, 1998 and Maurer, 1996). . Maurer *et al*. carried out an extensive study on the metabolism of racemic MDA (**4**), MDMA (**5**), MDEA (**6**), MBDB (**8**) and BDB (**7**), where the metabolites were identified in urine by GC-MS after enzyme hydrolysis, acetylation and methylation (Maurer, 1996). In phase I metabolism, the drugs were found to undergo *O*-dealkylation of the methylenedioxy group to form a dihydroxy derivative and *N*-dealkylation of the amine function to form the desmethyl analogue of the parent molecule (see Figure 11.2).

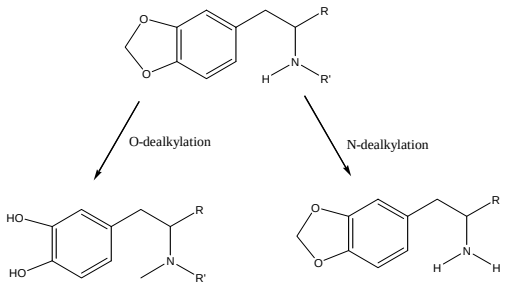


*Figure 11.2: The O-dealkylation and N-dealkylation of the methylenedioxy compounds (R=CH_3 for propanamines, R=CH_2CH_3 for butanamines), (R'=CH_3 for MDMA (**5**) and MBDB (**8**), R'=CH_2CH_3 for MDEA (**6**))*

Of course the primary amines MDA (**4**) and BDB (**7**) cannot undergo the latter pathway, as they have no *N*-alkyl group to be dealkylated. The consequence of this is that the primary amine MDA (**4**) will be present as a dealkylated metabolite in urine after ingestion of MDMA (**5**) and MDEA (**6**). Similarly BDB (**7**) will be present as the de-alkylated metabolite of MBDB (**8**). Hence the presence of the primary amine may not necessarily prove it was the ingested compound, as it may only be present as a result of metabolism. The phase II metabolism involves the formation of glucuronic acid and sulphate conjugates (Maurer, 1996).

The major metabolite in the case of MDMA (**5**) (Helmlin, 1996) and 4-MTA (**9**) (Kavanagh, 1999) in urine is the parent molecule. MDA (**4**) was detected in biological fluids using GLC (Midha, 1976). Lim *et al.* detected MDA (**4**) and MDMA (**5**) in bio-fluids using GC-MS (Lim, 1988 & Lim, 1989). MDMA (**5**) and MDA (**4**) were detected in bio-fluids by HPLC in the work of Garret *et al*. and in blood using HPLC with electrochemical detection by Michel *et al.* (Garret, 1991 & Michel, 1993). The detection of parent drug in the case MDMA (**5**), MDEA (**6**) and MBDB (**8**) was possible in samples of urine, and was used to differentiate the type of drug in the various samples (Maurer, 1996) using GC-MS. Kronstrand *et al*. detected MBDB in urine using GC-MS (Kronstrand, 1996). Kintz analysed for the parent drug and the desmethylated metabolite in an experiment used to follow the excretion of MBDB (**8**) and BDB (**7**) in urine, saliva and sweat (Kintz, 1997) with GC-MS incorporating heptafluorobutyric acid derivatisation. On the basis of these results it should be possible to determine whether methylenedioxypropanamines (MDA (**4**), MDMA (**5**) or MDEA (**6**)), methylenedioxybutanamines (BDB (**7**) or MBDB (**8**)) or thioamphetamines (4-MTA (**9**)) are being abused by analysing urine samples from individuals using these types of drugs, using GC-MS. Also as difficulty has been expressed in the past concerning the ability to distinguish the underivatised spectra of MDMA (**5**) and BDB (**7**) a derivatisation step, which might allow better spectral differentiation, may be desirable (Noggle, 1991).

The main objective of this study was to determine the types of amphetamines being abused by a group of recovering heroin addicts attending a drug rehabilitation programme in Dublin. It is widely accepted that many heroin users are polydrug abusers and a precondition of the methadone programme is the abstention from opiate drug abuse. However other drugs, such as amphetamines and benzodiazapines are being abused (Best, 2000; Schifano, 1998 & Browne, 1998). Urine samples were taken from methadone clinic clients, all from the same locality. One hundred samples which tested positive for amphetamine/ring-substituted amphetamine abuse by EMIT (enzyme multiplied immunoassay technique) immunoassay screen were further analysed. A GC-MS procedure, which incorporated a chemical derivatisation step, for the analysis of a series of amphetamine type compounds was established. The 100 samples, which had tested positive on screening, were liquid-liquid extracted and chemically derivatised and analysed using the GC-MS

procedure already established. It was hoped from the results it would be possible to see whether methylenedioxypropanamines (MDA (**4**), MDMA (**5**) & MDEA (**6**)), methylenedioxybutanamines (BDB (**7**) & MBDB (**8**)) or thioamphetamines (4-MTA (**9**)) were being abused by the cohort of clients.

Section 11.1: Instrumentation

GC-MS

The system consisted of a Hewlett Packard 5973 MSD quadrupole mass spectral detector coupled to a 6890 GC system and a 6890 Injector Autosampler. The column used was a HP5-MS (5%-phenyl-95%-dimethylsiloxane-copolymer, 30M × 0.25mm × 0.25μm film thickness). The system was computer controlled with the aid of Hewlett-Packard Chemstation software.

Immunoassay Analyser

All urine samples were tested by instrumental immunoassay on an Olympus AU 600 Analyser.

Miscellaneous Instruments

All weighing was carried out using a Sartorius BP110 S analytical weighing balance.

Section 11.2: Solvents & Blank Urine

Solvents

All solvents used were HPLC grade and 0.22 μm filtered.

Drug free urine

Drug free urine was collected from a drug free candidate outside of the drug treatment programme and stored at -18°C until required

Section 11.3: EMIT test procedure

Urine samples were taken, under strict supervision, from clients over a period of two months (July-August 1999), in a Drug Treatment Centre in Dublin. Urine samples were tested by instrumental immunoassay on a Olympus AU 600 analyser, according to manufacturers instructions, using the EMIT d.a.u. Monoclonal Amphetamine/Methamphetamine (Syva) kit. The staff of the Drug Treatment Centre conducted this procedure. EMIT positive samples were refrigerated (-18°C) until extraction (see Section 11.5).

Section 11.4:GC-MS procedure for the analysis of a series of derivatised amphetamine analogues

Preparation of analytical solutions

All reference solutions 1-7 were prepared by dissolving 2.0 mg/ml aliquots of the relevant compound(s) in 1 ml of drug free urine unless otherwise stated (see overleaf).

Reference Solution 1: Amphetamine (**3**)

Reference Solution 2: Methamphetamine (**30**)

Reference Solution 3: BDB (**7**)

Reference Solution 4: MBDB (**8**)

Reference Solution 5: MDA (**4**)

Reference Solution 6: MDMA (**5**)

Reference Solution 7: MDEA (**6**)

Reference Solution 8: 4-MTA (**9**)

Derivatisation procedure

Chemical derivatives of the reference standards (1-7) were prepared as follows. Each solution (2 ml) was made alkaline by the addition of conc. aqueous ammonia (0.5 ml) and extracted with 2 ml of petroleum ether. The supernatant petroleum ether was quantitatively transferred to a V-vial. The ether was then evaporated to dryness under a stream of nitrogen at room temperature. The residue

was reconstituted in toluene (80 µl). *N*-methyl-*bis*(trifuoracetamide) (MBTFA) (20µl) was then added to the vial immediately before injection into the chromatographic system (see below).

Chromatographic conditions

The GC-MS conditions used in the analysis are outlined in Table 11.4.1.

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	HP-5MS
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.25 µm
Transfer Line Temperature	280°C
Ionisation Mode	Electron Ionisation
Injected Volume	1µl
Injection Mode	Splitless
Initial Temperature (Hold Time)	90°C (1minutes)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	280°C (6 minutes)
Total Run Time	19.7 minutes
Detected Mass Range	40 – 600 m/z

Table 11.4.1: Chromatographic conditions used for the development of a GC-MS procedure for the analysis of a series of derivatised amphetamine analogues.

Analysis

Each of the reference solution extracts were chromatographed (in duplicate) using the above chromatographic system. The results of this section are discussed in section 12.

Section 11.5: Construction of mass spectral library.

Mass spectra of compounds analysed in section 11.4 were entered into the custom spectral library from chromatographic data generated in section 11.4. Library creation using Chemstation (Hewlett-Packard, 1986-1996) is fully explained in the software help tool. The help tool in the HP software was used in order to create and modify the library entitled Metab.l. An electronic copy of this library is included in Appendix II. This electronic copy requires Chemstation software or some other compatible software in order to function. The results of this section are shown in 13.

Section 11.6: Extraction and GC-MS analysis of EMIT positive urine

Section 11.6.1: Extraction of EMIT positive urine samples in preparation for GC-MS analysis

Sample collection and storage

Urine samples were collected as part of normal drugs of abuse screening at the Drug Treatment Centre, Trinity Court, Pearse Street, Dublin 2, during July and August 1999. 100 samples which had tested positive for Amphetamines by EMIT immunoassay in the treatment center were stored at -18°C until GC-MS analysis (see Section 11.3).

Derivatisation procedure

An aliquot of each urine sample (2ml) was made alkaline by the addition of conc. aqueous ammonia (0.5ml) and extracted with 2ml of petroleum ether. The supernatant petroleum ether was quantitatively transferred to a V-vial. The ether was then evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted in toluene (80 µl). MBTFA (20µl) was then added to the vial immediately before injection into a chromatographic system. The chromatographic system employed is described in Section 11.6.2.

Section 11.6.2: GC-MS analysis of EMIT positive urine sample extracts

Samples Analysed

The 100 EMIT positive urine samples that had been prepared in Section 11.3 were submitted for GC-MS analysis.

Chromatographic conditions

The GC-MS conditions used in the analysis are outlined in Table 11.6.1

GC-MS Parameter	GC-MS Condition
Carrier Gas	Helium
Carrier Gas Flow Rate	1 ml/min
Column	HP-5MS
Column Internal Diameter	0.25 mm
Column Length	30 M
Column Film Thickness	0.25 μm
Transfer Line Temperature	280°C
Ionisation Mode	Electron Ionisation
Injection Mode	Splitless
Initial Temperature (Hold Time)	90°C (1minutes)
Ramp Rate	15°C/min
Final Temperature (Hold Time)	280°C (6 minutes)
Total Run Time	19.7 minutes
Detected Mass Range	40 – 600 m/z

Table 11.6.1: Chromatographic conditions used for the development of a GC-MS procedure for the analysis of a series of derivatised amphetamine analogues.

Analysis

Each extract was chromatographed (in duplicate) under the GC-MS conditions described above. The results are discussed in Section 12.3.

The Drug Treatment Centre Board is responsible for the administration of much of the methadone treatment programme in Dublin. The programme is run through several drug clinics situated all over the city. Clients normally attend these clinics on a daily basis, where a Pharmacist dispenses a controlled amount of methadone. A pre-requisite of the programme is that clients do not continue to abuse drugs once they are accepted onto the programme, and instead rely only on methadone prescribed by the Clinicians of the Drug Treatment Centre Board. As the clients do not always adhere to this condition, screening programmes are necessary in order to detect non-compliance. EMIT immunoassay is a common screening procedure which is used by the Drug Treatment Centre to assess compliance with the conditions of the programme. If a client is in breach of the agreement then they are given a period of time to clear their system of the drug

(benzodiazepine, amphetamine etc.) before receiving further methadone maintenance.

Of the numerous urine samples taken over the course of July-August 1999 in one Dublin drug treatment centre 100 urine samples tested positive for amphetamine by EMIT II immunoassay. It was not discernible whether or not these samples actually contained amphetamine itself or some analogues of amphetamine. The reason for this uncertainity was the poor selectivity and cross reactivity exhibited by the immunoassay kits (Price, 1997). A positive immunoassay result could mean that one or more of a number of possible amphetamine type drugs are being abused. The d-amphetamine cut-off for the kit used is comparatively high, 1000 ng/ml (Syva®, 1998), when compared to other systems (e.g. FPIA-Amphetamine/Methamphetamine II, Abbott 300 ng/ml). Coupled with this is the fact that the detection period for MDA and MDMA is ca. 2 days (de la Torre, 1997). Hence, it is possible that many of the clients could have been using these drugs but evaded detection due to the fact that the concentration of abused drug was below the limit of detection of the screening system employed. The short half-life of the drugs in question may also have contributed to negative results on screening. As aforementioned the manufacturers of many of these systems advocate that any positive results be confirmed by GC-MS (see Section 10). Hence the objective of this study was to confirm that amphetamines (ring-substituted or otherwise) were being abused and more specifically to see whether methylenedioxypropanamines (MDA (**4**), MDMA (**5**) and MDEA (**6**), methylenedioxybutamines (MBDB (**8**)), thioamphetamines (4-MTA (**9**)) or non-ring substituted amphetamines (amphetamine (**3**) and methamphetamine (**30**)) were being abused.

Section 12.2: GC-MS procedure for the analysis of a series of derivatised amphetamine analogues.

In order to confirm the presence of amphetamine type compounds and more specifically the family of compounds (methylenedioxypropanamine/butanamines etc.), an analytical protocol was required that would allow for this type of determination. GC-MS seemed like the most logical option as it has the ability to unequivocally identify chemical compounds.

The target analytes chosen were considered the most likely amphetamine type compounds being abused in the Republic of Ireland, based on reference information (An Garda Siochana, 1997; An Garda Siochana, 1998). The compounds chosen were amphetamine (**3**), MDA (**4**), MDMA (**5**), MDEA (**6**), and MBDB (**8**). The compounds 4-MTA (**9**) and BDB (**7**) were included in this study along with the previously mentioned compounds. It was expected, based on the metabolism studies referred to in the introduction, that the parent drug would be excreted in the case of all of the aforementioned drugs and as such would be the target analyte. It is worth mentioning once more that it is not possible to identify whether the primary amines MDA (**4**) and BDB (**7**), if detected would be present as the *N*-alkyl metabolites of MDMA (**5**), MDEA (**6**), or MBDB (**8**) or whether the drug abuser additionally took them. Once the target analytes had been selected a GC-MS system capable of analysing them was required.

A GC-MS system that had been in use for some time in the Department of Pharmacology and Therapeutics, T.C.D. that involved chromatographic conditions that are applicable to most basic drugs was exploited. The system involved a HP-5 column (length: 30 M, i.d.: 0.25 mm, film thickness: 0.25 μm). The temperature programme used had an initial temperature at 90°C, which was held for 1 minute, before being ramped up to 280°C at a rate of 15°C/min. The temperature was then held at 280°C for 6 minutes (see Section 11.4 for full details). The method also employed a chemical derivatisation step using MBTFA.

A chemical derivatisation step was chosen based for two reasons.

1. Amines (the functional group common to all of the selected target compounds) do not give very featured (well fragmented) mass spectral fragmentation patterns and as such are not very good for confirmational analysis. It is much better if highly featured mass spectra can be obtained (Mc Lafferty, 1993)

2. Some of the analytes e.g. MDMA (**5**) and BDB (**7**) are isomers and concern was expressed in the past about the spectra of these compounds being too similar for the purposes of differentiation (Noggle, 1991).

The derivatisation in the case of the amines usually provides more featured fragmentation patterns when subjected to mass spectral analysis (Mc Lafferty, 1993).

Using the GC-MS system outlined (see Section 11.4) all of the compounds analysed, amphetamine (**3**), methamphetamine (**30**), MDA (**4**), MDMA (**5**), MDEA (**6**), BDB (**7**), MBDB (**8**) and 4-MTA (**9**) were sufficiently separated (based on visual inspection) (see Figure 12.1, overleaf). The retention times were determined for each of the standards which had been extracted from drug free urine (Table 12.2.1 below). The peaks were identified by comparison of the retention times of the standards containing the individual standards (reference solutions 1-7) with the retention time of the standards in the mixture (reference solution 9). Figure 12.1 was prepared by plotting the extracted ion abundance (most intense mass spectral ion for each of the analytes) versus time (minutes) from reference solution 9. The most intense ions (140 m/z, 154 m/z, 265 m/z, 135 m/z, 137 m/z and 168 m/z) were individually plotted and superimposed using the HP Chemstation software resulting in the merged chromatogram (see Figure 12.2.1, overleaf).

