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# **Characterisation and Profiling of Ecstasy Tablets**

A thesis submitted for the Degree of  
Doctor of Philosophy

By

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## **Dedication**

To the soul of my father who had always encouraged me to seek knowledge, to my mother for her great love and prayers, and to my wife for her patience and support.



# **ABBREVIATIONS**

CE, capillary electrophoresis

FTIR, fourier transform infrared

GC-FID, gas chromatography-flame ionisation detector

GC-IRMS, gas chromatography- isotope ratio mass spectrometry

GC-MS, gas chromatography-mass spectrometry

IR, infrared

KBr, Potassium bromide

LLE, liquid-liquid extraction

MDA, 3,4-methylenedioxy-amphetamine

MDEA, 3,4-methylenedioxy-ethylamphetamine

MDMA, 3,4-methylenedioxy-methamphetamine

MDP-2-P, 3,4-methylenedioxy-phenyl-2-propanone

MECC, micellar electrokinetic capillary chromatography

NMR, nuclear magnetic resonance

PFK, pentafluorokerosene

PMK, phenyl methyl keton

RSD, relative standard deviation

SPE, solid phase extraction

SPME, solid phase microextraction

## SUMMARY

In addition to identifying the presence of a specific controlled drug in an exhibit and measuring its concentration, forensic drug laboratories are requested in certain cases or as a routine to provide additional information that may be helpful to the investigation process. On the basis of their chemical and physical characteristics, seized drugs may be profiled and linked to common sources or routes of distribution.

Chapter 1 is an introduction to the illicit drug production from cultivation to manufacturing and trafficking. Chapter 2 describes the role of the drug chemist and includes characterisation of seizures, that is identification, quantification, and comparison of illicit drugs. Chapter 3 provides a literature review of the different analytical methods used in the area of drug profiling.

This project has been on the subject of drug profiling with focus on the ring-substituted amphetamine, 3,4 methylenedioxymethamphetamine (MDMA) or ecstasy as it is widely called. Among the main objectives of this study was the development and optimisation of a new extraction procedure, solid phase extraction, for impurities in seized ecstasy tablets. The instrumental analysis of impurities found in ecstasy tablets usually require a preliminary process to extract, isolate, and concentrate these impurities from the total tablet content. In the process, interfering

materials are removed, and the required substances are concentrated into a solvent that is suitable for introduction into the instrument. Chapter 4 describes the development of a solid phase extraction (SPE) procedure and also an evaluation of a comparison procedure of liquid-liquid extraction (LLE) and SPE for extracting impurities in ecstasy tablets for profiling purposes. Solid phase extraction of impurities in ecstasy tablets proved to be more efficient than the traditional liquid-liquid extraction. SPE provided impurity peaks with higher intensities than did LLE and a shorter extraction time.

Another area of research was the use of infrared technology as an additional tool for profiling ecstasy tablets as seen in Chapter 5. Infrared method can serve as an elimination or screening step for gross clustering or grouping of exhibits.

In chapter 6 the synthesis of MDMA using different synthetic routes to acquire authentic samples of impurities which are usually present in street samples were performed. These authentic samples were analysed and their mass spectra and retention indices were used to identify impurities in actual street samples to determine their route of synthesis. In chapter 7 ecstasy tablets confiscated within the UK were analysed to establish their route(s) of synthesis using the data of the authentic compounds synthesised earlier.

The main contributions of this project were:

1. Developing a solid phase extraction procedure as an alternative to the conventional liquid-liquid extraction procedure. SPE provided extraction with no cross

contamination of phases and no emulsion problem, as with LLE, due to the presence of fatty acids in ecstasy tablets.

2. Developing a simple and fast infrared method as a screening or elimination tool for ecstasy profiling.
3. Study of the synthetic routes of ecstasy samples within the UK with the aid of route-specific authentic impurity compounds synthesised in-house.

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# **1. ILLICIT DRUGS**

## **1.1. INRODUCTION**

It is widely accepted that illicit drug traffic and abuse are international problems. These problems are international because they cannot be successfully dealt with by a country without the co-operation and assistance of others. The contemporary economic, political and social developments have significant impact on the illicit drug phenomenon. New globalising economic regulations and advances in technology have resulted in a more interdependent world. Criminal organisations involved in illicit drugs respond to these opportunities created by a more relaxed global market of free trade to increase the illicit drug trafficking. Another factor for the rise of international drug production and distribution was the political situation in many different countries around the world including domestic instability and conflicts. The collapse of state structures in many countries has left an institutional vacuum which has been exploited by drug trafficking organisations. Illicit drug revenues or the drugs themselves are usually exchanged for arms to support the different groups involved in armed conflicts.

## **1.2. ILLICIT DRUG PRODUCTION**

Drugs of abuse available in the illicit market can be divided into two general categories with respect to origin, natural, from plants, and synthetic, from precursor chemicals. Natural drugs may be further subdivided into semi-synthetic (e.g. heroin or LSD), isolative (e.g. cocaine or hash oil) or plant material (e.g. marijuana).

### 1.2.1. Plant-based drugs

Plant-based drugs have the largest share of drugs consumed illicitly [1]. In addition to the cultivation of cannabis, the plants used in the illicit production of heroin and cocaine are opium poppy and coca leaf respectively.

#### 1.2.1.1. Illicit cultivation

The illicit cultivation percentage as a share of the total arable land has expanded dramatically in recent years due to the higher profit margins than with licit crops. Figure 1.1 [1] shows the countries with the largest cultivation areas of illicit crops relative to arable land in the early 1990s.