Analyte (TFA Derivative)	Retention Time (minutes)
Amphetamine (**3**)	5.83
Methamphetamine (**30**)	6.75
MDA (**4**)	8.50
BDB (**7**)	9.08
4-MTA (**9**)	9.16
MDMA (**5**)	9.41
MDEA (**6**)	9.75
MBDB (**8**)	9.83

Table 12.2.1: Retention times) for amphetamines analysed.

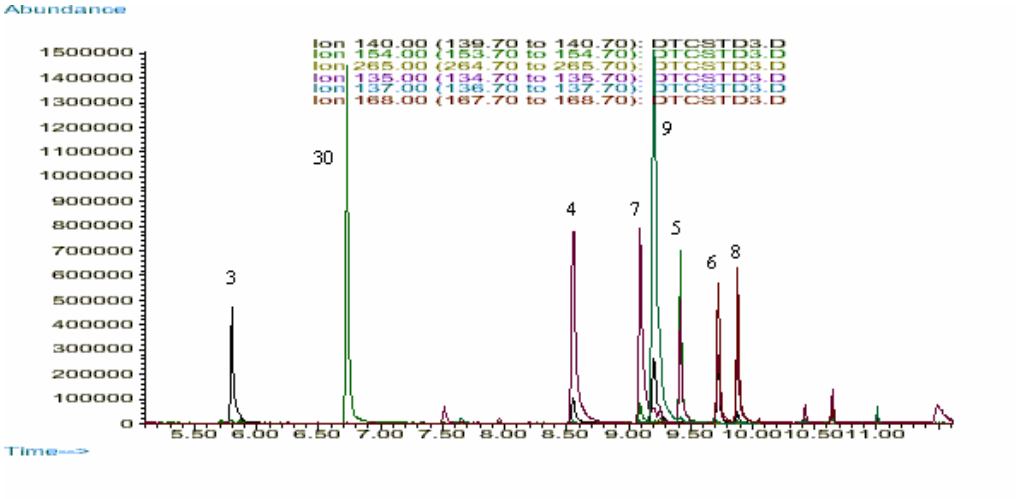


Figure 12.2.1: Extracted ions abundance (x-axis) plotted against Time (minutes). The most intense ions (140 /z, 154 m/z, 265 m/z, 135 m/z, 137 m/z and 168 m/z) were individually plotted and merged using the HP Chemstation software resulting in the merged chromatogram above. Peaks are annotated with compound reference number. Note all compounds are TFA derivatised

The mass spectra of each of the analytes consisted of well fragmented mass spectra at high mass for the MBTFA derivatives (see Table 12.2.2).

Analyte (TFA Derivative)	Main Mass Spectral Peaks m/z (relative abundance %)
Amphetamine (**3**)	140 (*100*), 118 (*92*), 91 (*66*), 69 (*31*)
Methamphetamine (**30**)	154 (*100*), 118 (*36*), 110 (*36*), 91 (*32*), 69 (*20*)
MDA (**4**)	135 (*100*), 162 (*57*), 77 (*31*), 275 (*18*)
BDB (**7**)	135 (*100*), 176 (*50*), 289 (*15*), 77 (*17*)
4-MTA (**9**)	137 (*100*), 164 (*88*), 69 (*49*), 277 (*44*), 122 (*39*)
MDMA (**5**)	154 (*100*), 168 (*100*), 135 (*73*), 110 (*44*), 97 (*34*), 280 (*14*)
MDEA (**6**)	168 (*100*), 162 (*63*), 140 (*38*), 138 (*42*), 77 (*19*), 303 (*7*)
MBDB (**8**)	168 (*100*), 176 (*85*), 135 (*59*), 303 (*11*)

Table 12.2.2: Most significant ions in the mass spectra acquired for the respective chromatographic peaks. Note: the subscript of the mass ion refers to the mass spectral peak abundance

The proposed fragmentation patterns for amphetamine (**3**), methamphetamine (**30**), MDA (**4**), MDMA (**5**), MDEA (**6**), BDB (**7**), MBDB (**8**) and 4-MTA (**9**) are shown in Figures 12.2.2, 12.2.3,

12.2.4, 12.2.5, 12.2.6, 12.2.7, and 12.2.8 respectively. Typically what happens is an alpha cleavage to form a benzyl moiety (ring substituted or otherwise) yielding two significant ions, the benzyl and he amine fragment. The formation of styrene type molecule (ring substituted or otherwise) also yields a significant fragment in the mass spectra of these compounds.

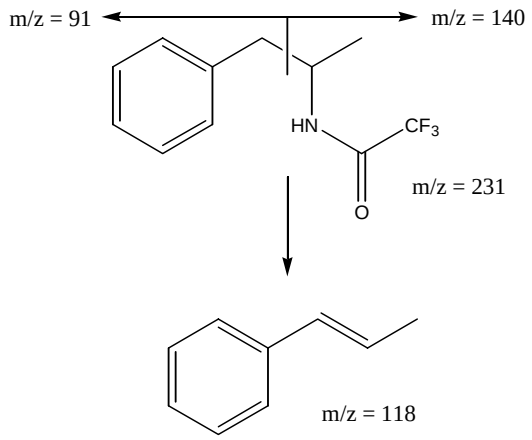


Figure 12.2.2: Proposed fragmentation pattern of the amphetamine-TFA derivative.

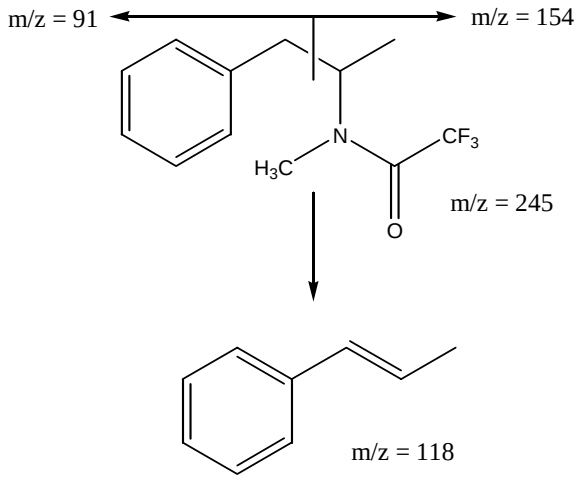


Figure 12.2.3: Proposed fragmentation pattern of the methamphetamine-TFA derivative.

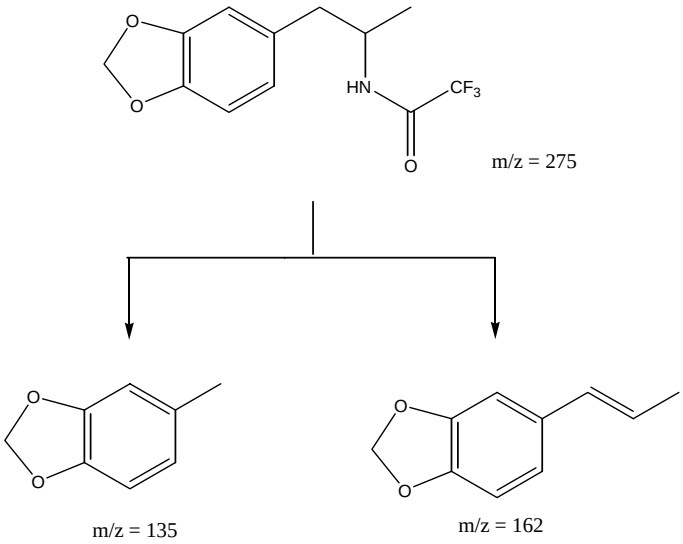


Figure 12.2.4: Proposed fragmentation pattern of the MDA-TFA derivative.

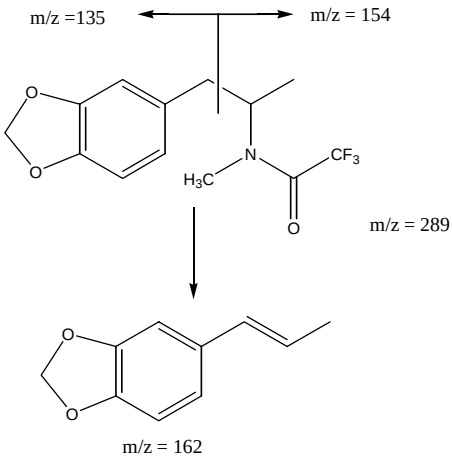


Figure 12.2.5: Proposed fragmentation pattern of the MDMA-TFA derivative.

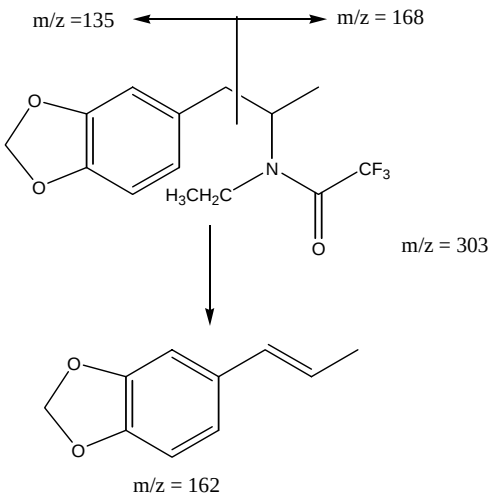


Figure 12.2.6: Proposed fragmentation pattern of the MDEA-TFA derivative.

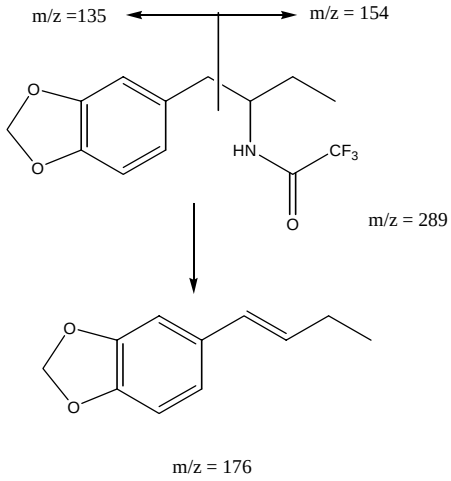


Figure 12.2.7: Proposed fragmentation pattern of the BDB-TFA derivative.

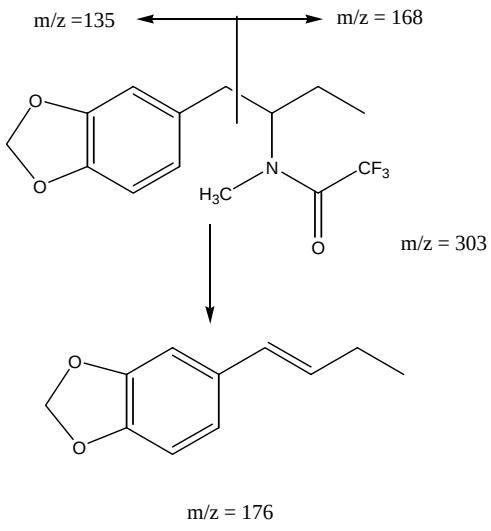


Figure 12.2.8: Proposed fragmentation pattern of the MBDB-TFA derivative.

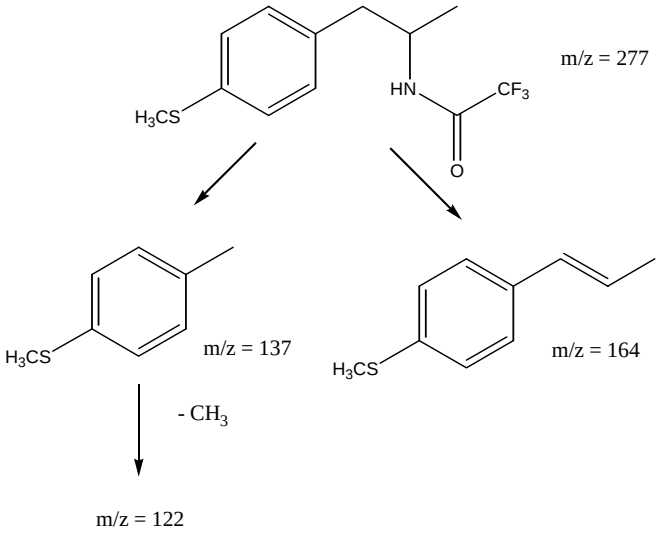


Figure 12.2.9: Proposed fragmentation pattern of the 4-MTA-TFA derivative.

Now that a suitable chromatographic system had been established, which was capable of identifying the target molecules in urine, it could be applied to the analysis of the EMIT amphetamine positive urine samples acquired from the Drug Treatment Centre. The mass spectra of the compounds were entered into a specially constructed mass spectral library, which would be used when analysing

future samples. The title of this library is metab.l and an electronic copy of it is available in Appendix II.

Section 12.3 GC-MS analysis of EMIT positive urine sample extracts

Using the GC-MS system described in section 12.2 the EMIT positive urine samples were extracted and analysed by GC-MS, as described in Section 11.4. The MBTFA derivatives of the target analytes, amphetamine (**3**), methamphetamine (**30**), MDA (**4**), MDMA (**5**), MDEA (**6**), BDB (**7**), MBDB (**8**) and 4-MTA (**9**), were searched for manually using the HP Chemstation interface in the 10 EMIT positive samples. Peaks were positively identified if the retention time and the mass spectrum (at that retention time) both matched.

In 21 out of 100 samples analysed, amphetamine (**3**) was the only drug detected. The amphetamine (**3**) peak was observed eluting at 5.83 minutes and the mass spectrum matched that of the amphetamine (**3**) peak on visual inspection. An example is shown below for sample 20 with the major selected ions extracted (140 m/z, 154 m/z, 265 m/z, 135 m/z, 137 m/z and 168 m/z) and superimposed (see Figure 12..3.1).

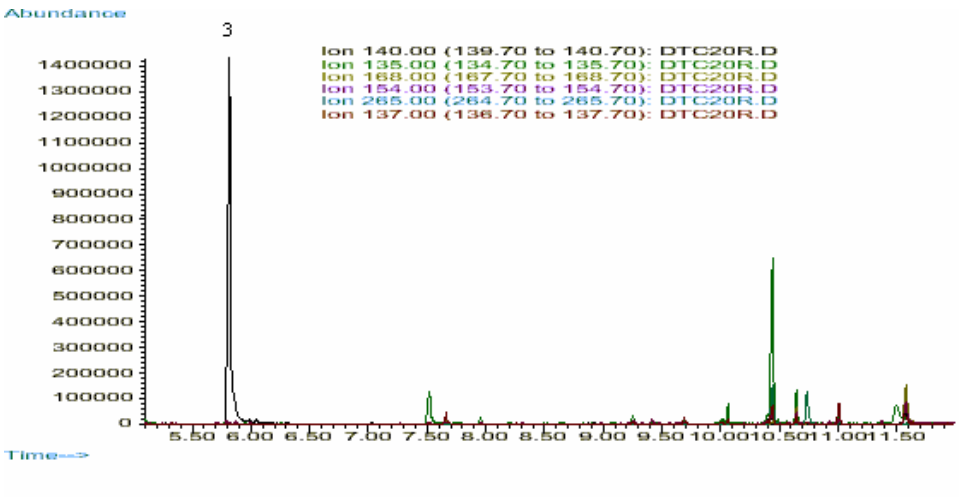


Figure 12.3.1: GC-MS chromatograms of selected ions (140 m/z, 154 m/z, 265 m/z, 135 m/z, 137 m/z and 168 m/z) used for monitoring EMIT positive sample 20. The amphetamine (3) peak is annotated above the relevant peak.

The spectrum for the compound eluting at 5.83 minutes was subjected to a search of the specially constructed library and the result is shown below (see Figure 12.3.2).

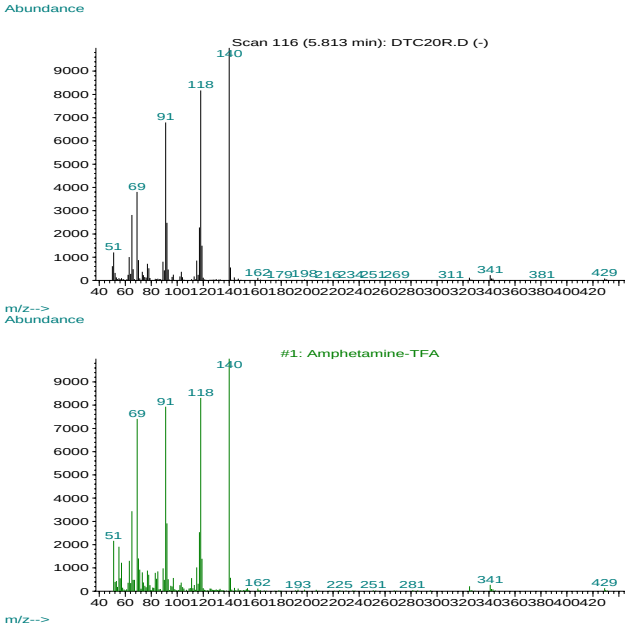


Figure 12.3.2: Comparison of spectrum of compound eluting at 5.83 minutes in the extract of sample 20, with the spectra of the specially constructed library.

As can be seen the spectra are a good match for each other, the quality algorithm which mathematically estimates the quality of the match between the two was greater than 90, which is very good (Kavanagh, 2001).

MDMA (**5**)/MDA (**4**) was the only significant substituted amphetamine in 54 of the 100 samples. Sample 21 serves as good example of this (see Figure 12.3.3). In this sample MDMA (**5**) and MDA (**4**) were detected at 9.4 minutes and 8.5 minutes respectively. These retention times matched the retention times for the standards and the mass spectra were compared with the spectra in the library.

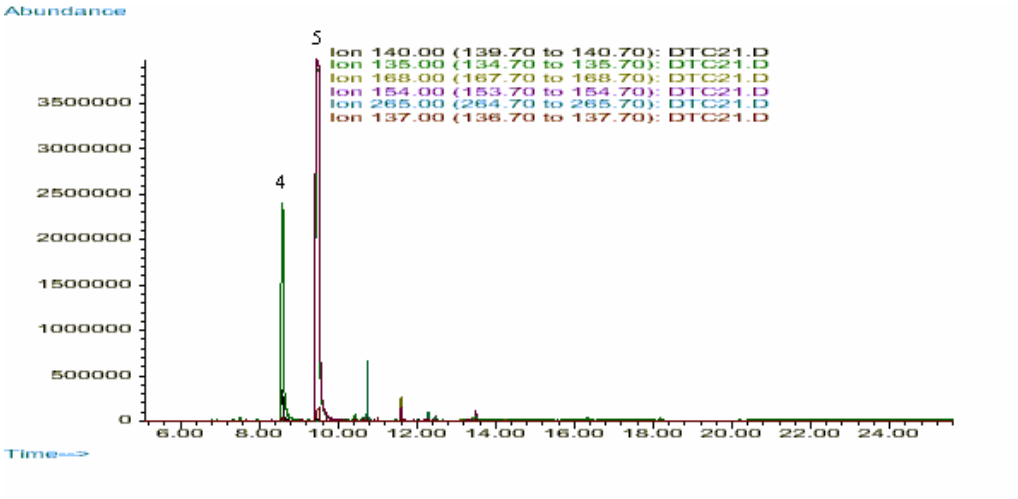


Figure 12.3.3: GC-MS chromatogram of sample 21, showing both MDA (4) and MDMA (5).

Both spectra were identified as MDA-TFA and MDMA-TFA respectively (see Figure 12.3.4) and the quality algorithm returned a value exceeding 90 in each case.

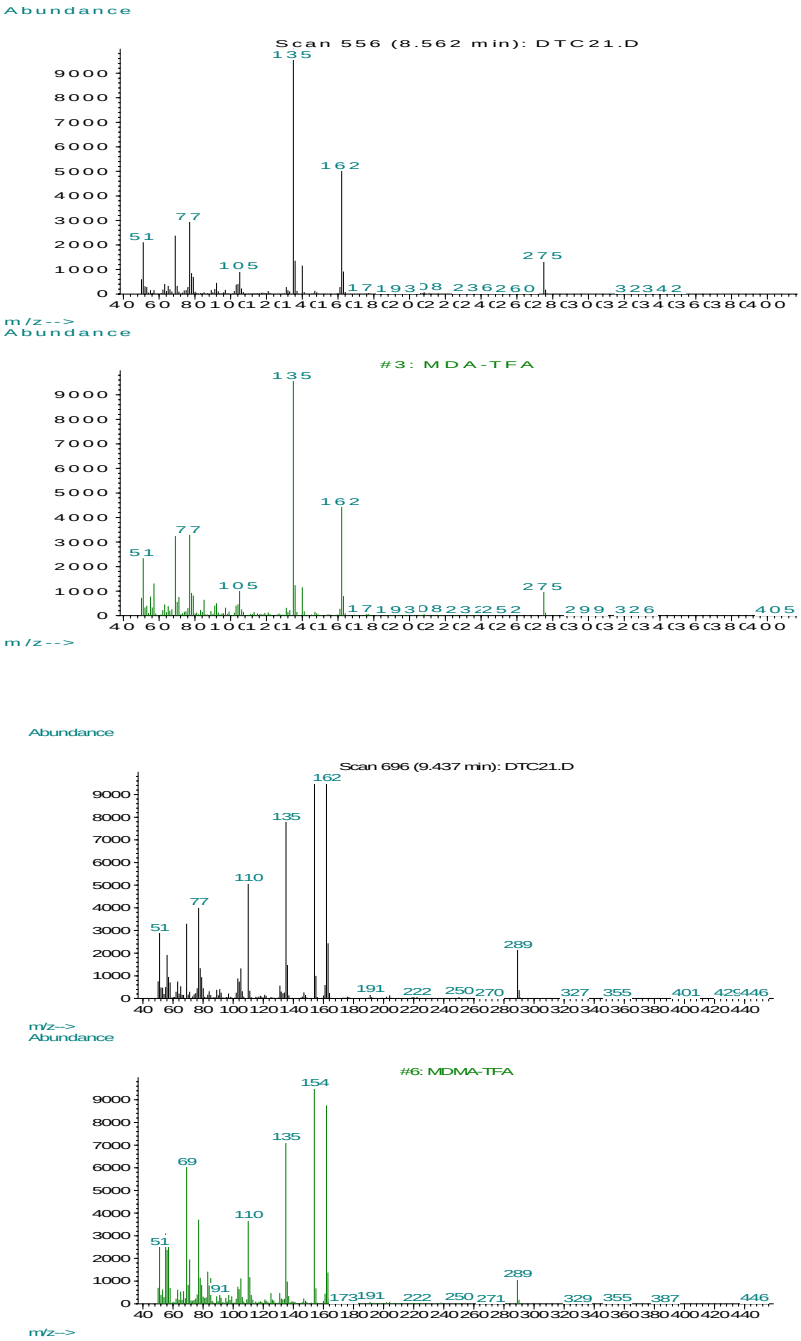


Figure 12.3.4: The two uppermost Spectra: MDA-TFA spectra compared to spectra eluting at same time in sample 21, The two lower Spectra: MDMA-TFA spectra compared to spectra of compound eluting at same time.

In all 24% of the samples showed both amphetamine and MDMA/MDA. A typical sample which was found to contain both is shown below (Figure 12.3.5) for sample 27.

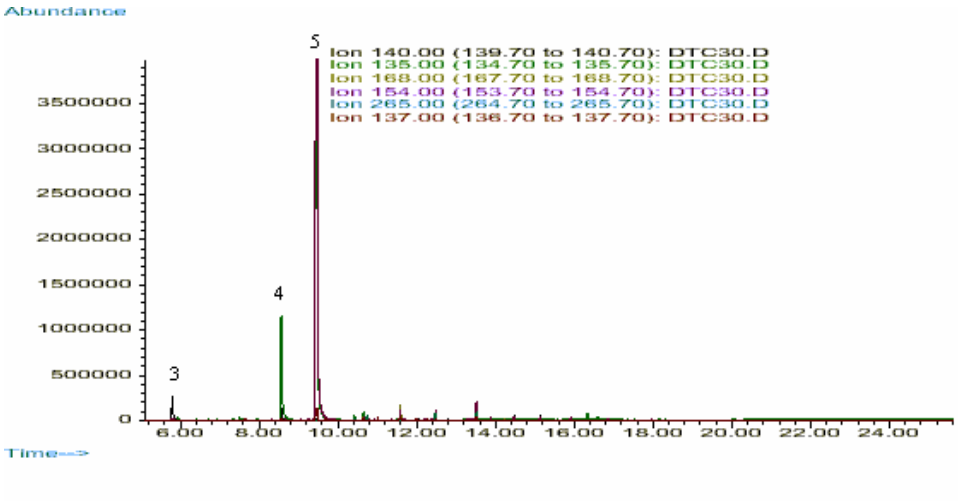


Figure 12.3.5: GC-MS chromatogram of sample 27 extract. The peaks at 5.8 minutes, 8.5 and 9.4 minutes were identified as amphetamine (3), MDA (4) and MDMA (5) respectively.

The findings indicate that propanamine ring-substituted amphetamines (MDA (**4**) and MDMA (**5**)) and non-ring substituted amphetamines, and in this case specifically amphetamine were being abused. There was no observed occurrence of MDEA (**6**). There was no detection of MBDB (**8**) or BDB (**7**), which would indicate that the particular cohort monitored, was not abusing methylenedioxybutanamines. Thioamphetamines (4-MTA (**9**)) were not detected either. There was one incidence of the anorectic, phentermine, in the samples analysed. A pie chart summarising the results is shown below (see Figure 12.3.6).

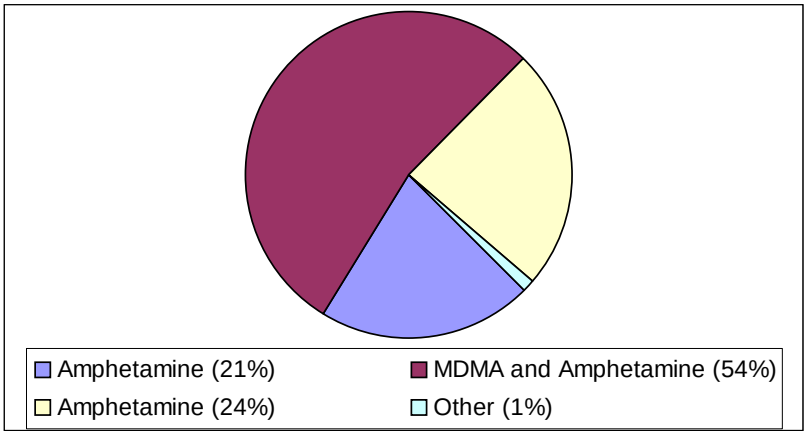


Figure 12.3.6: Pie chart summarising results of the analysis of 100 EMIT positive samples by GC-MS

The fact that there were no BDB (**7**), MBDB (**8**) or MDEA (**6**) present was not surprising as Irish Police (An Gardai) records also reflect this observation. It would appear that the majority of ''Ecstasy'' seized in the Republic of Ireland in 1998 was almost exclusively MDMA (**5**) and only a fraction of cases involved MDEA (**6**) and MBDB (**8**) (An Garda Siochana, 1998). In that same year there no reported seizures of BDB (**7**).

In Germany MDMA (**5**) and MDEA (**6**) seem to be the most significantly abused, with little MBDB (**8**) or MDA (**4**) being encountered (Sondermann, 1999). It is also reported that 95% of all drugs sold as ''Ecstasy'' in Germany contain only one compound and that only 5% of formulations appeared as mixtures, which may give anecdotal credence to the possibility that the MDA (4) in the samples was of metabolic origin. However King *et al.* (1997) described the significant occurrence of MDA (**4**), MDMA (**5**) and MDEA (**6**) in the U.K. with some samples seen as mixtures of MDMA (**5**) with MDEA (**6**) and MDMA (**5**) with MDA (**4**).

It has been shown in the past that 4-MTA (**9**) cross-reacts with the particular immunoassay system that was employed here (Kavanagh, 1999). The absence of 4-MTA (**9**) was probably due to the limited number of seizures in Europe, coupled with the high incidence of 4-MTA (**9**) associated fatality (EMCDDA, 1999), which may have lead to a decline in its use.

It is worthwhile pointing out that the extent of ring-substituted amphetamine abuse exceeds that of the non-substituted form. Amphetamine abuse among first-contact patients of the drug treatment programme in 1997 and 1998 in the Dublin area was ca. 1% (O'Brien, 1999). This low level of amphetamine abuse has been a feature of the drug scene in Ireland since the 1970's (Corrigan, 1987). The extent of ring-substituted amphetamine abuse for first-contact patients for the same region was 4.8% in 1997 and 3.7 in 1998 (O'Brien, 1999).

Although not particularly renowned for its abuse potential the appetite suppressant, phentermine, will rarely induce euphoria but can cause insomnia (Perrine, 1996). The occurrence of the Phentermine sample highlights the potentials for cross-reactivity in immunoassay systems and bolsters the need for reliable and confirmatory GC-MS procedures. It has been shown that both amphetamine and MDMA (**5**)/MDA (**4**) abuse can be confirmed by GC-MS with trifluoroacetyl derivatisation and that such abuse is occurring in the clients of the Dublin Methadone programme.

Section 12.4: Conclusion

A rapid GC-MS procedure incorporating flash derivatisation has been established for a series of amphetamines and their analogues which can be used in urinalysis to identify the type of amphetamines being abused. It also shows that in one locality in the Republic of Ireland a small group of drug users, who would most likely be exposed to similar market trends as the general populace, are being exposed

to methylenedioxypropanamines which have been around for many years and not to newer analogues such as thioamphetamines and butanamines.

Section 13.1: Historical Background

The plant *Cannabis sativa* L is among one of the world's oldest and best known plants/illicit drugs. It has been used for millennia for its fibre and intoxicating properties (Nahas, 1984). It was known to the Chinese and many other ancient civilisations as far back as the second millennium BC, when the plant was first cultivated for both its fibre and medicinal properties (Camp, 1936; Nahas, 1984). Although the plant certainly originated in Asia, its precise origin has yet to be identified (Nahas, 1984). It is thought that there was an early diffusion over a vast area stretching from the Caspian Sea to the southern Himalayas then extending into China and western Siberia, where today in the Altai valleys it can still be found in its wild state (Crescini, 1971). *Cannabis sativa* was one of the first non-food industrial plants to be cultivated by man (Mignoni, 1997/1998). Cannabis is currently controlled in most countries including the Republic of Ireland, under the terms of the Misuse of Drugs Acts 1977 and 1984 (Irish Government, 1977; Irish Government, 1984)

Section 13.2: Botany

Many botanists believe *Cannabis sativa* to be a single non-stabilised species and it was thought in 1949 that there were as many as 100 varieties or races (Bailey, 1949); this number may be far greater today. Another opinion exists however that there are three distinct species, *Cannabis sativa* of temperate United States and the rest of the world, *Cannabis indica* of central Asia, and *Cannabis ruderalis*, a more northern species growing in Russia and Siberia (Schultes, 1980). For the purposes of this thesis *Cannabis*, where referred to, will be considered as being one species, *Cannabis sativa* L. The many varieties of *Cannabis sativa* L. have been categorised further into three phenotypes which are described as the drug-type (containing high levels of psychoactive material), the intermediate–type (intermediate levels of psychoactive material) and the fibre-type (containing low levels of psychoactive material). The details of this categorisation are discussed in more detail below.