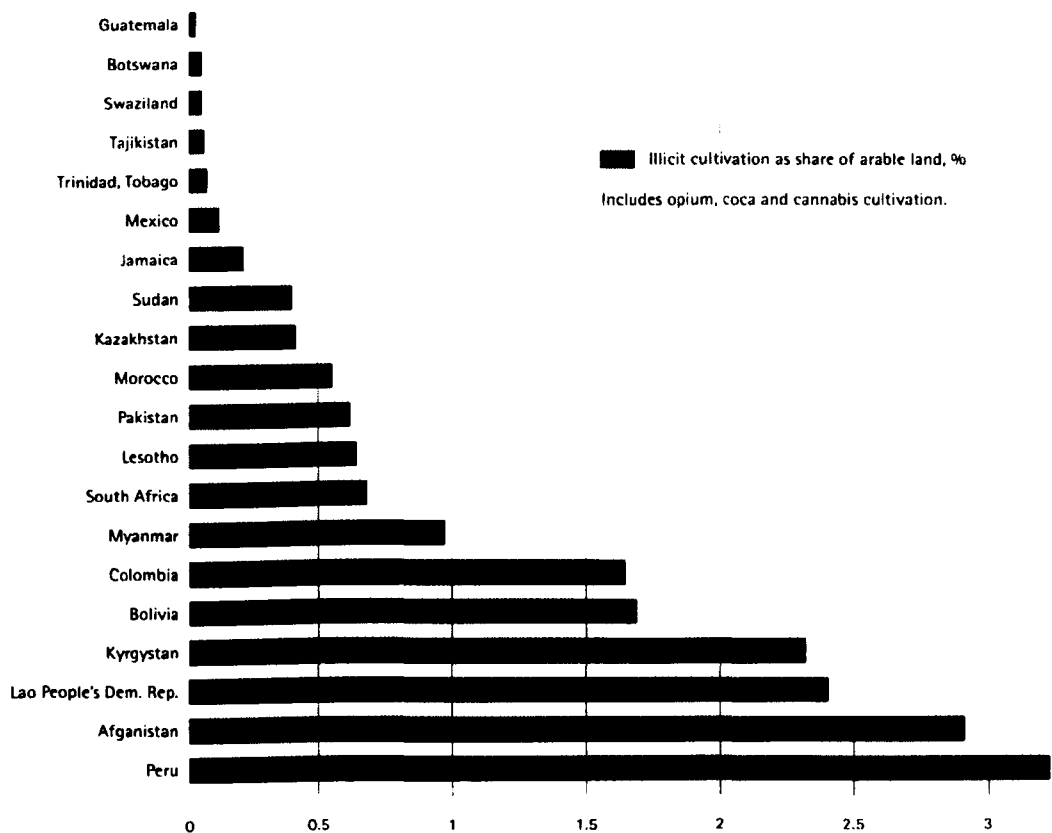


Figure 1.1. Illicit cultivation as share of arable land in the early 1990s

Almost 90 % of the world illicit production of opiates originates in two main areas, the Golden Triangle (Lao PDR, Myanmar, Thailand) and the Golden Crescent (Afghanistan, Pakistan, Iran) [2]. Myanmar and Afghanistan are the two main countries of illicit opium poppy cultivation. Other countries with a lesser extent of illicit cultivation are Columbia, Mexico and countries of Central Asia. Only about a third of the total illicit production of opium gum, which has reached 5000 tonnes in 1996, is thought to be consumed as opium [1]. The rest is used in the manufacture of heroin in clandestine laboratories.

Virtually all of the illicit cocaine produced comes from the Andean regions of three South American countries, Bolivia, Peru and Columbia, which together account for more than 98 % of the world cocaine supplies [3]. Most of the coca leafs used to produce cocaine are grown either in the valleys of Bolivia or Peru [4]. The most important supplier of coca paste and cocaine base to cocaine hydrochloride laboratories in Colombia is the world's leading cultivator of illicit coca, Peru. The amount of coca cultivation in Peru is more than that of the other leading coca producing countries, Bolivia and Colombia [4]. In 1996 about 300,000 tonnes of coca leaf were produced with Peru accounting for half of the total global cultivation area.

Illicit cannabis cultivation is not limited to certain areas as is the situation with coca leaf and opium poppy. However, there are certain regions and countries where mass cultivation and production is practised including Jamaica, Morocco, Columbia, the USA, the Central Asian Republics of the Commonwealth of Independent States, Mexico, Afghanistan, Pakistan and the Republic of South Africa. Figure 1.2 [1] illustrates global trends in the production of coca leaf, opium poppy and cannabis.

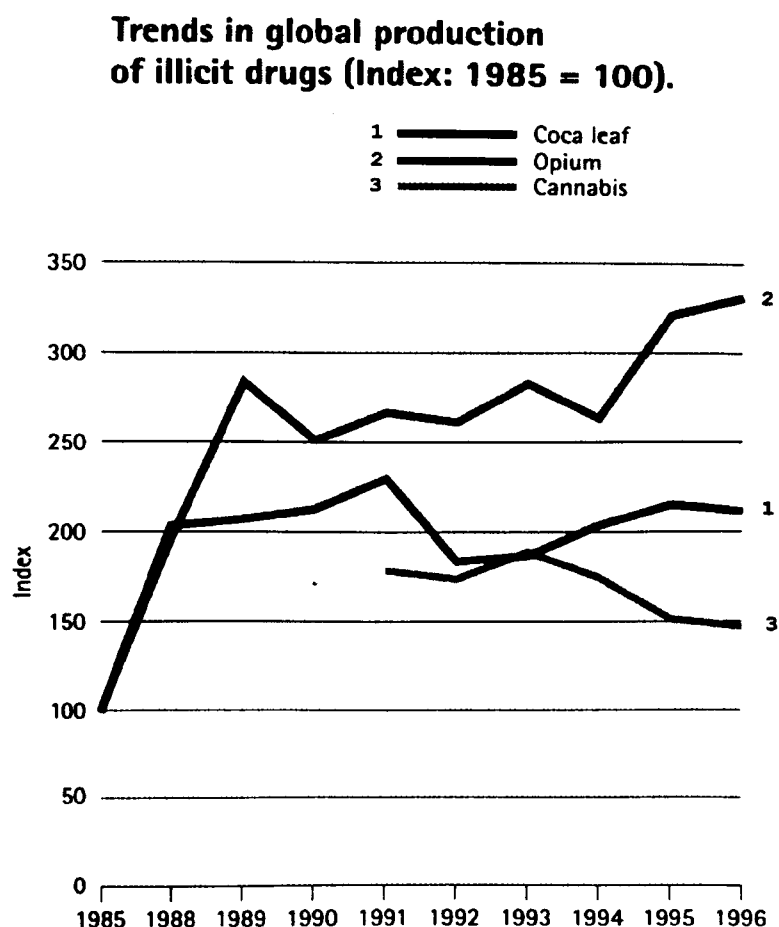


Figure 1.2. Trends in the production of coca leaf, opium poppy and cannabis

#### ***1.2.1.2. Illicit production***

After cultivating and harvesting of the illicit crops (coca leaf and opium poppy), the production stage of cocaine and heroin takes place. A series of laboratories are commonly utilised in processing a drug with each laboratory performing a specific stage or stages of production. The manufacturing of injectable heroin employs six distinct stages of increasing technical complexity [4, 5]. The first stage involves the use of lime and ammonia to extract morphine from opium poppy. The second step is the conversion of morphine into heroin, which begins with the bonding of morphine and acetic acid into an impure form of diacetylmorphine (heroin). Removal of impurities from the compound using water and chloroform is the third

step. The final and most difficult stage involves the use of ether and hydrochloric for the conversion of heroin into powder form. A high level of technical skill is required here to avoid igniting the volatile ether gas.

Cocaine production is done in three different stages of clandestine processing, pasta lab, base lab, and crystal lab as they are called by the clandestine community [6]. The coca leaf must first be processed into coca paste, then coca base. Only then can the resultant compound be converted into the powdery white substance called cocaine hydrochloride. A further stage produces crack cocaine. Traditionally, each stage was completed in separate laboratories, but recently this has changed and the whole process is condensed into direct leaf-to-base laboratories, by-passing the isolation of coca paste [6]. While the paste, base and direct leaf-to-base laboratories are usually in the form of isolated, widespread cottage industry including many individual units all over the coca-producing regions of South America, the crystal laboratories (conversion of coke base to cocaine hydrochloride) are normally larger, more sophisticated and centralised [6].

A new trend in illicit production is the shift in production sites. In the past most heroin was manufactured in Europe near the source of chemical precursors, while today much of the production process takes place within the cultivating area. [4]. Cocaine processing has also shifted to the immediate growing area. This new shift is perhaps a way to increase the unit value of the trafficked substance [4].

### **1.2.2. Synthetic drugs**

Since the mid-1980s the world has faced a wave of synthetic stimulant abuse. The principal synthetic drugs manufactured clandestinely are the amphetamine-type stimulants (ATS) which include the widely abused amphetamine and meth-

amphetamine, as well as the more recently popularised methylenedioxy-methamphetamine (MDMA), known as ecstasy. Lysergic acid diethylamide (LSD) is a synthetic drug also produced clandestinely, whereas sedatives, another type of synthetic drug which includes barbiturates and benzodiazepines, are typically diverted from licit channels. ATS are now consumed in almost every region of the world as seen in figure 1.3. Synthetic drug abuse, particularly ATS are on the increase worldwide. For example, methamphetamine abuse is more widespread than that of cocaine in the countries of the Asia/Pacific region and used more often than heroin in many other countries. In several European countries consumption of ecstasy and amphetamine is greater than that of heroin and cocaine [1].