In Latin, the word *Cannabis* means hemp, and denotes the genus of the hemp family of plants. The species name, *sativa*, means planted or sown, and indicates the nature of the plant's growth (Nahas, 1984). *Cannabis sativa* is closely allied to *Humulus*, the genus of the hop plant (curiously enough

another industrial plant, which looms large in connection with another intoxicant-alcohol). *Cannabis* and *Humulus* are the only two genera of the family *Cannabaceae (order Urticales)*, but *Cannabis* is the only plant in nature containing the "cannabinoid" chemicals (see chemistry below) (Nahas, 1984). The plant is a herbaceous annual plant, which grows from a seed for a season and dies down; it grows again the following spring from its own hardy seed (Metchalfe, 1950).

Cannabis is a dioecious plant, producing in a single sowing an equal proportion of female pistillate flowers and male staminate ones (Arnoux, 1966a &1966b). Hermaphrodite plants with both male and female flowers also occur occasionally under unfavourable conditions. *Cannabis sativa* also has the ability, rarely observed in nature, to reverse its sex after being transplanted. This phenomenon is sometimes referred to as ''the confused sexuality of the plant'' and is thought to depend on the length of the daylight exposure. further individual plant parts have the ability to unpredictably change their sex as the year goes on (Schaffner, 1923 & 1931). Soil nitrogen levels are also thought to play a part in this confusion (Talley, 1934).

The morphological characteristics of this plant are distinct allowing for easy identification. A picture of a female plant is shown in Figure 13.2.1. A slender stalk, 5 –7 cm long supports the characteristic palmate leaves, with a narrow groove along the upper side. Each leaf presents 5 – 11 dark green leaflets radiating from the top of the stalk (Nahas, 1984). The soft-textured leaflets, which may be as long as 25cm, are most often narrow and shaped like a lance finely sharpened to a narrow point; the edges of the leaflets are regularly dentate like the blade of a saw, with deep ridges running diagonally from the centre to the periphery. The upper side of the leaf is dark green, contrasting with the lighter colour of its underside (Nahas, 1984).

The male flower clusters, with their pollen-laden stamina standing out from the leaves on the individual flowering branches, consist of five whitish or greenish hairy petals 3 mm long and five pendulous stamens.

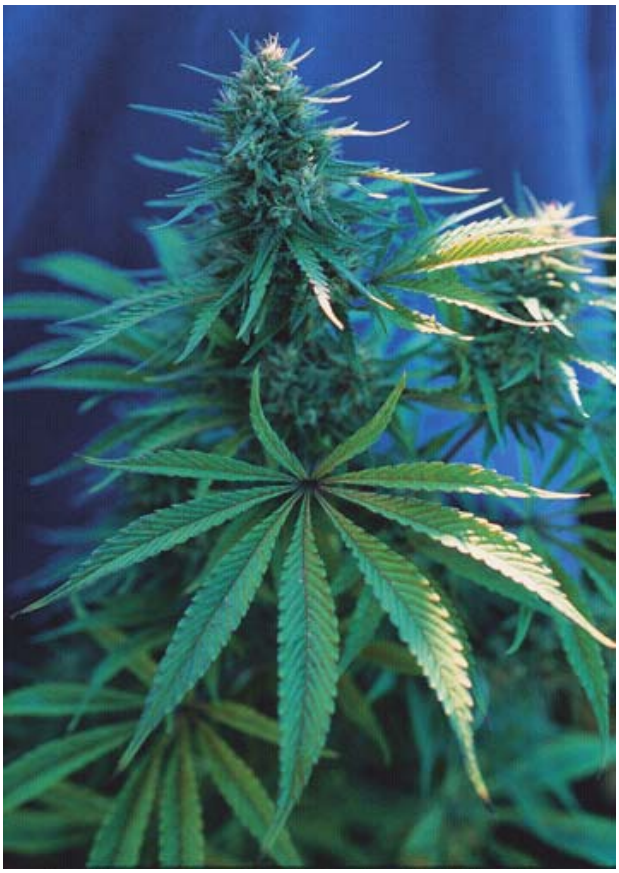


Figure 13.2.1: Female plant of the species Canabis sativa.

The female flower clusters are usually more densely packed and do not extend beyond the leaves. Each flower presents a green organ, the bract, a tubular sheath, which surrounds the ovary and out of which project two long pollen-catching stigmas. The bract is covered with hairs of which there are two types; capitate stalked or sessile *glandular* hairs and cystolythic or non-cystolythic *covering* hairs. The stalkless, circular glands secrete drops of resin. The bract, after pollination, produces a fruit, which is an achene; a hard shell containing a single seed. Female plants in general are taller and stockier than their male counterparts. , where the flowers are more tightly packed around the stem.

Both the male and female flowers produce a resin in the trichomes on the bract, which surrounds the flower. It is in these trichomes that the bulk of psychoactive biosynthesis occurs. The trichomes contain a sticky resin, which is thought to be present as a means of protecting the plants from water loss in hot

and dry climate (Perrine, 1996). Female plants generally produce more resin than males and hence they are considered to be more psychoactive than male plants (Perrine, 1984).

Cannabis is also capable of assuming astonishing variation of shape and size which has often lead to doubt being cast about whether there is only one species. A plant grown in India will look very different to one grown in America. If there is only one species these responses may be due to environmental factors such as soil, climate, human manipulation and also to *Cannabis sativa's* genetic plasticity. The plant is extremely tolerant of harsh environmental conditions and is known to thrive well in any type of soil (Nahas, 1984).

Section 13.3: Chemistry

The main chemical constituents of the plant, *Cannabis sativa*, are the cannabinoids. These compounds are unique to *Cannabis* and have not been found anywhere else in nature. Over 400 chemical compounds have been identified in varying quantities in *Cannabis*; more than 60 of these have been identified as cannabinoids (Turner, 1980). Chemically, cannabinoid structures are discernible as a terpene joined to an alkyl-substituted resorcinol. However for purposes of nomenclature the Chemical Abstracts Service (CAS) views the system as a benzopyran ring rather than a substituted terpene, leading to two numbering systems, both of which are widely employed. For the purpose of this thesis the benzopyran numbering system is used. Broadly speaking the term cannabinoid refers to the C21-compounds present in the plant and includes their transformation products and related analogues.

It took many years and unsuccessful attempts to identify the euphoric constituent in *Cannabis sativa* (Perrine, 1996). However, in the 1960's the main psychoactive constituent was identified as Δ^9-tetrahydrocannabinol (THC) (**72**) as a result of the work of Gaoni and Mechoulam (1964). Two other important cannabinoids, which are found to exist in abundance in *Cannabis sativa*, are cannabinol (CBN) (**73**) and cannabidiol (CBD) (**74**). The structures of THC (**72**), CBN (**73**) and CBD (**74**) are shown below in Figure 13.31. CBN (**73**) is not actually biosynthesised by the plant, it is the oxidative breakdown product of THC (**72**) and therefore one would not expect to find it in a fresh plant, but

rather in an aged plant (Nahas, 1984). CBN (**73**) and CBD (**74**) are not known to be euphoriants (Nahas, 1984).

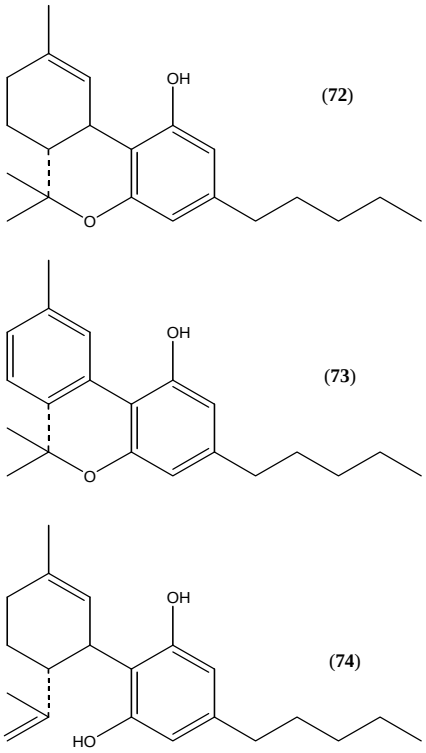


Figure 13.3.1: Structures of Δ^9-THC (72), CBN (73) and CBD (74)

Section 13.4: Pharmacology

The pharmacological activity of THC (**72**) is now known to be mediated through two types of receptors whose endogenous ligand is anandamide. The receptors are known as CB_1 and CB_2. The CB_1 receptors occur in the brain and mediate for the characteristic effects of cannabis (feeling of relaxation and well

being, increased visual and auditory perception, analgesia and depression of motor activity) (Nocerino, 2000). So far the CB_2 receptors have only been found outside the central nervous system (Nocerino, 2000). The pharmacology of the cannabinoid receptors, their agonists and antagonists have been extensively reviewed (Adams, 1996; Ameri, 1999; Glass, 1998 & Reggio, 1999).

Section 13.5: Potency and Administration

Cannabis preparations are largely derived from the female plant due to the fact that it is believed to produce the highest levels of THC (**72**) of the two sexes (Perrine, 1996). The cannabinoids are distributed throughout the whole plant but the THC (**72**) content is highest in the flowering tops of the plant (which contain the resinous glandular trichomes), declining in the leaves, lower leaves, stems and seeds (Martin, 1997/1998). Marijuana or *Cannabis* herb (''weed'', ''spliff''', ''grass'' etc.) is prepared from the dried flowering tops and leaves of the plant (THC (**72**) content 0.5%-5.0%) (Martin, 1997/1998). Hashish (hash, Lebanese Gold, Pakistani black) consists of dried cannabis resin and compressed flowers (THC (**72**) content 7%-14%) (Martin, 1997/1998). Hashish oil, derived directly from the plant via solvent extraction and distillation, may contain 15%-50% THC (**72**) (Martin, 1997/1998). Sinsemilla is a form of *Cannabis*, where all male plants are culled from any females early in the growth cycle. The females then produce higher levels of THC (**72**) (Nahas, 1984). This effect may be due to the fact that the unfertilised female plants do not have to channel their energies into reproduction. Levels of THC (**72**) up to 20% have been recorded (Adams, 1996). New varieties have also appeared on the black market (e.g. ''Nederwiet'', ''Skunk'') which have been specially bred through clandestine research to produce high levels of THC (**72**) (up to 20%) (Adams, 1996).

Cannabis can be eaten or smoked in order to experience its euphoric effects, however it is generally smoked as this results in more immediate effects. Cannabis may be smoked in a ''joint'' which is hand made cigarette (with or without tobacco), or in a water pipe (bong) (Hall, 1998).

Section 13.6: Medicinal Properties and Adverse Effects

The discovery of the cannabinoid receptors has paved the way for much research into the synthetic manipulation of the cannabinoid backbone in an attempt to discover useful drugs that may be used in the treatment of many diseases.

However, cannabis is used in modern medicine for the treatment of emesis in chemotherapy where Dronabinol (THC (**72**)) and Nabilone (a synthetic variant of THC (**72**)) which has increased water solubility) are available on prescription as anti emetics (Perrine, 1996).

As well as being useful anti-emetics, cannabinoids appear to have therapeutic value as antispasmodics, analgesics and appetite stimulants and may also have potential in the treatment of epilepsy, glaucoma and asthma (Ashton, 1999). The biomedical effects (Martin, 1997/1998; Ashton, 1999; Gurley, 1998; Marmor, 1998; McGinn, 1999; Edwards, 1999; Hollister, 1998 & Ling, 2000) and the debate surrounding their use (Grinspoon, 1999; Clark, 2000; Morris, 1997 & Gowing, 1998) have been studied and reviewed by many authors.

As mentioned, all drugs including cannabis have adverse affects. These effects have been reviewed (Hall, 1998; Ashton, 2000, Ashton 2001 & Holdcroft, 2000). Some of the main areas for concern amongst the medical community with regard to adverse effects are chronic effects such as immunosuppression, chronic bronchitis, negative reproductive effects, negative behavioural effects, dependence syndromes, impaired cognitive function and psychosis (Hall, 1998). Acute effects such as panic attacks, anxiety, paranoia, elevated blood pressure and impaired cognitive function have been reported (Hall, 1998).

Section 13.7: Cannabis and Chemical Variation.

As mentioned in the botany section above, the many varieties of *Cannabis sativa* L. are categorised further into three chemically different types or phenotypes. There is the drug-type (containing high levels of THC (**72**)), intermediate –type (intermediate levels of THC (**72**)) and fibre-type (low-level of THC (**72**)). For the purpose of this thesis hemp will refer to fibre-type cannabis, while herb will be the

term used to describe intermediate-type and drug-type cannabis. The psychoactive material referred to is THC (**72**). The level of a particular cannabinoid, be it THC (**72**) or CBD (**74**) are used to differentiate varieties of cannabis which are chemically different (Nahas, 1984).

The three phenotypes are quantitatively defined as follows.

- Drug type (THC (**72**) > 1% and CBD (**74**) = 0); plants growing in warm climates: Mexico, South Africa
- Intermediate type (THC (**72**) > 0.5% and CBD (**74**) > 0.5%); plants grown in warm climates around the Mediterranean.
- Fibre type (THC (**72**) < 0.3% and CBD (**74**) > 0.5%); Plants grown in temperate climates such as Ireland, France.

A method of classifying variants according to the following ratio of the identified cannabinoids has been suggested (Fetterman, 1971).

$$\text{Phenotype ratio} = \frac{(\%\text{THC} + \%\text{CBN})}{(\%\text{CBD})}$$

If the ratio is >1.0, the preparation is classified as drug "phenotype"
It the ratio is <1.0, the preparation is classified as fibre "phenotype."

However de Meijer *et al.* (1992) acknowledged the limitations of the approach by Fetterman *et al.* which failed to allow for an intermediate variety. de Meijer *et al.* (1992) used the following criteria for the classification of phenotypes according to cannabinoid content, including provision for an intermediate phenotype (Table 13.2).

Phenotype	[THC] (%)	[CBD] (%)	[THC](%) / [CBD] (%)
Fibre type	< 0.5	≥ 0.5	< 1

Intermediate type	≥ 0.5	≥ 0.5	
Drug type	≥ 0.5	< 0.5	> 1

Table 13.2: Phenotype characterisation criteria as defined by de Meijer et al. (1992).

CBN (**73**) was excluded from the study as the authors used values from fresh plants, which had extremely low levels of CBN (**73**).

The ideal, with regard to research conducted clandestinely for the purpose of illicit drug production has been to develop drug-type varieties with ever increasing THC (**72**) levels (Szendrei, 1997/1998). However the other extreme has been to develop fibre type cannabis with low levels of THC (**72**) and high levels of CBD (**74**) for industrial use (paper, rope etc.) (Szendrei, 1997/1998). Although the growth of drug-type *Cannabis* plants is prohibited, the growth of hemp is permitted by the E.U. and practised in the Republic of Ireland under license from the Dept. of Health and Children (European Union, 1989).

There is also an EU subsidy, which is available to farmers interested in growing hemp and since its introduction the amount of hemp harvested in the EU has increased from 5,840 hectares in 1980-1982 to 45,000 hectares in 1999 (Mignoni, 1997/1998). Ireland has been involved in the EU programme since 1998 (Maguire, 1999). The main compliance required for the licence is that the THC (**72**) content of the hemp must not exceed 0.3% in order to comply with EU Council Regulations (European Union, 1984).

Section 13.8: Extraction and Detection of Cannabinoids.

The cannabinoids are easily extracted from plant material or resin using any of a variety of organic solvents. The stability of the cannabinoids in different solvents has been reviewed (Gough, 1983), however petroleum ether seems to be a suitable choice if one intends to analyse the extract soon after extraction.