As opposed to drugs from plants, synthetic drugs can be manufactured from chemicals which can be found or produced almost anywhere in the world. Therefore trafficking distances are much less than the distances required in the production of plant-based drugs. This nature of illicit manufacturing will not only reduce risk, but reduce trafficking and input costs as well, resulting in much larger gross profit margins [7]. Although the illicit production for both plant-based and synthetic drugs behave similarly in certain ways, such as the same characteristics of illegality and risk, they do have crucial differences in that geographical determinants are much less important.

Because of the above differences of the nature of the illicit synthetic industry within plant-based manufacturing, greater profits will result, and the coming years will witness significant expansion in the illicit synthetic drugs industry and consequently higher consumption patterns.





Figure 1.3. Global illicit use of ATS

#### 1.2.2.1. Clandestine manufacture of synthetic drugs

The discovery of clandestine drug laboratories is becoming increasingly common. These laboratories have increased the mobility of the individuals involved. It is not uncommon for them to cook up a batch, make a sale, discard the equipment and chemical residues onsite, and move on to continue the process at another location. The problem of clandestine laboratories for manufacturing illicit drugs is experienced in many countries around the world. Amphetamine and methamphetamine manufacturing premises have been seized in Denmark, Iran, Mexico, the Netherlands, Belgium, the United States, Germany, Korea, Sweden, Canada, and the United Kingdom [8]. Detection of the number of laboratories where drugs are produced illegally can serve as an indicator of illicit drug production. For example, methamphetamine was the drug produced in 84% of the seized clandestine laboratories in the US in the 1990s, 94% of Colombian illegal laboratories produced cocaine, and most Polish laboratories manufactured heroin

from poppy straw [1]. ATS laboratories detected worldwide exceeded those of both heroin and cocaine over the period 1991 – 1994. Around 55% of the total seized laboratories worldwide involved in ecstasy production were located in Europe.

Illicit drug manufacturing poses great adverse effects and can have dangerous consequences including placing people at risk of exposure to toxic chemicals. Chemicals used in the manufacturing process can be corrosive, explosive, flammable, toxic and possibly radioactive. Exposure can occur via absorption, inhalation, ingestion or injection. Generally there are two health hazards associated with clandestine laboratories, criminal hazards (e.g., violence) and chemical hazards (e.g., use of toxic or flammable substances) [9].

#### ***1.2.2.2. Environmental problems***

Environmental problems associated with illicit cultivation are numerous although relatively little attention has been given to this issue [4]. Numerous scientific studies conducted to date describe the negative consequences related to the loss of the world's tropical forest resources. The cultivation of illicit narcotic crops such as coca, opium poppy and cannabis has contributed to the forest removal and water and soil pollution. Illicit cultivation has a share in the tropical deforestation and environmental damage. In Peru, the leading illicit coca cultivator, there are around 125,000 hectares devoted to coca cultivation [4]. Growers commonly plant their illicit crops in fragile forest environments in remote areas. Moreover, chemicals used by many growers at all stages of illicit drug cultivation and production are dumped onto the land and into small streams, rivers and water bodies polluting almost all the surrounding areas.

### 1.3. TRAFFICKING AND DISTRIBUTION

Type of drug usually governs the trafficking pattern worldwide. This involves many different transit countries or forms of transport, some trafficking routes are complex and reflect the criminal efforts to avoid law enforcement authorities (figure 1.4).

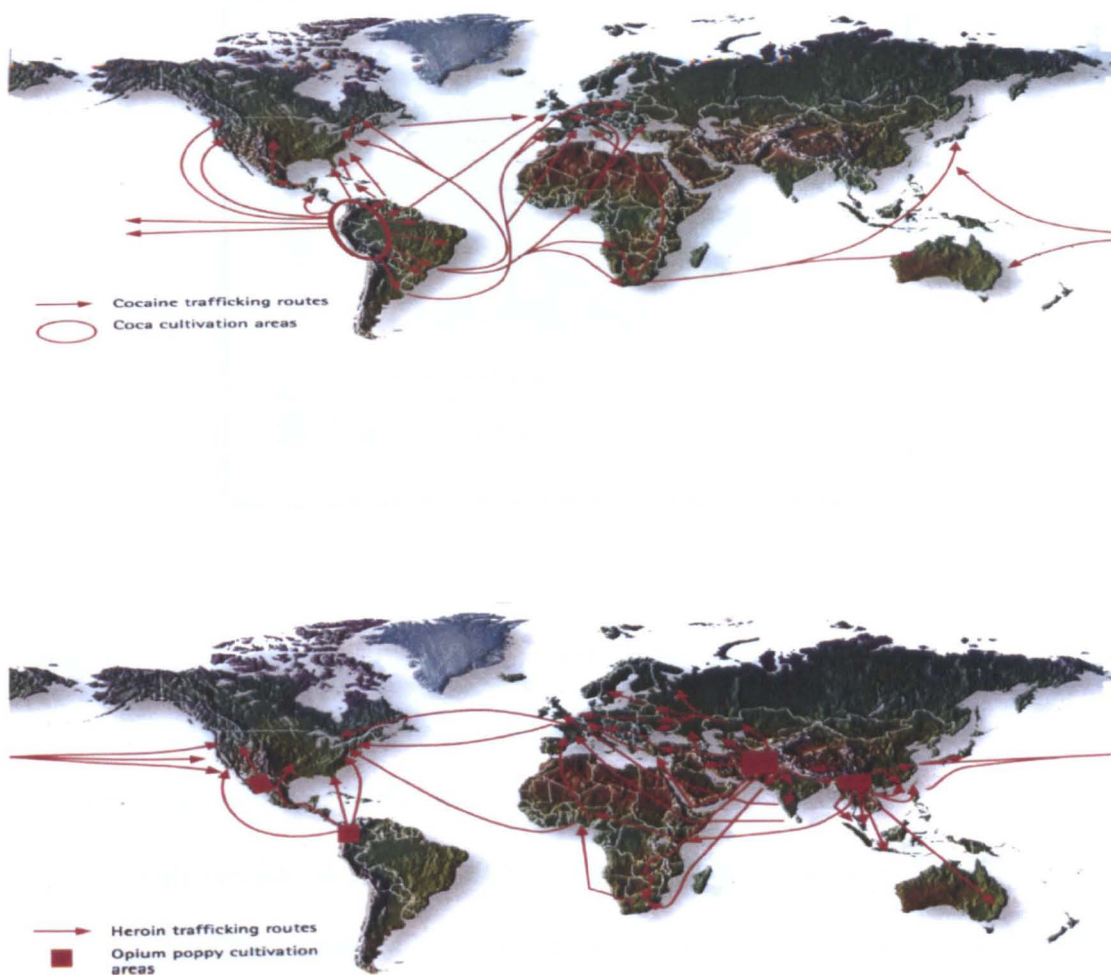


Figure 1.4. International trafficking of cocaine and heroin [1].

Trafficking is the crucial link in the chain between illicit drug production and consumption. The distribution stage of the illicit drug industry accounts for the highest percentage of the gross profit as seen in figure 1.5.