Thin layer chromatography has also played an important role in the identification of the plant *Cannabis sativa* L. and the products derived from it. With spray reagents such as Fast Blue B produce specific colours which also help to identify specific cannabinoids (Segelman, 1976). Nowadays techniques such as Gas Chromatography with Flame Ionisation Detection (GC-FID), Gas Chromatography with Mass-Spectrometry (GC-MS) and High Performance Liquid Chromatography with Ultra-Violet Detection (HPLC-UV) are the main tools used for the identification and investigation of *Cannabis sativa* L. plants and products.

In Cannabis, THC (72**) is present as its carboxylic acid (THCA). GC analysis will give the 'total' THC (**72**) present, i.e. the THC (**72**) originally in the sample, together with that formed by decarboxylation of the cannabinoid acid at elevated temperatures in the GC (Gough 1983). As most Cannabis products are smoked, GC analysis mimics the smoking process and gives a better idea of the potency of the crude drug under investigation (Baker, 1981). Otherwise the samples have to be derivatised before GC analysis in an attempt to preserve the acids (Turnet, 1974). GC has been used to good effect by many workers in the determination of THC (**72**), CBD (**74**) and CBN (**73**) (Stephanidouu, 1993, 1998 & 2000; Rasmussen, 1975; Fairbairn, 1974; Rowan, 1977 and Zeeuw, 1972). The official method for the determination of THC (**72**) in hemp, as published by the EU, is in fact a GC method (European Union, 1989). Originally this method used a packed column method and has only recently been upgraded to a capillary column method (European Union, 2000).**

GC-MS has also been used in the determination of cannabinoids in the *Cannabis sativa* L. (Yotoriyama, 1992; Lercker, 1992 and Ross, 2000).

HPLC-UV analysis has been used with good effect in the analysis of cannabis especially in the analysis of both neutral cannabinoids and their acid derivatives (Baker, 1980; Baker, 1981; DeBruyne, 1994; Kanter, 1979; Smith, 1975; Smith, 1976 and Turner, 1982).

DNA testing has also begun to appear in the literature as a means of positively *identifying Cannabis sativa* L. and its derived products (Linacre, 1998, Gigliano, 1998).

Section 13.8.1: Quantitation of the Cannabinoids.

Quantitation of THC (**72**), CBN (**73**) or CBD (**74**) via GC-FID is normally achieved via external standardisation, using standard solutions of THC (**72**), CBN (**73**) or CBD (**74**) respectively. However Poortman-van der Meer and Huizer (1999a) cast doubt on the accuracy of using THC (**72**) as a standard for a number of reasons. Firstly THC (**72**) is an oil and is supplied as a solution in ethanol, provided at a concentration specified by the supplier. The analyst has to prepare working standards from this solution introducing a possible error. Secondly, THC (**72**) is unstable and the solutions are generally labelled to state that the concentration is approximately 95% (contributing significant uncertainty to the measurement). Ideally analysts would be using a standard which is a solid crystalline material that can be acquired in high purity. However when one is using a flame ionisation detector then the method known as the effective carbon number (ECN) method can be considered as an approach to minimising these potential inaccuracies.

This approach was studied extensively by Sternberg et al. (1962) and who claimed that it is possible to predict a molecule's response in a FID detector. As THC (**72**), CBN (**73**) and CBD (**74**) have the same number of carbons, their relative response factors should not differ by much. Therefore, if one predicts the response for THC (**72**), CBN (**73**) and CBD (**74**) then one could feasibly use either CBD (**74**) or CBN (**73**) and a correction factor for THC (**72**) as an indirect standard for the determination of the other molecules. CBD (**74**) and CBN (**73**) are stable crystalline solids and are therefore very suitable for use as indirect standards in the quantitation of THC (**72**). Poortman-van der Meer *et al*. (1999a) found that the predicted and experimental response factor values for THC (**72**), CBN (**73**) and CBD (**74**) were in agreement and hence the use of CBD (**74**) as a means of quantifying THC (**72**) was justifiable. The following equation applies:

$$\text{Weight THC} = 1.002 \times \left(\frac{\text{Weight CBD}}{\text{Peak Area CBD}} \right) \times \text{Peak Area THC}$$

Section 13.9: Objectives

The objectives of the work presented here were to determine the levels of THC (**72**), CBN (**73**) and CBD (**74**) in samples of cannabis resin and cannabis herb seized and/or grown in the Republic of Ireland. The Garda National Drugs Unit supplied the samples to the Department of Pharmacognosy.

The Department of Agriculture, Food and Forestry supplied samples of hemp, grown on farms holding licenses from the Department of Health and Children, to grow hemp. The THC (**72**) content of these samples was determined in order to validate compliance with EU regulations (European Union, 1989), requiring a content of less than 0.3% THC (**72**)). The CBD (**74**) and CBN (**73**) content were also determined in order to determine the phenotype of the *Cannabis sativa* L. analysed.

The method of analysis was petroleum ether extraction followed by GC-FID analysis. It was hoped that for the first time ever a comment could be made on the potency of cannabis seized in the Republic of Ireland. From the data generated the phenotype of the cannabis product analysed was determined.

In addition the THC (**72**) content as estimated using THC (**72**) itself as the standard, was compared to the THC (**72**) content estimated using CBD (**74**) as an indirect standard for all of the samples analysed and any statistical differences assessed.

Section 14.1: Instrumentation & Solvents

GC-FID

The GC-FID instrument used in all of the experiments detailed below was a Perkin Elmer Sigma 3 Gas Chromatograph with a flame ionisation detector attached. The column used was a 2 metre glass packed column, with a 3% OV-17 stationary phase. Data output from the system was collected with the aid of a Shimadzu CR-6A integrator.

Miscellaneous Instruments

All weighing was carried out using a Sartorius BP110 S analytical weighing balance. A generic flask shaker was used to agitate samples for long periods of time as required. A Heidolph, Labrota 4000 rotary evaporator was used to evaporate solvents under reduced pressure as required. A series of microlitre syringes were used to measure small volumes of fluid (10 μl, 25 μl and 100μl).

Solvents

All solvents were HPLC grade unless otherwise stated.

Section 14.2: Preparation of standard curves for quantitation of THC (72), CBD (74) and CBN (73).

Preparation of analytical standards for preparation of standard curves used in the analysis of samples 1-18.

Preparation of CBD (**74**) standard: A 5 mg/ml CBD (**74**) standard was prepared by dissolving 5 mg of CBD (**74**) (Sigma-Aldrich) in 1 ml of ethanol.

Preparation of THC (72**) standard: A working standard of THC (**72**) was prepared by dissolving the 200 µl of 25 mg/ml THC (**72**) (Sigma-Aldrich) in ethanol. The resulting solution was a 5 mg/ml THC (**72**) standard.**

Preparation of Internal Standard solution: A 10 mg/ml internal standard solution was prepared by dissolving 10 mg of Androstene-3, 17-dione (Sigma-Aldrich) in 1 ml of ethanol.

Preparation of combined standard: The 10 mg/ml internal standard solution, and the 5 mg/ml THC (72**) standard were used to prepare the analytical standards. The details of the volumes of each solution required to achieve the desired concentrations of the analytical standards are outlined below in Table 14.2.1. The standards were made up in 500 µl vials, which were sealed with the septum caps that could be crimped closed. The standards were refridgerated until required for analysis. New standard solutions were made up every two weeks.**

Volume of 5 mg/ml THC (**72**) standard	Volume of 10 mg/ml AD internal standard	Volume of Blank ethanol	Final Volume	Final concentration of each cannabinoid	Final concentration of AD internal standard
20µl	40µl	340µl	400 µl	0.25 mg/ml	1.00 mg/ml
40µl	40µl	320µl	400 µl	0.50 mg/ml	1.00 mg/ml
60µl	40µl	300µl	400 µl	0.75 mg/ml	1.00 mg/ml
80µl	40µl	280µl	400 µl	1.00 mg/ml	1.00 mg/ml
100µl	40µl	260µl	400 µl	12.50 mg/ml	1.00 mg/ml

Table 14.2.1: Volumes required and concentrations achieved for the preparation of cannabinoid standards

Preparation of analytical standards for preparation of standard curves used in the analysis of samples 18-87.

Preparation of CBD (**74**) standard: A 5 mg/ml CBD (**74**) standard was prepared by dissolving 5 mg of CBD (**74**) (Sigma-Aldrich) in 1 ml of ethanol.

Preparation of CBN (73**) standard: A 5 mg/ml CBN (**73**) standard was prepared by dissolving 5 mg of CBN (**73**) (Sigma-Aldrich) in 1ml of ethanol.**

Preparation of THC (72**) standard: A working standard of THC (**72**) was prepared by dissolving the 200 µl of 25 mg/ml THC (**72**) (Sigma-Aldrich) in ethanol. The resulting solution was a 5 mg/ml THC (**72**) standard.**

Preparation of Internal Standard solution: A 10 mg/ml internal standard solution was prepared by dissolving 10 mg of Androstene-3, 17-dione (Sigma-Aldrich) in 1 ml of ethanol.

Preparation of combined standard: The 10 mg/ml internal standard solution, and the 5 mg/ml THC (72**) standard were used along with the 5 mg/ml CBN (**74**) and the 5 mg/ml CBD (**73**) standards to prepare the combined analytical standards. The details of the volumes of each solution required to achieve the desired concentrations of the analytical standards are outlined below in Table 14.2.2. The standards were made up in 500 µl vials, which were sealed with the septum caps, which were closed by crimping. The standards were refrigerated until required for analysis. New standard solutions were made up every two weeks.**

Volume of 5 mg/ml THC (**72**) standard	Volume of 5 mg/ml CBD (**74**) standard	Volume of 5 mg/ml CBN (**73**) standard	Volume of 10 mg/ml AD internal standard	Volume of Blank ethanol	Final Volume	Final concentration of each cannabinoid	Final concentration of AD internal standard
20µl	20µl	20µl	40µl	320µl	400 µl	0.25 mg/ml	1.0 mg/ml
40µl	40µl	40µl	40µl	280µl	400 µl	0.50 mg/ml	1.0 mg/ml
60µl	60µl	60µl	40µl	240µl	400 µl	0.75 mg/ml	1.0 mg/ml
80µl	80µl	80µl	40µl	200µl	400 µl	1.00 mg/ml	1.0 mg/ml
100µl	100µl	100µl	40µl	160µl	400 µl	1.25 mg/ml	1.0 mg/ml

Table 14.2.2: Volumes required and concentrations achieved for the preparation of cannabinoid standards

Preparation of individual 0.5 mg/ml standards of THC (72), CBD (73), CBN (74) and AD

One in ten dilutions of the previously prepared 5 mg/ml standards of THC (**72**), CBD (**73**), CBN (**74**) were prepared by dissolving 100 µl of respective standard in 900 µl of ethanol. A 5 mg/ml solution of standard AD was prepared by making a one in twenty dilution of the 10 mg/ml standard with ethanol.

Chromatographic Conditions

The chromatographic conditions used are outlined in Table 14.2.3 below.

Parameter	Value
Column Type	Glass Packed
Column Length	2 metres
Column i.d.	2 mm
Column o.d.	6 mm
Stationary Phase	3% OV-17
Support Phase	Chromosorb G-AW-DMCS, 80-100 mesh.
Mobile Phase	Nitrogen
Mobile Phase Flow Rate	30 ml/min
Oven Temperature	235°C
Injector Temperature	265°C
Detector Temperature	265°C
Injection Volume	1 µl
Chart Speed	2 mm/min

Table 14.3: Chromatographic conditions used for the analysis of the Cannabinoid standard solutions.

Analysis

Each of the individual solutions containing either 5 mg/ml THC (**72**), CBD (**74**) or CBN (**75**) were analysed in duplicate in order to determine chromatographic data for each of the standards. The analytical standards (0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml, 1.00 mg/ml and 1.25 mg/ml of THC (**72**), CBD (**74**) and CBN (**73**)) were analysed in duplicate as required during the course of the work. The results obtained are discussed in section 15.

Section 14.3: Preparation and Analysis of Cannabis products using GC-FID.

It is worth noting at this point that the method outlined below for the extraction of cannabinoid material from hemp is the same as that set down by the E.U. Commission Regulation No 1164/89 (Annex C).

Origin of Cannabis products.

The Garda National Drugs Unit (GNDU) provided the herbal samples and the resin samples. The Department of Agriculture, Food and Forestry provided the hemp samples.

Preparation of Cannabis products.

All cannabis products (hemp, resin, and herb) were firstly dried in an oven at 40°C until constant weight. The products were then powdered in a mortar and pestle. The resulting triturates were reduced to semi-fine powders with the aid of a 1,000 meshes per cm^2 sieve as required. The powders were transferred to a sealed glass vial, which was stored in the dark until further processed. The samples were generally extracted and analysed by GC-FID within 48 hours of powdering.

Preparation of analytical solutions.

A 1mg/ml androsten-3,17-dione (I.S.) was prepared by dissolving 100mg of androsten-3,17-dione in 100 ml of ethanol. This solution was used for the reconstitution of cannabis residues after extraction (see section 14.2.4).

Extraction of cannabis products.

The amount of powder taken for extraction depended on the type of Cannabis product (resin, hemp or herb) under analysis. The weights taken for each are shown in Table 14.3.1 (below).

Cannabis Product	Amount taken (mg) for extraction
Resin	100 mg
Herb	50 mg
Hemp	2000 mg

Table 14.3.1: Amounts of powdered Cannabis product taken for extraction.

The extraction followed the same path in the case of each product-type. The required amount of product was accurately weighed out into a plastic weighing boat (see Table 14.3.1). The contents of the weighing boat were quantitatively transferred to a 250ml ground glass stoppered conical flask with the aid of 5 ml of petroleum ether. A further 30ml of petroleum ether was added and the flask was stoppered. This was carried out in duplicate for each sample. The flasks were then shaken mechanically for 30 minutes in the case of resin and herb. The flasks were shaken for 24 hours in the case of hemp samples. Once the extracts had been shaken for the required amount of time they were taken from the shaker and filtered into a 10 ml round bottomed flask. The solvent was then evaporated under reduced pressure using a rotary evaporator. The round bottomed flasks were allowed to cool before having the residue reconstituted in Petroleum Ether.

In the case of herb and resin extractions, residues were reconstituted in 2 ml of 1.00 mg/ml androsten-3,17-dione internal standard . In the case of hemp extractions, residues were reconstituted in 10 ml of 1.00 mg/ml androsten-3,17-dione internal standard. Solutions were refrigerated until required for analysis.

Chromatographic Conditions

The chromatographic conditions are as follows (see Table 14.3.2).

Parameter	Value
Column Type	Glass Packed
Column Length	2 metres
Column i.d.	2 mm
Column o.d.	6 mm
Stationary Phase	3% OV-17
Support Phase	Chromosorb G-AW-DMCS, 80-100 mesh.
Mobile Phase	Nitrogen
Mobile Phase Flow Rate	30 ml/min
Oven Temperature	235°C
Injector Temperature	265°C
Detector Temperature	265°C
Injection Volume	1 µl
Chart Speed	2 mm/min

Table 14.5: Chromatographic conditions used for the analysis of the Cannabis product extracts.

Analysis

Extraction residues of resin, herb and hemp which had been reconstituted in the appropriate volume of 1 mg/ml androstene-3,17-done were analysed in duplicate under the chromatographic conditions outlined. The results are discussed in section 15.

Section 15.1: Assessment of suitability of GC-FID method for the analysis of the cannabinoids.

The objective of the work presented here was to quantify the three key cannabinoids (THC (**72**), CBN (**73**) and CBD (**74**)) in samples of cannabis resin and herb supplied by the Garda national drugs unit and in hemp supplied by the Department of Agriculture, Food and Forestry. The analytical method, employed for this task utilised GC-FID. The method chosen for the analysis was an official method of the EU for the analysis of THC (**72**) in hemp (European Union, 1989). Many other workers (Turner, 1984 & Parker, 1974) have used a similar method not only for THC (**72**) analysis, but also for CBN (**73**) and CBD (**74**) in cannabis resin and cannabis herb. The method was set up and assessed for system suitability (resolution, peak tailing, efficiency and linearity), the results of which follow. The official EU method has been modified by using a capillary column method in the last year (European Union, 2000), and would presumably be considered a better method. Time did not permit a comparison of the original and revised protocols but the future work in this area would have to be conducted using the revised method.