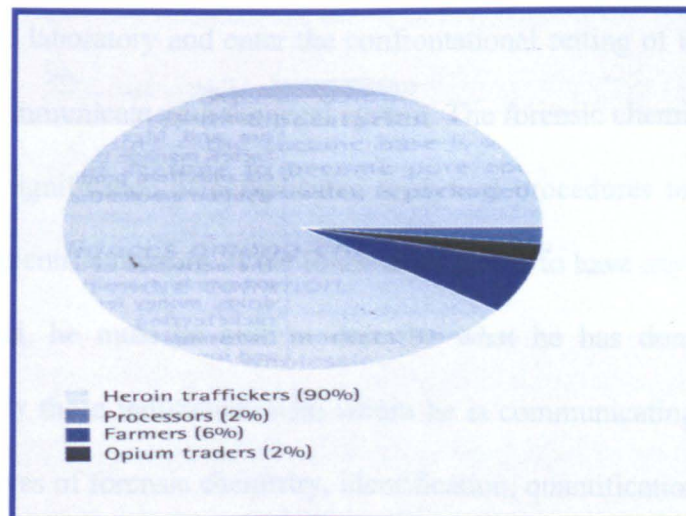


Figure 1.5. Gross profit of different levels in the chain of heroin industry [1].

The most important cost in this large industry in terms of number of participants and turnover with considerable profit margins, is risk, both personal and to the product. Because of the illicit nature of this trade the transactional cost accounts for the highest proportion of the total cost [1]. Production and distribution of illicit drugs have seen notable increase despite defections and arrests. This is mainly attributed to two main factors, high profit margins and ability to innovate and maintain operations. The illicit drug industry has shown high level of organisation including the use of advance technologies and new methods of smuggling.

## **2. ROLE OF THE DRUG CHEMISTRY LABORATORY**

### **2.1. INTRODUCTION**

Every examination made by a forensic chemist has potential legal consequences. Forensic chemists must be prepared to depart from the familiar natural science setting of the laboratory and enter the confrontational setting of the courtroom and be able to communicate with the legal system. The forensic chemist must be able to explain the significance of complicated analytical procedures to individuals with little or no scientific training. If the forensic analyst is to have any credibility on the witness stand, he must be able to describe what he has done in terminology understood by those individuals with whom he is communicating. There are three main objectives of forensic chemistry, identification, quantification and comparison of different exhibits.

### **2.2. IDENTIFICATION OF CONTROLLED SUBSTANCES**

The materials sent to the drug section of a forensic laboratory may be divided into two classes. The first class comprises pharmaceutically manufactured legal preparations, usually tablets or capsules suspected to be illegally possessed or misused, and illicitly prepared tablets. The second class contains materials which lack characteristic external properties, usually powders and sometimes solutions. The most dangerous drugs generally belong to this class as well as clandestinely manufactured tablets.



Controlled substances sold on the street are usually mixed with adulterants and diluents in a crude and mostly unspecified manner. In some laboratories, the analysts are required to identify and quantitate both the controlled substances and the adulterant drugs and diluent materials. Once the analysis has been completed, it must be documented. The final report must be clear and accurate, with all conclusions supported by analytical data. The data may be in the form of notations on paper in the analyst's writing or on instrumental printout. Each time a report is signed, the analyst places his reputation and credibility before the strict test of the court and the careful look of his peers. Discovering a mistake after the report has been submitted to the court is not good.

Whenever a controlled substance is identified, the possibility exists that an individual could be imprisoned or suffer some other legal consequences as a result. There is, therefore, an absolute requirement for high degree of certainty in the identification of controlled substances. An example of this requirement is seen when the law in some countries requires certain conclusions as to whether an exhibit is the base or salt form for sentencing purposes, as is the case with cocaine seizures. In this case, a variety of instrumental techniques can be used to distinguish cocaine HCl from cocaine base. FTIR spectroscopy is commonly used in many laboratories for this purpose. The IR spectra of cocaine HCl and cocaine base are quite different and easily distinguished.

Most street drug samples contain adulterants and diluents. Adulterants are chemicals added to illicit drugs which can give some sort of physiological response. This response can range from very mild to quite severe. Diluents are chemicals added to controlled substances which are used more as fillers than to elicit a physiological response. They can be added to affect the colour and composition for

the sake of satisfying the user. Adulterants and diluents are usually added to the controlled substance mixture by those involved in illicit distribution. Samples taken from large scale (kilogram seizures) will be relatively pure. Adulterants are encountered, in increasing proportion, as the illicit drug progress down the distribution chain from the main supplier to the dealers to the users.

Identification of adulterants and diluents may or may not be a requirement as a part of the identification scheme in the forensic science laboratory. In most cases, the requirements of the legal system will be limited to the identification of the controlled substances. While adulterants are, in most cases, organic substances with similar chemical properties and can therefore be detected and identified in a similar manner as to the controlled drugs, diluents such as sugars, carbohydrates, starch, sodium chloride and calcium carbonate, can be simply identified by microscopic procedures. Chemical separation, however, is also fairly easy because most diluents are usually insoluble in solvents such as diethyl ether or hexane, and only slightly soluble in methanol. Once separated by organic extraction from the main drug mixture, they can be identified using IR if only one diluent is present or HPLC in the case of a mixture of diluents.

Even if the identification of all adulterants, diluents and other by-products are not required in the final report generated by the analyst, such information can prove useful in evaluating trends and possible distribution patterns.

## **2.3. QUANTIFICATION**

There are several reasons why quantitative analyses of drugs are needed. Firstly, in some legislative systems, the penalty for drug crimes is affected by the

concentration of the controlled substances in seizures. Secondly, sudden changes in the drug content (purity) may imply serious threat to the user, especially as far as such highly toxic drugs as heroin and cocaine are concerned. In order to monitor these changes, quantitative analysis of street drugs are needed. Thirdly, such analyses will also establish possible common origins of drug seizures. Matching seizures may then be subjected to closer comparison [10] using methods described in the following section and in chapter 3.

A number of different methods can be used to quantitate controlled substances. Capillary column GC and HPLC are probably the two most utilised instrumental methods to accomplish this task. The most common analytical method for quantitation involves the use of an internal standard, providing a consistent concentration of a known chemical in solution. The internal standard method is especially advantageous because the detector response for the internal standard to the drug can be checked for each injection.

The basic demands for quantitative analysis are accuracy and precision. The ability to measure the true value or the closeness of the measured value to the true value for a sample is the accuracy of the method [11-15]. Accuracy is usually determined in several ways:

- by analysing a sample of known concentration such as a certified reference material and comparing the measured value to the true value or,
- by comparing the results from the new method with results from an established reference method.

The precision of an analytical method is the amount of scatter in the results obtained from multiple analysis of a homogeneous sample. It is the degree of agreement among



individual test results when the procedure is applied repeatedly to multiple aliquots of a sample.

Precision can be divided into three components, 1) *system precision*: the precision of the actual measurement independent of the sample preparation process, 2) *method precision*: this step involves the sample preparation considerations (at least six aliquots from a homogenous sample mixture should be analysed), 3) *day-to-day precision*: allows for a complete assessment of method performance.

An accurate method of low precision will give a mean result close to the true value. However, a large number of determinations would be needed to achieve this and that is a disadvantage from a practical point of view. It may be said therefore, that good accuracy requires good precision.

In quantitative analysis the analyst needs to know how the response measured depends on the analyte concentration. The linearity assessment determines the procedure's ability to obtain test results which are proportional to the concentration of the analyte in the sample within a given range either directly or via a well defined mathematical transformation [12, 16].

## 2.4. COMPARATIVE ANALYSIS

Two different kinds of controlled substance analysis are routinely conducted in the forensic science laboratory. The first is identification, to identify a controlled substance by name. The second, less common, type of analysis is comparative analysis. Its purpose is to determine a commonality of source. A comparative analysis will include a comprehensive examination of the sample's chemical and

physical characteristics, with a goal of demonstrating, with a high degree of certainty, a common origin for two or more samples.

In forensic examination of illicit drugs, it is possible to state with a high degree of certainty that two exhibits of a white powder share a common source. The wording in stating such a conclusion is critical. Words must be carefully selected in order to convey the conclusion clearly and concisely, without overstepping the scientific certainty that exists. The following quote, about two samples of cocaine, is from the transcript of drug trial held in 1991. It illustrates the appropriate language to be used on such occasions.

*After a review of all analytical data, it can be stated that with a high level of scientific certainty and beyond a reasonable doubt that a close chemical relationship exists between (the two samples) strongly suggesting that they were derived from the same manufacturing process...and that they were probably derived from the same batch [17].*

Before undertaking a detailed examination of two samples, a broad overview is desirable. The colour and granularity of the exhibits should be examined, and then the components of the sample identified and quantitated. The second procedure involves the evaluation of the process by-products and trace materials in the exhibits. It is important to realise that for successful evaluation of two exhibits to determine commonality of source, each exhibit must be analysed in the same way using the same methodology and instruments, and chemicals and solvents from same containers.

Controlled substances such as heroin and cocaine are the simplest to compare because they are derived from botanical substances (opium poppy and coca leaf) [18]. Many naturally occurring by-products from the plants are carried through the processing stages of the drugs, and these can be used to confirm the existence of a common source.

The investigation of relationship in the field of drug analysis is an important task in forensic chemistry for court evidence and drug intelligence. Common source of drug seizures is typical example of such relationships. The methods used for this purpose were reviewed [19, 20] and may be divided into groups according to the following scheme.

- A. Physical or “ballistic” methods for comparing items having characteristic external properties, e.g. tablets and capsules.
- B. Chemical analysis
  - i. Inorganic chemical methods for the display of trace element profiles.
  - ii. Organic chemical methods.
    - (a) Identification of impurities characteristic of the synthetic route (key impurities) which may establish relationship with precursor chemicals.
    - (b) Comparison based on the analysis of the chemical composition for the establishment of common sources.
      - 1. Comparison based on chemical signatures (impurity profile)
      - 2. Comparison based on the analysis of known main constituents.

Other methods have also been reported including isotope ratio determination to cluster many different seizures into smaller groups or determine the country of

origin for some drug samples, enantiomeric determination using chiral columns or chiral agents, and qualitative and quantitative determination of residual solvents in illicit drugs. Chapter 3 contains detailed discussion on these methods.

## **2.5. CLANDESTINE LABORATORIES**

Clandestine laboratories are generally divided into two types. The first is the operational clandestine laboratory. This laboratory, usually operating in secrecy, is engaged in the production of controlled substances, precursors to controlled substances, or controlled substances analogues. The second is the non-operational clandestine laboratory. This usually is a storage facility that is under investigation because of information obtained from precursor and essential chemical monitoring [21].

The forensic chemists may be asked to provide assistance in preparing search warrants based on available information, such as knowledge that certain chemicals and pieces of analytical equipment such as gas cylinders, and glassware, such as large triple neck round bottom flasks, have been purchased [22]. This sort of information is critical in determining what kind of synthesis is taking place. The forensic scientist will also provide technical advice regarding the importance of specific safety considerations and offer suggestions on handling situations such as on-going reactions [21].

After the clandestine laboratory site has been secured by the appropriate law enforcement authorities, the forensic scientist may enter the site to evaluate the environment and decide on the most appropriate action. The forensic analyst will begin to sample, package, and mark evidence containers. This process will usually

proceed slowly and methodically to ensure accuracy and completeness [8, 22]. Once the clandestine laboratory has been seized and the evidence collected, the forensic analyst will proceed to the laboratory in an attempt to identify as many of the samples as deemed necessary for the required legal action. This may mean identifying all exhibits that were seized, or it may mean that only those exhibits required to form a conclusion as to an identification of the final product are necessary. The extent of the analysis can be more of a legal question than a scientific question. The forensic scientist should be able to provide the basics of the reaction mechanisms. This information will be based on the chemicals at the site and those identified in the reaction mixture. He should also be able to provide the theoretical yield of the final product based on the amounts of the chemical precursors.

After the work in the laboratory has been completed, the forensic scientist has the responsibility of assisting the legal authorities in understanding what was happening in the clandestine laboratory, what was being synthesized and details of the synthetic method. The forensic analyst must recognise his responsibilities as an expert witness and provide factual information in as much detail as necessary.

### 3. ILLICIT DRUG COMPARISONS / PROFILING

#### 3.1. INTRODUCTION

Drug exhibits in crime laboratories are often present as illicit preparations or as counterfeits of brand-name pharmaceutical products. They are usually encountered in various forms such as powders, tablets or capsules.

Many drug traffickers and dealers mix their supply, which may be already cut, with physiologically active or inactive substances. Various unintentional impurities such as starting materials, synthesis by-products and residual solvents are also present as a result of poor quality control in clandestine laboratories. Substances usually found in illicit drugs other than the active constituent are classified as follows:

**1. Cutting agents:** adulterants are pharmacologically active substances added to compensate for the lack of the illicit drug (e.g. diamorphine). Among the most common are paracetamol, caffeine, phenobarbital, and local anaesthetics [23,24]. Diluents on the other hand are pharmacologically inactive substances added to drugs to increase bulk (e.g. sugars, starch, corn flour, and talc powder) [23,24].

**2. Impurities:** They are substances found in illicit drugs in trace amounts and are of two origins, natural: occur naturally as constituents of natural drugs such as opium and coca leaf; synthetic: constituents in the starting materials, impurities of side products and unreacted starting materials [23]. As a chemical reaction proceeds, various impurities are formed which accumulate because of the lack of quality control in illicit manufacture and are present in the finished drug product [5].

Drugs of abuse can be divided into two general categories with respect to origin; natural, from plants, and synthetic, from precursor chemicals. Natural drugs may be further subdivided into semi-synthetic (e.g. heroin or LSD), isolative (e.g. cocaine or hash oil) or plant material (e.g. marijuana or khat).

In addition to identifying the presence of a specific controlled drug in an exhibit and measuring its concentration, forensic drug laboratories are requested in certain cases or as a routine to provide additional information that may be helpful to the investigation process. On the basis of their chemical and physical characteristics, seized drugs may be profiled and linked to common sources or routes of distribution.

The characterisation and classification of illicit drug samples is an important objective for drug intelligence. It establishes relationships between seizures and may provide evidence of links between dealers and users. Not only does this allow the matching of samples in local cases of dealing but also provides intelligence information on the origin and distribution of samples which may be of use in investigating major trafficking operations. The overall purpose for the comparative analysis [25] is to drive a strategic and/or tactical intelligence. Strategic (macro level), intelligence refers to the derivation of general processing origins, for example, where and how a seized drug was manufactured. This allows for determining trafficking trends (Figure 3.1). Tactical (micro-level) intelligence refers to the derivation of case specific links, relating samples to a common batch and allowing for sample/sample comparison (Figure 3.2).