When the analysis began the only standard available was THC (**72**). Subsequently a CBD (**74**) standard became available. According to Poortman-van der Meer *et al*. CBD (**74**) is a more useful standard as it can be used as a standard to quantitate CBD (**74**) and also as a surrogate standard in the accurate quantitation THC (**72**) and CBN (**73**)(1999a).

The chromatographic parameter values are presented in Table 15.1.1, and are the averages of six replicate injections of a 0.5 mg/ml standard, where the relative standard deviations were calculated to be less than 4% in all cases. The peaks were identified by injection of standard solutions containing individual components (e.g. THC (**72**) in ethanol etc.). The peak area ratios of CBD (**74**), THC (**72**) and CBN (73) with respect to the area of AD, for six replicate injections of the 0.5 mg /ml standard are shown in Table 15.1.2.

Peak	Peak Identity	$t_{R\ mins}$	t_R Relative to AD	Capacity Factor	$Resolution_{peaks}$	Asymmetry	Theoretical Plates
1	CBD (**74**)	7.68 mins	0.37	29.71	--	1.0	943.22
2	THC (**72**)	10.41 mins	0.50	40.66	$2.74_{1,2}$	1.0	1735.55
3	CBN (**73**)	12.96 mins	0.62	50.85	$2.55_{2,3}$	1.1	1194.95
4	AD	20.84 mins	--	82.35	$3.94_{3,4}$	1.1	1736.72

Table 15.1.1: Chromatographic parameters for six replicate injections of 0.5 mg/ml combined THC (72), CBN (73), CBD (74) and 1.0 mg/ml androstene dione standard.

Cannabinoid	*CBD (**74**)*	*THC (**72**)*	*CBN (**73**)*
Peak Area Ratio	*0.48*	*0.38*	*0.45*

Table 15.1.2: Peak area ratios of CBD (74), THC (72) and CBN (73) with respect to the area of AD, for six replicate injections of the 0.5 mg/ml standard.

A typical chromatogram of the combined standard is shown in the Figure 15.1.1 below for the 0.5 mg/ml combined standard, which also contained androstene dione at a level of 1.0 mg/ml AD.

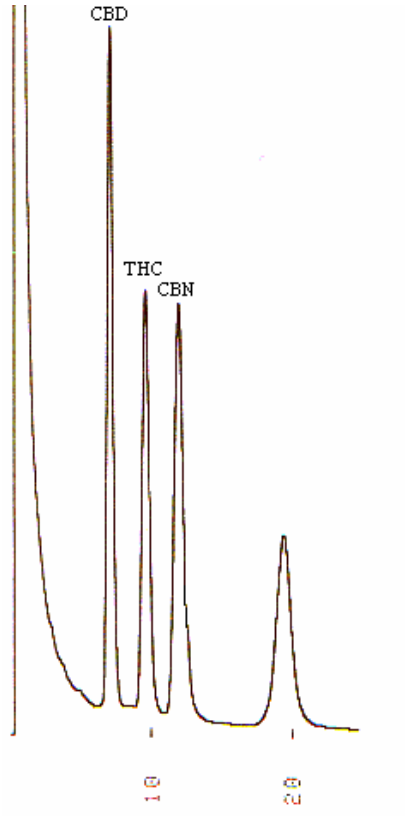


Figure 15.1.1: 0.5 mg/ml THC (72), 0.5 mg/ml CBD (74) and 0.5 mg/ml CBN (74) combined standard which also contained androstene dione at a level of 1.0 mg/ml AD, in ethanol.

*The first compound to elute from the mixture after the solvent front, in the example chromatogram shown in Figure 15.1.1, was the CBD (**73**), which eluted at circa 7.68 minutes (relative retention w.r.t. AD was 0.37). The next compound to elute was the THC (**72**), which had a retention time of 10.5 minutes (0.50 relative to the internal standard, AD). The final cannabinoid to elute was the CBN (**73**), eluting at a retention time of 13.07 minutes (0.62 relative to androstene dione). The final peak to elute was the androstene dione peak itself, which eluted at 21.02 minutes. The resolution was adequate in the case of all of the peaks, as the calculated value exceeded 2 units in each case, which allowed adequate separation and easy integration (Dolan, 2000). The peak tailing was acceptable as the values were below 1.2 units (Snyder, 1997). The capacity factors seemed high (see Table 15.1.2), when compared with previous work presented in this thesis for HPLC analysis, however one must be mindful that the non-retained volumes in GC can be much lower with such high flow rates. On visual inspection of the*

example chromatogram (Figure 15.1.1) it is clear that the peaks have eluted within an acceptable time. Although one can see peak broadening for the AD peak, integration of the peak was achieved without difficulty. The column efficiency values seemed low for packed column GC, as one would expect values in the region of 4000-5000 plates per 2 metre column (Baugh, 1993).

15.1.1: Assessment of the precision of the method.

The precision of the method was assessed by analysing the retention times and peak areas for the six replicate injections. The relative standard deviation for the peak areas and retention times are shown below in Table 15.1.1.1.

Parameter	Peak 1 (**73**)	Peak 2 (**72**)	Peak (**74**)	Peak 4 (**A.D**.)
t_R R.S.D	0.03 %	0.01 %	0.02 %	0.01 %
Area R.S.D.	2.78 %	2.41 %	1.76 %	2.12 %

Table 15.1.1.1: Relative standard deviations of peak area and retention time for CBD (74), THC (72), CBN (73) and AD.

As can be seen in all cases the relative standard deviations were low (< 3%) and so the method was considered to be precise for the analysis of the cannabinoids.

15.1.2: Assessment of the selectivity of the method.

The selectivity of the method was not assessed as it was an official method published by the European Union (European Union, 1989).

15.1.3: Assessment of the linearity of the method.

*The linearity of the method for each of the standards is illustrated with the combined THC (**72**), CBD (**74**) and CBN (**73**) at the levels 0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml, 1.0 mg/ml and 1.5 mg/ml (each containing AD at 1.0 mg/ml). The data used to construct a standard curve is shown in Table 15.1.2.1, below, and the standard curve is shown in Figure 15.1.2.1. The averaged peak area ratio for duplicate injections was calculated for each peak in the chromatograms, relative to the AD peak. The peak area ratio values were plotted against concentration and are shown in Figure 15.1.2.1 below. The R^2 value was used to assess the linearity between a group of points on a graph and will have a value close to one when a set of data show a linear relationship. The R^2 values calculated for each of the fitted trendlines using Excel was 0.9993 in the case of CBD (**74**), 0.9992 in the case of THC (**72**) and 0.9996 in the case of CBN (**73**). This high degree of linearity is excellent, however as one can see the slope of the THC (**72**) is somewhat lower, 0.0008, than the value, 0.001, recorded for both the CBD (**72**) and CBN (**73**) curves.*

Concentration (mg/ml)	CBD peak area ratio	THC peak area ratio	CBN peak area ratio
0.25	0.258	0.207	0.249
0.50	0.52	0.427	0.506
0.75	0.782	0.634	0.764
1.00	1.052	0.85	1.007
1.25	1.281	1.036	1.242

Table 15.1.2.1: Peak area ratios for cannabinoid standard curve.

*The example shown is not an isolated incident, the slope of the THC (**72**) standard curves were consistently lower than the slopes of the other two standard curves for the CBD (**74**) and CBN (**73**).*

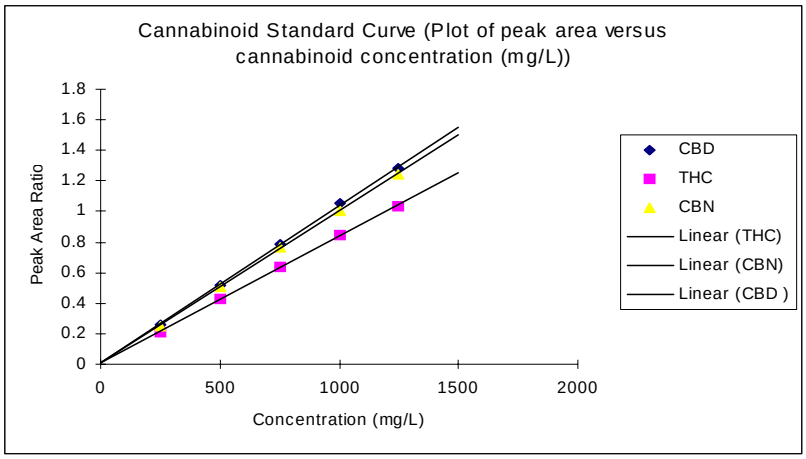


Figure 15.1.2.1: Cannabinoid standard curve for THC (72), CBN (73) & CBN (74).

This was a cause for concern, as the contribution of Poortman-van der Meer and Huizer (1999a) suggested that the responses for all three should be quite similar (i.e. one would expect similar slope values). It was decided that the oily liquid nature of the THC (**72**) standard was most likely the cause in this instance, as the CBN (**73**) and CBD (**74**) were solid and thus were more easily prepared to concentration with known weights of the relevant standard. The THC (**72**) standard was purchased from Sigma-Aldrich and had already been prepared to a particular concentration (25 mg/ml). Further the label states that purity of this unstable standard is approximately 95% which is poor when one considers that this is supposed to be a primary standard, which may be used for the quantitation of a drug. There is simply too much room for error hence the CBD (**74**) would be used to estimate the THC (**72**) concentration, where possible. It is obvious this was not possible for samples 1-19, as the only standard curve prepared during the analysis of those samples was based on the THC (**72**) standard. The standard curves prepared for THC (**72**) analysis of sample 20 onwards were adjusted using the following formula (Poortman-van der Meer, 1999a) (Equation 15.1.2.1).

$$\text{Equation 15.1.2.1: Concentration THC} = 1.002 \times \left(\frac{\text{Concentration CBD}}{\text{Peak Area CBD}} \right) \times \text{Peak Area THC}$$

Using the CBD (**74**) as a replacement standard for THC (**72**) should have provided a more accurate result. How this was done is illustrated below for the curve shown in Figure 15.1.2.1. The actual data

that was recorded for each of the three standards over the concentration range investigated is shown in Table 15.1.2.1 (above).

By inserting the peak area ratio data for CBD (**74**) and THC (**72**) into equation 15.1.2.1 at each specific concentration a corrected set of concentrations for the peak area ratios of THC (**72**) is calculated. The values calculated here are shown in Table 15.1.2.2.

Corrected THC (**72**) concentration (mg/ml)	Observed peak area ratio for THC (**72**)
0.201	0.207
0.411	0.427
0.609	0.634
0.810	0.85
1.013	1.036

Table 15.1.2.2: Data for corrected THC (72) standard curve.

A standard curve is plotted showing the uncorrected THC (**72**) data (Table 15.1.2.1) and the corrected THC (**72**) data (Table 15.1.2.2). The corrected THC (**72**) standard curve is shown in red. (See Figure 15.1.2.2).

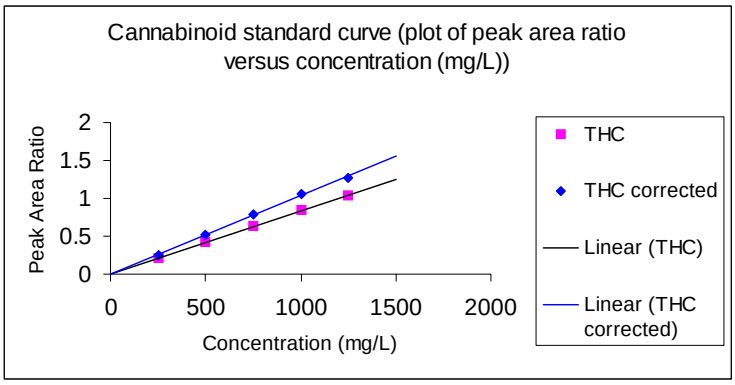


Figure 15.1.2.2: Standard curve for THC uncorrected and corrected.

A plot of the corrected THC (72) data and the CBD (74) and CBN (73) data is shown below. The trendlines for CBD (74) and THC (72) are superimposed (See Figure 15.1.2.3).

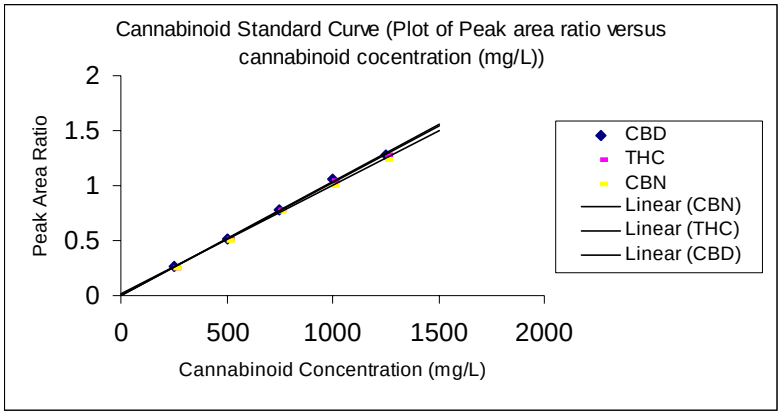


Figure 15.1.2.3: Corrected cannabinoid standard curve for THC (72), CBN (73) & CBN (74).

If one compares Figure 15.1.2.2 and Figure 15.1.2.3 one can clearly see that the slope of the THC standard curve has increased and the response is brought closer to that of the CBN (**73**) and the CBD (**74**) as one would expect. The slopes of the corrected THC (**72**) curve, the CBN (**73**) curve and the CBD (**74**) curves are 0.001 in all cases.

This correction was applied to all standard curves used for the quantitation of THC (**72**) from sample 20 onward (**72**). As mentioned above no CBN (**73**) standard curve was available for samples 20-29 however by measuring the peak area ratio for the CBN (**73**) peak and inserting it into equation 15.1.2.2, a corrected standard curve for CBN (**73**) could be derived.

$$\text{Equation 15.1.2.2: Concentration CBN} = 1.00 \times \left(\frac{\text{Concentration CBD}}{\text{Peak Area CBD}} \right) \times \text{Peak Area CBN}$$

A THC (**72**) standard curve was prepared for use during the analysis of samples 1-19, but no CBD (**74**) or CBN (**73**) standards were available when those samples were being analysed. The amount by which the corrected curves for THC (**72**) deviated from the actual value provided a crude correction of the THC (**72**) content in these samples. It was considered futile to try to predict the CBN (**73**) and CBD (**74**) contents of these samples, as the error in the values would very likely be large.