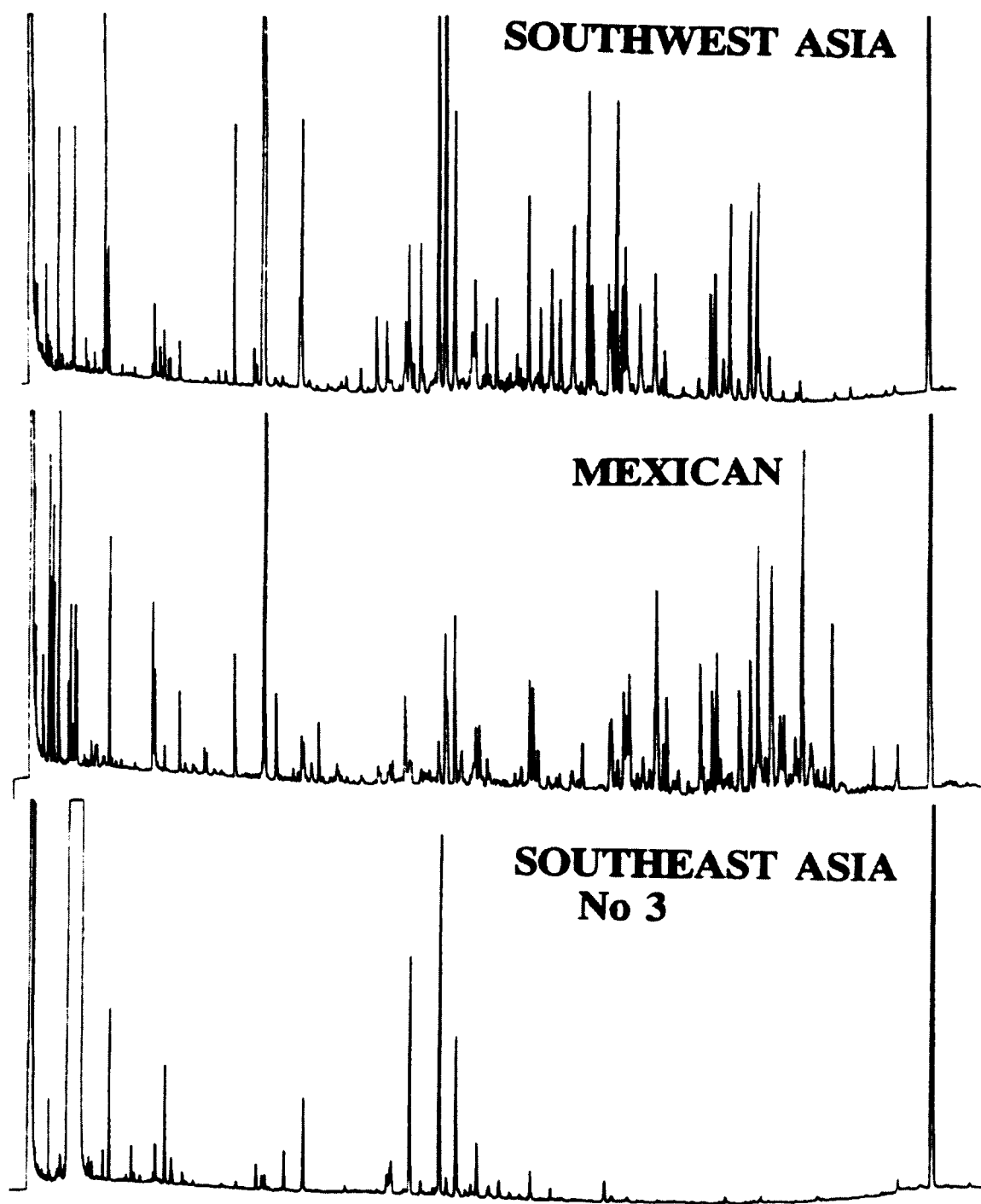


Figure 3.1. Strategic intelligence. Impurity profiles for heroin samples from different regions. [25]

Scientists, specialising in illicit drug analysis in criminal or other analytical laboratories have been using many different approaches in the area of illicit drug characterisation / profiling since the early 1970s. These approaches varied



according to the drug nature and the analytical philosophy or needs. Samples coming into laboratories in tablets or capsule form are usually subjected to physical analysis or so called “ballistics”. Other exhibits such as powders are subjected to chemical analysis, which can be organic or inorganic. Inorganic analysis of illicit drugs is concerned with the determination and comparison of the elemental content in exhibits. Organic analysis on the other hand is more varied with different types of analysis and approaches available to the forensic chemist. There are many different types of organic analysis performed by analytical laboratories, which are involved in illicit drug characterisation and profiling:

1. Determination and quantification of impurities or minor components present in the seized drug, which originate from precursors, natural raw materials, solvents used in the synthesis, and side-reaction and intermediates.
2. Measurement of naturally occurring isotopic ratios.
3. Determination of diastereoisomeric or enantiomeric composition of drug exhibits
4. Determination of residual solvents for comparative analysis
5. Identification and quantification of the principal controlled drug(s) and cutting agents present in the exhibit.
6. Drug profiling using infrared spectrometry

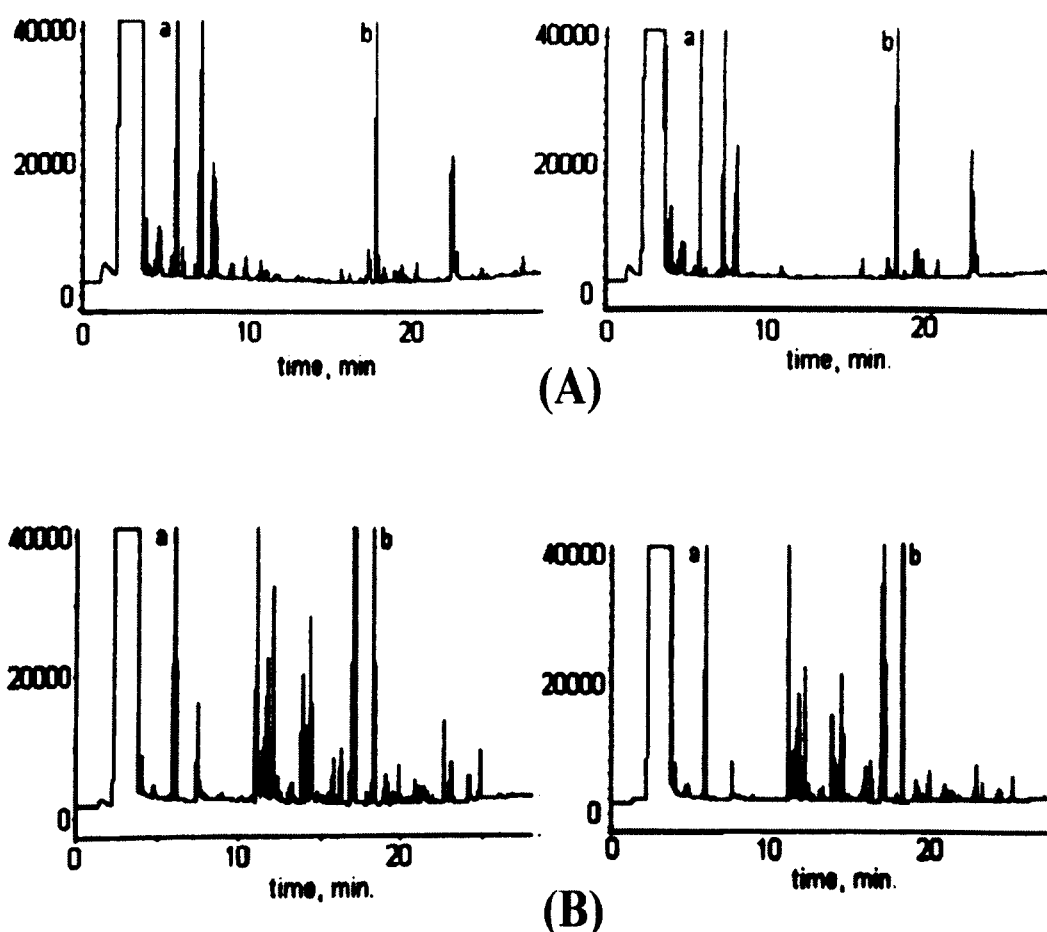


Figure 3.2. Tactical intelligence. (A) Two samples from different cases identified as coming from the same batch. (B) Two samples from same case identified as coming from same batch.

This chapter provides a review of published reports carried out by many workers in the field of illicit drug characterisation and profiling. Most of the reports cited were concerned with heroin, cocaine, and amphetamines (amphetamine, methamphetamine, and 3,4-methylenedioxyamphetamine and analogues). Reports reviewed include experimental analysis used such as analytical instruments and systems, extraction procedures, and computer software including statistical analysis.

### 3.2. PHYSICAL METHODS “BALLISTICS”

These methods are used for comparing items having characteristic external properties, e.g. tablets, capsules and fractured hashish cakes. Physical analysis of illicit drug preparations is largely limited to tablets and capsules, although occasionally other seizures received considerations. For tablets, the examination involves firstly the determination of gross characteristics of the preparation such as shape, diameter, thickness and weight. Other features, such as the groove depth and width, bevell angle and groove or score angle, can be studied by the use of microscopic means [26]. Secondly, low power microscopy is used to detect minute “punch” marks which are transferred to the tablet from the punch used during the manufacturing procedure; these serve to identify a tablet with a particular set of punches. The marks on the tablet are a result of the damaged areas of the punch faces. These marks are normally seen on the groove faces and bevelled edges of a tablet. Capsules on the other hand are less easy to identify and examinations are usually limited to the determination of size, shape, locking features, colour, etc.

Only physical tests that involve accurate measurements are of value for the comparison of illicitly produced tablets. The success of a physical analysis of drug exhibits depends on considering some critical requirements [19], such as adequate numbers of exhibits, proper handling (e.g. storing at stable conditions, maintaining physical integrity) and building up a database of authentic materials. Normally, two or more physical analysis are performed on the same exhibit resulting in an orthogonal technique for establishing connectivities [27].

### 3.3. CHEMICAL METHODS

These methods are normally used for the analysis of items lacking characteristic external properties such as powders or solutions, but have also been applied to other samples such as tablets and plants. Chemical analysis for the characterisation of illicit drugs normally involves the following procedures:

#### 3.3.1. Inorganic analysis

A study of inorganic impurities provides additional information to the impurity profiling of illicit drugs. Elemental analysis of drugs can be helpful in giving information regarding geographic origin, synthetic route, and type of refining treatment used plus direct sample/sample comparison [28]. Inorganic analysis is a simple and effective method for profiling illicit drugs [29]. Trace elements (inorganic impurities) analysis provides some advantages over organic analysis including the following:

1. no storage decomposition as is the case with many organic substances [30],
2. difficulty comparing and classifying samples based on their organic impurities rather than their inorganic impurities if the drug exhibit is very pure [31],
3. high cost of maintaining organic impurity profiling methods and acquiring data [32].

Study of the elemental concentrations in drug exhibits can provide unique information regarding the raw materials used and manufacturing procedures employed [33].

Trace element profiling has been successfully applied to many different matrices including food products [30], where the geographic origin determination analysis was performed for orange juice, pistachio, and macadamia nuts, diamond, gold and drugs of abuse, i.e. methamphetamine (figure 3.3 ) [29, 34, 35], heroin [28, 32] and sodium gamma hydroxy butyrate (GHB) [29].

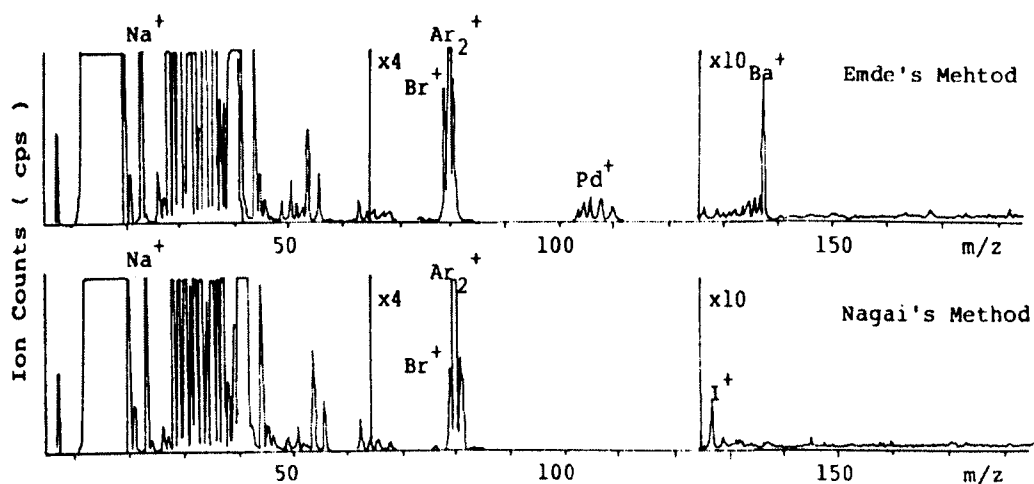


Figure 3.3. ICP spectra for methamphetamine hydrochloride [36]

Inductively coupled plasma, ICP with atomic emission spectrometry, AES [33] or mass spectrometry, MS [28, 29, 31, 32, 34, 35] was the analytical tool predominantly used for trace elements analysis. Heroin sample were prepared by dissolving in nitric acid [28,29,32], or hydrochloric acid after being dry-ashed [33], methamphetamine samples were dissolved in deionised water [31, 34, 35] and cocaine samples in HCl. Other instruments such as atomic absorption spectrometry (AAS) [34] and ion chromatography [31] were also used. Statistical analysis usually follows elemental analysis to provide a better means of presenting data in the form of cluster analysis [34] as shown in Figure 3.4 or dendograms [32] as illustrated in Figure 3.5.

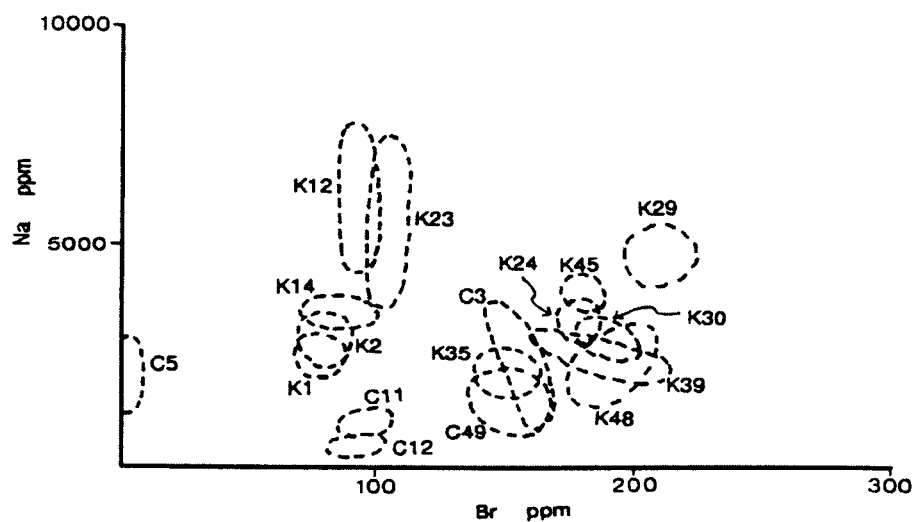


Figure 3.4. Classification of methamphetamine sample by Br and Na concentration.[34]