Section 15.2: Cannabinoid content of the Cannabis products

15.2.1 Cannabinoid content of Hemp.

*The hemp grown under licence from the Department of Health and Children was analysed for content of THC (**72**), CBN (**73**) and CBD (**74**). In all 21 samples were analysed, 13 harvested in 1998 and 8 harvested in 1999. The samples were grown in the counties Cavan, Roscommon, Sligo, Clare, Kerry, Mayo and Tipperary. The varieties selected for cultivation were all registered EU varieties of* Cannabis sativa*. The main variety, which was grown, was Fedora 19 but there was also one case of Ferimon and one case of Futura. All of these varieties are well known fibre type plants, producing low levels of psychoactive drug (Mignoni, 1997/1998). The plants were harvested in September/October each year. Quantitation of the cannabinoids in the sample was achieved using a standard curve derived from all 3 cannabinoids which were individually identified on the basis of retention time relative to the internal standard included in the re-constitution solution (see Section 14.2). A typical chromatogram obtained with a hemp sample is shown below (see Figure 15.2.1.1 A).*

■

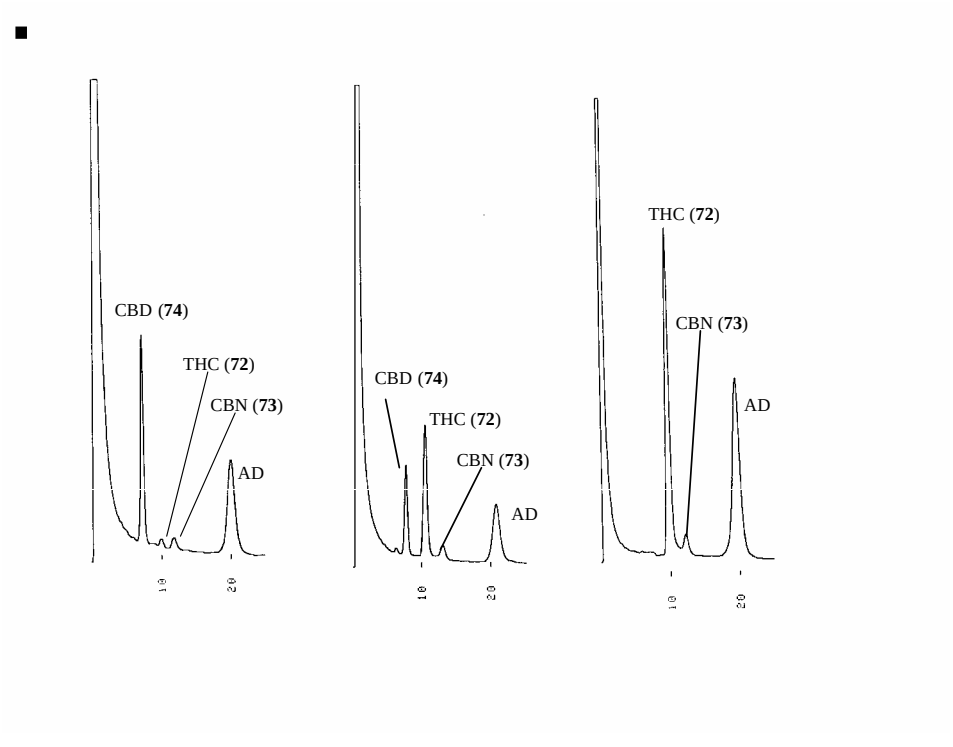


Figure 15.2.1.1: Typical chromatograms for hemp (A), resin (B) and herb (C).

*A peak area ratio for each cannabinoid with respect to the AD peak was calculated. The peak area ratios for the respective cannabinoids were interpolated from the respective graphs. In the case of THC (**72**) the standard curve was corrected using CBD (**74**) standard curve data, according the equation of Poortman-van der Meijer* et al. *(1999a) and the peak area ratio for the THC (**72**) (relative to AD) was interpolated from the corrected graph. The method for correcting the THC (**72**) data has been outlined in detail above. Once interpolated, a value in mg/L was returned, this value was converted in % cannabinoid via the following equation (Equation 15.2.1.1):*

$$\%\text{Cannabinoid} = \left(\frac{\text{Interpolated Value mg/L} \times \text{Reconstitution volume} \times 10^{-3}\,\text{L}}{\text{Weight of plant material taken for analysis}} \right) \times \frac{100}{1}$$

Equation 15.2.1.1: Calculation used to estimate percent cannabinoid.

The content of THC (**72**), CBD (**74**) and CBN (**73**) for the 21 samples of hemp analyzed are shown in Table 15.2.1.1 below. Samples 68-76 were supplied and analysed as part of the 1999 years harvest. Samples 77-88 were supplied and analysed as part the 1998 harvest.

Sample Number	*Variety*	*% THC (**72**)*	*% CBN (**73**)*	*% CBD (**74**)*
68	*Fedora 19*	*0.05*	*0.08*	*0.80*
69	*Fedora 19*	*0.03*	*0.06*	*0.95*

70	*Fedora 19*	*0.06*	*0.12*	*1.22*
71	*Fedora 19*	*0.17*	*0.08*	*1.68*
72	*Ferimon*	*0.08*	*0.13*	*1.68*
73	*Fedora 19*	*0.18*	*0.06*	*1.19*
74	*Fedora 19*	*0.01*	*0.09*	*1.26*
75	*Futura*	*0.03*	*0.08*	*0.58*
76	*Fedora 19*	*0.01*	*0.02*	*1.29*
77	*Fedora 19*	*B.L.O.Q*[*]	*0.15*	*2.00*
78	*Fedora 19*	*B.L.O.Q*[*]	*0.07*	*2.86*
79	*Fedora 19*	*0.05*	*0.03*	*1.70*
80	*Fedora 19*	*B.L.O.Q*[*]	*0.04*	*1.41*
81	*Fedora 19*	*0.04*	*0.07*	*0.78*
82	*Fedora 19*	*0.06*	*B.L.O.Q*[*]	*1.80*
83	*Fedora 19*	*0.02*	*0.04*	*2.15*
84	*Fedora 19*	*0.27*	*B.L.O.Q*[*]	*2.72*
85	*Fedora 19*	*0.01*	*0.10*	*1.99*
86	*Fedora 19*	*B.L.O.Q**	*B.L.O.Q*[*]	*2.67*
87	*Fedora 19*	*B.L.O.Q*[*]	*B.L.O.Q*[*]	*1.13*
88	*Fedora 19*	*0.12*	*B.L.O.Q*[*]	*1.89*

Table 15.2.1.1: THC (72), CBN (73) and CBD (74) contents of hemp grown in the Republic of Ireland 1998-1999 (* B.L.O.Q. refers to the fact that the cannabinoid was below the limit of quantitation).

*As can be clearly seen from the results (see Table 15.2.1.1) the THC (**72**) content for the hemp was never greater than 0.3% which is the limit set down by the EU (1984). Therefore all of the farmers who had grown the hemp for industrial purposes in the Republic of Ireland satisfied the licence and it would appear that no drug type plants were being grown on these farms on the strength of the above results. Some of the values were below the level of quantitation (B.L.O.Q.). The average amount of THC (**72**) in the samples was 0.06% THC (**72**) with a range of 0.01% to 0.27%. The level of CBN (**73**) was also quite low in most cases with the average of 0.06% (range 0.02% to 0.15%) calculated. CBD (**74**) was the major cannabinoid and was found at a average concentration of 1.61% CBD (**74**), calculated for the 21 samples analysed. The range of CBD (**74**) content was found to be 0.18% CBD (**74**) to 2.86% CBD.*

*Hemp normally has levels of CBD (**74**) greater than 0.5% (Nahas, 1984). Stefanidou* et al. *found that of 29 herbal cannabis plants analysed 16 were hemp, with an average CBD (**74**) content of 0.20% (range 0.0004% to 1.43%) (1993). In a study by de Meijer* et al., *out of 97 accessions of* Cannabis sativa *evaluated for cannabinoid content, 60 were considered to be of the fibre type (1992). The average THC (**72**) content was found to be 0.22%, with a range of 0.06% to 0.42% THC (**72**). The average CBD (**74**) content was found to be 1.18%, with a range of 0.55% to 2.19% CBD (**74**). Among the 60 samples which were analysed were Futura (average THC=0.23%, average CBD=1.49%, n=2), Ferimon (average THC=0.17%, average CBD=1.16%, n=1) and Fedora 19 (average THC=0.26%, average CBD=1.41%, n=1). When one compares data of de Meijer et al. (1992) and Stefanidou et al. (1993) to the data presented here it is clear that the CBN (**73**) and THC (**72**) levels were lower than the averages found by de Meijer* et al. *Further the average CBD (**74**) content of hemp grown in the Republic of Ireland is higher than that of the 60 accessions evaluated by de Meijer* et al. *(1.18% CBD versus 1.61% CBD). A bar chart graph of CBD (**74**) content versus variety and origin (Irish hemp or hemp analysed by de Meijer et al. (1992)) is shown below for the three varieties grown in the Republic of Ireland (see Figure 15.2.1.2). As can be seen from the chart, the CBD (**74**) content of the Ferimon and the Fedora varieties were comparable, while the CBD (**74**) content for the Futura variety was lower for Irish hemp.*

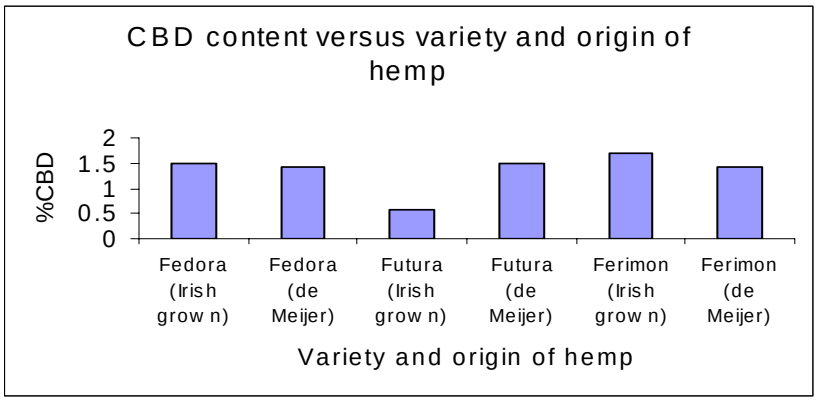


Figure 15.2.1.2: A bar chart graph of CBD (74) content versus variety an origin (Irish hemp or hemp analysed by de Meijer et al. (1992)) for the three varieties grow in the Republic of Ireland.

15.2.2 Cannabinoid content of Herbal Cannabis

*There were very few herb samples submitted for analysis by the Garda National Drugs Unit (n=5) compared to the resin samples (n=62). This indicates that resin is the prevalent form of cannabis being abused in the Republic of Ireland. This would also have been the case in the U.K., but in recent times clandestine cultivation of herbal cannabis has increased dramatically (Bone, 1997/1998), and it may not be long before an increased amounts of in Irish grown herbal cannabis appear on the market. Three of the samples (Early '98, Rosslare '97 and Dutch '97) were seized from individuals attempting to enter the State in Rosslare ferry port. Another sample referred to here as ''skunk'' was seized in Dublin in 1998. The final sample, which was analysed, was seized again in Dublin but in this case the plant was being cultivated domestically. This sample was presented in a plastic bag bearing a label which read ''Cannablitz'' As with the hemp analysis all of the samples were analysed after the adoption of the cannabinoid standard containing THC (**72**), CBN (**73**) and CBD (**74**). The correction procedure for the THC (**72**) standard curve was also applied. The values obtained from the standard curves were converted into % cannabinoid as per section 15.2.1.The results for the individual for the five samples analysed are shown below (see Table 15.2.1.2). A typical chromatogram of a herbal sample is shown in Figure 15.2.1.1 C, above.*

Sample Number	*Name/Origin*	*%THC (**72**)*	*%CBN (**73**)*	*%CBD (**74**)*
40	*Early '98*	*9.54*	*0.72*	*B.L.O.Q.**
41	*Rosslare '97*	*4.78*	*0.68*	*B.L.O.Q.**
41A	*Skunk*	*9.48*	*B.L.O.Q.**	*B.L.O.Q.**
42	*Dutch '97*	*5.86*	*1.52*	*0.48*
43	*Cannablitz*	*1.36*	*0.33*	*0.18*

Table 15.2.1.2: THC (72), CBN (73) and CBD (74) contents for herbal samples analysed.

*Although the sample size is small it is of interest that the THC (72) content ranged from 1.36% to 9.54%. The average THC content (**72**) is 6.20% (standard deviation 3.44), and the CBN (**73**) content is much lower at 0.65% (standard deviation 0.57), which indicates that the samples are not to old or have been very well preserved (Ross, 1997/1998). The Dutch '97 sample, had a high level of CBN (**73**) (1.52%) compared to THC (**72**) (5.86%). The CBD content was low, as one would expect for drug-type herb with an average level of 0.13% (standard deviation 0.21). Traditional herbal cannabis would generally contain 0.5-5.0% (Martin, 1997/1998). It is obvious that three of the samples, Early '98, Dutch '97 and ''Skunk'' exceed the level suggested. Hence, these can only be described as either Sinsemilla-type (there was no evidence of seeds in any of the plants) or Nederweed-type, as these types of plants are the only ones capable of generating so much THC (**72**) (Adams, 1996).*

*The ''Cannablitz'' had a low level of THC (**72**) in comparison to the other samples. All the other samples were received as intact flowering tops and leaves. The Cannablitz on the other hand had been processed and was coarsely powdered. It contained a lot of stalk which would have diluted the sample somewhat as the stalk of the cannabis plant does not contain a high level of THC (**72**). However it seems less likely that Cannablitz was Sinsemilla or Nederweed and more likely that it was some traditional variety of cannabis herb. Some work carried out on the THC (**72**) content of cannabis seized in the Republic of Ireland in 1981 suggested that no high THC (**72**) content varieties were encountered at that time (O'Brien, 1981) as the average THC content of eight herbal samples analysed was 1.36 (range 0.18%-2.9%). Hence, the above results provide proof that high THC (**72**) content varieties of cannabis are now available in the Republic of Ireland. Also if one aggregates the %THC (**72**) and the %CBN (**73**) one gets an idea of the initial levels of THC (**72**). In this case the average goes from 6.2% (THC only) to 6.85% (THC +CBN). This is high when compared to the average content of traditional marijuana observed by Elsohly et al. (2000) over the period 1980-1997 (rising from 1.5 % THC in 1980 to 4.2% in 1997). THC (**72**) contents of up to 11.53% have been recorded for Sinsemilla over the same period (Elsohly, 2000). Niesink reported that the average THC (**72**) content in Dutch herb was 7.5%, which compares well with our observations (Niesink, 2000). King reported that the THC (**72**) content of the flowering tops of herb, seized in the U.K. in 1999, was 9.6%, which is high when compared to the average reported here (20101). This average compares well with the THC (**72**) contents of the Early '98 and the Skunk samples.*

15.2.3 Cannabinoid content of Resin

In all 62 samples of resin were analysed. The results are presented in three sections.

1. *Samples 30-39 & 44-67 (THC (**72**), CBD (**74**) and CBN (**73**) standard curves prepared)*
2. *Samples 20-29 (Only THC (**72**) and CBD (**74**) standards curves prepared)*
3. *Samples 1-19 (Only THC (**72**) standard curve prepared)*

Cannabinoid content of samples 30-39 & 44-67.

*A typical chromatogram for a resin sample is shown in Figure 15.2.1.1 B. The peak area ratios for the CBD (**74**) and CBN (**73**) were obtained straight from the graph and converted into % values as per section 15.2.1. The THC (**72**) curve was corrected as per section 15.1. The results of percentage (corrected) THC (**72**), percentage CBN (**73**) and percentage CBD (74) for samples 30-39 and 44-67 are shown below (Table 15.2.3.1).*

Sample No.	*%THC (**72**)*	*% CBN (**73**)*	*% CBD (**74**)*	*Sample No.*	*% THC (**72**)*	*% CBN (**72**)*	*% CBD (**72**)*
30	*3.05*	*3.20*	*1.41*	*51*	*0.40*	*0.45*	*1.51*
31	*2.61*	*0.78*	*2.01*	*52*	*5.52*	*1.02*	*2.66*
32	*0.44*	*1.08*	*0.07*	*53*	*0.54*	*1.96*	*1.62*
33	*1.65*	*2.40*	*0.56*	*54*	*0.53*	*2.52*	*1.23*
34	*0.70*	*2.70*	*1.89*	*55*	*0.68*	*1.04*	*1.49*
35	*1.07*	*3.10*	*2.16*	*56*	*0.84*	*2.47*	*2.11*
36	*0.58*	*2.82*	*2.20*	*57*	*0.84*	*2.07*	*2.13*
37	*2.37*	*1.37*	*2.45*	*58*	*2.04*	*2.07*	*1.20*
38	*5.15*	*1.14*	*2.66*	*59*	*0.38*	*2.93*	*2.28*
39	*0.44*	*2.16*	*1.72*	*60*	*0.68*	*2.34*	*2.16*
44	*0.21*	*1.15*	*1.18*	*61*	*0.32*	*3.78*	*3.24*
45	*0.80*	*2.73*	*1.94*	*62*	*0.40*	*2.66*	*1.67*
46	*0.88*	*2.93*	*2.03*	*63*	*0.88*	*3.34*	*4.40*
47	*1.07*	*2.63*	*2.20*	*64*	*0.83*	*2.12*	*11.54*
48	*0.31*	*0.65*	*0.48*	*65*	*0.36*	*2.25*	*1.50*
49	*1.40*	*4.59*	*4.29*	*66*	*0.68*	*0.74*	*1.42*
50	*0.50*	*1.77*	*1.85*	*67*	*1.96*	*2.59*	*2.11*

Table 15.2.3.1 : Cannabinoid content of samples 30-39 and 44-67.

Cannabinoid content of samples 20-29.

*For these samples, the THC (**72**) standard curve was corrected using the CBD (**74**) standard curves and the CBN (**73**) values were predicted using the CBD (**74**) standard curve. The results of percentage (corrected) THC (**72**), percentage CBN (**73**) and percentage CBD (**74**) for samples 20-29 are shown below (Table 15.2.3.2).*

Sample Number	*%THC (**72**)*	*% CBN (**73**)*	*% CBD (**74**)*
20	*3.84*	*0.86*	*2.00*
21	*2.20*	*1.89*	*2.10*
22	*2.33*	*2.68*	*2.05*
23	*2.64*	*2.31*	*2.49*
24	*1.48*	*1.72*	*1.54*

25	*0.44*	*2.16*	*2.30*
26	*0.50*	*3.19*	*3.12*
27	*1.34*	*1.95*	*1.84*
28	*2.02*	*2.02*	*2.01*
29	*0.72*	*1.65*	*1.61*

Table 15.3.2.2: Cannabinoid content of samples 20-29.

THC content of samples 1-19.

*As previously mentioned it was only possible to determine the amount of THC (**72**) in these samples, as only a THC (**72**) standard curve was prepared during the analysis of these samples. The deviation between the uncorrected THC (**72**) estimations of samples 30-39 & 44-67 and the corrected THC (**72**) values was found to be 22.9%. Subtracting the two values (corrected and uncorrected) from each other and dividing by the number of samples gave this average. The actual THC (**72**) contents as calculated by the THC (**72**) standard curves were multiplied by 1-0.229 (0.771) in order to correct these figures. This is very crude but in the absence of a reliable standard curve the only option was to correct the values on the basis of more reliable historical data. The corrected THC (**72**) contents for the resin sample 1-19 are shown in Table 15.3.2.3.*

Sample Number	*% THC (**72**)*	*Sample Number*	*% THC (**72**)*
1	6.22	*11*	1.54
2	0.19	*12*	3.85
3	0.87	*13*	3.62
4	4.26	*14*	2.56
5	2.53	*15*	3.12
6	3.72	*16*	3.83
7	2.76	*17*	3.13

8	2.21	*18*	1.02
9	4.13	*19*	1.88
10	1.49		

Table 15.3.2.3: corrected THC (72) content of samples 1-19.

Cannabinoid content of Resin samples seized in the Republic of Ireland.

*The average THC (**72**) content found in all samples of cannabis resin analysed was calculated, and found to be 2.11% (standard deviation, 1.45%, range 0.4%-8.08%). The average amount of CBN (**73**), excluding samples 1-19, was found to be 2.13% (standard deviation 1.14%, range 0.31%-4.59%). Finally the average amount of CBD (**74**), excluding samples 1-19, was found to be 2.19% (standard deviation, 1.65%, range 0.07%-4.38%). The level of THC (**72**) reported in resin samples varied from 7%-14% (Martin, 1997/1998), however Adams* et al. *(1996) claimed that values from 2%-20% have been recorded. The level of THC found in resins was low in comparison to reported values. Conversely the CBN (**73**) levels (2.13% average) suggested that the samples had aged significantly (Fairbairn, 1976). Aggregation of the percentage THC (**72**) and CBN (**73**) content perhaps better reflects the original average THC (**72**) content present when the resin was first produced. In these circumstances average THC (**72**) content in the resin at the time of preparation would be 4.24% which seems reasonable when this figure is compared to those recorded by Elsohly (2000) in the USA. He reported that the average THC (**72**) content of resin analysed during the period 1980-1997 was 5.52% and the combined CBN (**73**) and THC (**72**) content was 7.00% in the USA for the same period. Resin samples analysed by the Forensic Science Service in the U.K. in 1999 were found to have an average THC (**72**) content of 5.0%, which again is high when compared with the values reported here (King, 2001). There was no CBN (**73**) data available for these samples (King, 2001).*

The CBD levels reported here were also high (2.19% (standard deviation, 1.65%, range 0.07%-4.38%)) when one considers the fact that the resin samples contained a reasonable amount of THC

*(**72**). This observation may indicate that the resin was actually collected from an intermediate type plant. This theory will is investigated further in the following section.*

It is notable that the work of O'Brien et al. *1981 found lower levels of CBD (1.04%) and THC (0.9%) for a number of resin samples analysed, than are reported here. The level of CBN found in the same samples was 1.4%, which again is lower, but the high levels of CBD would suggest that the resin samples analysed by O'Brien et al. may also have been from an intermediate type variety of* Cannabis sativa.

15.3 Phenotype ratio of cannabis hemp, herb and resin seized in the Republic of Ireland.

The phenotype ratio concept, as described in the introduction, was applied to the samples of herb, hemp and resin (excluding sample 1-19). The formula that was suggested by Fetterman (1971) was applied here where the following equation allows the determination of a phenotype (Equation 15.3.1).

$$\text{Phenotype ratio} = \frac{(\%THC + \%CBN)}{(\%CBD)}$$

Equation 15.3.1

The criteria of de Meijer *et al.* (1992) were used for the classification of phenotypes according to cannabinoid content, including provision for an intermediate phenotype (Table 15.3.1). Note that de Meijer *et al.* excluded CBN (**73**) as the samples analysed by this group were all fresh, however it is acceptable to include CBN (**73**) in our case.

Phenotype	%THC + %CBN	%CBD	%THC+%CBN / %CBD
Fibre type	< 0.5	≥ 0.5	< 1
Intermediate type	≥ 0.5	≥ 0.5	
Drug type	≥ 0.5	< 0.5	> 1

Table 15.3.1: Criteria for the classification of phenotypes according to cannabinoid content (de Meijer et al. 1992).

Giroud et al. *(1999) used the same formula as Fetterman and de Meijer. However Giroud,* et al. *calculated the logarithm (base 10) of equation 15.3.1 in order to simplify the graphical interpretation of the data he presented. The equation now changes to 15.3.2*

$$\text{Phenotype ratio} = Log_{10}\left(\frac{(\%THC + \%CBN)}{(\%CBD)}\right)$$

Equation 15.3.2

Now, if one uses equation 15.3.2 the criteria shown in table 15.3.1 must be changed to take the logarithm into account. The criteria of de Meijer et al. now change to the criteria shown in Table 15.3.2.

Phenotype	%THC + %CBN	%CBD	Log(%THC+%CBN / %CBD)
Fibre type	< 0.5	≥ 0.5	< 0
Intermediate type	≥ 0.5	≥ 0.5	
Drug type	≥ 0.5	< 0.5	> 0

Table 15.3.2: Log_{10} of criteria for the classification of phenotypes according to cannabinoid content (de Meijer et al., 1992).

A plot of %THC + %CBN versus the result of equation 15.3.2 (log (%THC +%CBN /%CBD) is very useful in visually comparing the phenotype of the subjects under analysis . If we look firstly at the plot for the hemp samples (see Figure 15.3.1).

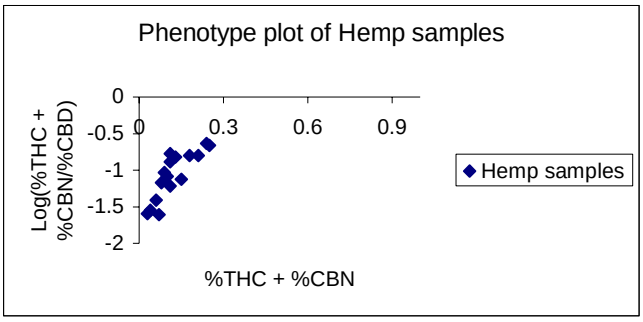


Figure 15.3.1: Phenotype plot for hemp samples

*One can see from the plot that all of the sample points in Figure 15.3.1 are less that zero on the y-axis (log (%THC +%CBN /%CBD). This indicates that the samples are of the fibre type (see Table 15.3.2). It is noteworthy that all of the hemp samples had CBD (**74**) levels above 0.5%, satisfying the %CBD criterion outlined in Table 15.3.2.*

The resin samples are also plotted in this way and are shown in Figure 15.3.2, below.

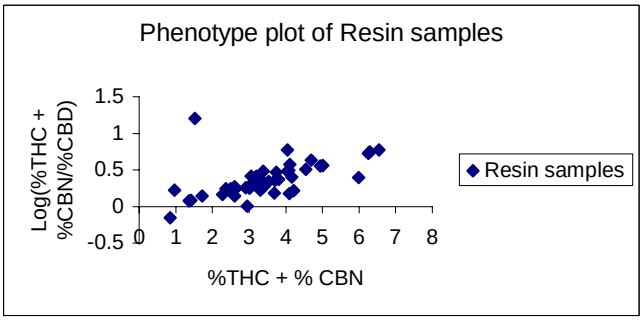


Figure 15.3.2: Phenotype plot for Resin samples

*One can see from Figure 15.3.2 that all of the resin samples have greater than zero on the y-axis (log (%THC +%CBN /%CBD) except one. Further all samples contained levels of CBD (**74**) greater than 0.5 %. The combination of both of these criteria indicates that the samples are intermediate-type. The one sample which does not fit into any of the three categories outlined in Table 15.3.2 could be*

*considered unusual, it must be derived from a variety closely related to hemp as it had a high levels of CBD (**74**) (1.51%).*

*Note that if the CBD (**74**) values were below the limit of quantification as was the case in 3 of the herb samples or if there was no measurable amount of both THC (**72**) and CBN (**73**) then those samples were omitted. The phenotype plot of the two herbal samples that could be included is shown below (Figure 15.3.3).*

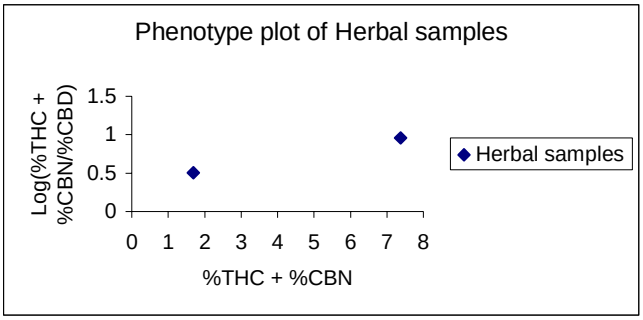


Figure 15.3.3: Phenotype plot for Resin samples

*The other three herbal samples had such low levels of CBD (**74**) (below the limit of quantitation) that there would be no denominator for equation 15.3.2. However the fact that there was no quantifiable CBD (**74**) and that the levels of THC (**72**) were high (9.54%, 4.78% and 9.48%) indicates that these samples were indeed of the drug-type variety.*

The combined plot of Figures 15.3.1, 15.3.2 and 15.3.3, for herbal, hemp and resin samples respectively is shown below (Figure 15.3.4).

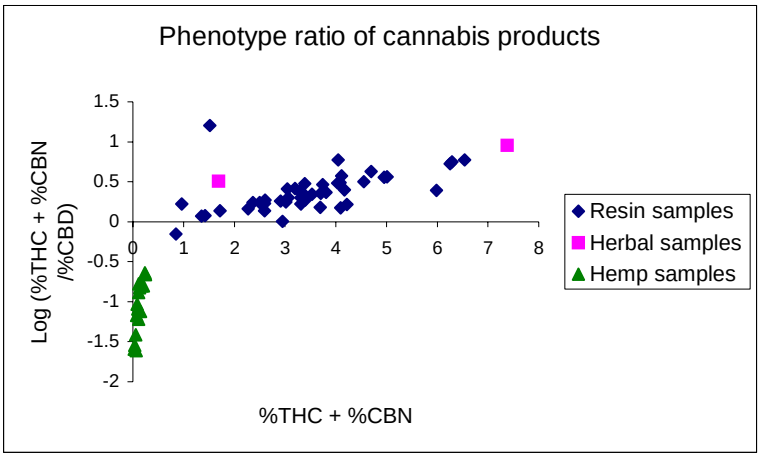


Figure 15.3.4: Plot phenotype ratio for herbal, hemp and resin samples

15.4: The THC content of Joints.

Buchanon *et al.* (1998) estimated the amount of cannabis resin and the amount of cannabis herb in ''joints' seized by the Irish Gardai over the period 1980 to 1996 (1998). It was found that the average amount of cannabis resin found in hand made cigarettes was 102 mg, while the amount of herb was found to be 260 mg (Buchanon, 1998). If one applies the amounts of THC (**72**) quoted in the introduction then the following ranges are likely to be administered by the *cannabis* smoker (see Table 15.3.5).

Cannabis product	Range of THC (**72**), in a ''Joint'', according to the estimates of Buchanon *et al*. (1998)
Cannabis Herb	0.51 mg – 5.1 mg
Cannabis Resin	18.2 mg –36.4 mg
Skunk/Sensimilla	Up to 52 mg

*Table 15.3.5: The estimate THC (**72**) content range in ''joints'' based on the results of Adams et al. (1996), Buchanon et al. (1998) and Martin et al. (1997/1998).*

Hall et al. *(1998) claim on the basis of Australian research that a typical joint may contain from 0.5 g to 1.0 g (of Herb presumably) resulting in a content of THC (**72**) between 35 mg- 70 mg for traditional herb and up to 100 mg for ''Skunk'' /Sinsemilla type drug.*

*As one can see from the Table 15.3.5 the THC (**72**) content could be quite varied. However it is thought that only a small amount of cannabis is required (e.g. 2-3 mg of available THC (**72**)) to produce a brief pleasurable euphoria (high), but more may be needed to satisfy a regular user (Hall, 1998). The THC (**72**) delivered varies between 20% and 70% (Hall, 1994), its bioavailabilty ranging from 5%-24% (Adams, 1996).*

*Using the THC (**72**) levels presented here an estimate of the amount of THC (**72**) likely to be experienced in the Irish situation can be estimated. The estimated amount of THC (**72**) in a joint containing 260 mg of herbal cannabis would be 16.2 mg, but could range from 3.5 mg- 21.84 mg. The amount of THC (**72**), which is likely to be found in a ''joint'' prepared using cannabis resin, would be 4.3 mg, but could range from 0.40 mg- 8.2 mg. When one compares this to the value of 16.2% THC in a joint containing herbal cannabis, one can see that the difference in dose is very large. These high doses may increase the likelihood of the precipitation of some of the acute adverse effects mentioned in the introduction to this chapter, especially among naive or first time users (Hall, 1999). It is noteworthy that there is no information available on the amount of either herb or resin used when individuals attempt to smoke cannabis using a pipe, hence no estimation can be made as to the amount of THC (**72**) administered via this route in the Republic of Ireland.*

15.5: Conclusion

Hemp samples grown under license from the Department of Health and Children satisfy the criteria of the licence (%THC <0.3%) and all the samples were deemed to be of the fibre-type phenotype. High potency varieties of cannabis herb are being traded on the black market in the Republic of Ireland, and all samples analysed would appear to be of the drug phenotype. The resin samples analysed would all appear to be of the intermediate phenotype and do not seem to have a %THC content which is comparable with samples analysed in the USA but not with samples analysed in the Netherlands. The work presented here goes only part of the way to estimating the potency of cannabis products seized and/or grown in the Republic of Ireland. Further work is needed in order to both improve the quality if the data presented here but also as a means of monitoring changes (if any) in the potency of cannabis products seized and/or grown in the Republic of Ireland

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