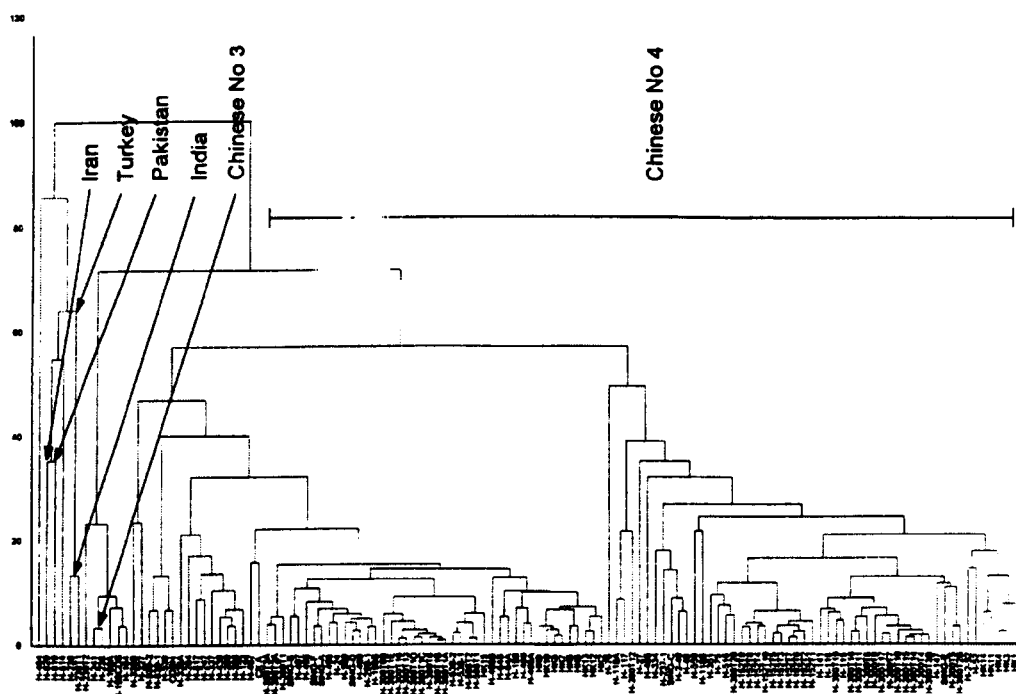


Figure 3.5. Dendrogram from individual analysis from separate heroin seizures [32]

### 3.3.2. Organic Analysis

#### 3.3.2.1. Analysis of impurities (minor components)

Clandestinely manufactured drugs often contain impurities in trace amounts. As a chemical reaction proceeds, various impurities are formed which may accumulate because of the lack of quality control in illicit manufacture [37] as seen in Figure 3.6. Impurities are of two origins; natural (constituents of natural drugs such as opium and coca leaf), and synthetic (constituents in the starting materials, precursors, side products, solvents and intermediates) [5]. The determination and quantification of these impurities provides a great deal of information to the drug analyst for the characterisation and comparison of drug exhibits. It reflects precursor chemicals, synthetic routes, common origin, and trafficking trends.

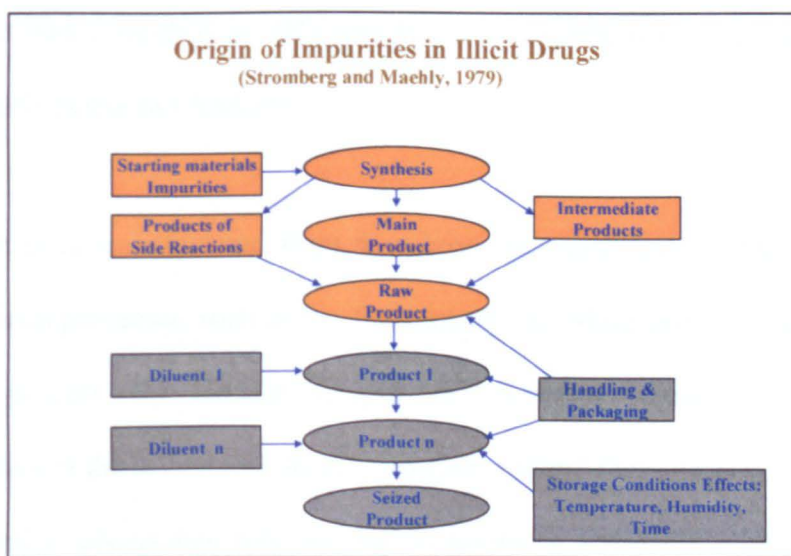


Figure 3.6. Origin of impurities in illicit drugs [37]

##### 3.3.2.1.1. Identification of impurities

Identification of impurities present in clandestinely manufactured drugs often provides information on the precursor chemicals used and synthetic routes employed. Certain impurities identified in illicit drugs are route specific impurities

(e.g. intermediates). Identification of impurities for the purpose of establishing the synthetic route has been reported for amphetamine [38-44], methamphetamine [45-49], 3,4-methylenedioxy-methylamphetamine, MDMA, [50-56], heroin [23, 57-60], and cocaine [24, 61-64].

### Heroin:

Heroin is manufactured via the direct precursor, morphine, from the dried milky juice obtained from the ripe flower pods of *Papaver somniferum*, opium. Opium contains many natural alkaloids including morphine, narcotine (noscapine), codeine, papavarine and thebaine.

Morphine was first isolated from opium in 1805 while the first synthesis of diacetylmorphine (heroin) was reported in 1874. Due to its wide abuse early this century, it was controlled in 1925 and the first clandestine manufacture of heroin subsequently began in China [5].

Since heroin is manufactured from morphine, the latter is extracted from opium using various processes, such as the Thiboumery and Mohr process, the Robertson-Gregory process, the Barbier process, the Heumann process, the Schwyzer modification of the Robertson-Gregory process, and the Merk process. The majority of clandestine laboratories followed the Thiboumery and Mohr process [5]. Once the morphine is produced, synthesis of heroin follows (Figure 3.7).



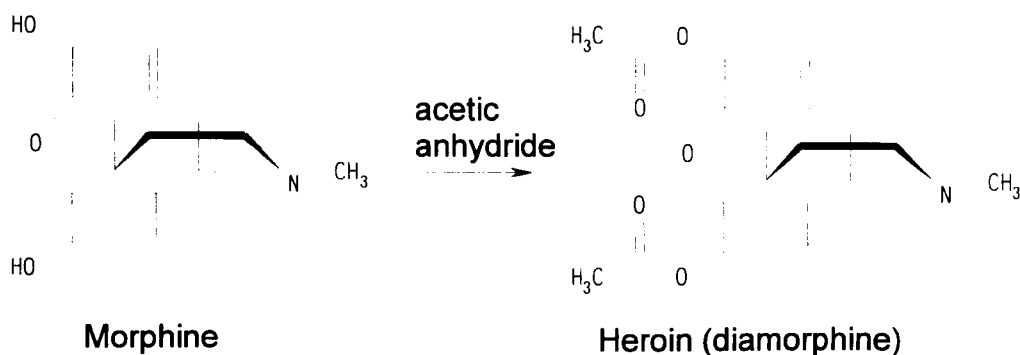
SYNTHESIS OF HEROIN [52] :

Figure 3.7. The acetylation of morphine to produce heroin.

Because of lack of quality control in the clandestine manufacturing of heroin and with no purification steps used for the final product, many impurities originating from opium, formed during synthetic reactions and/or coming from chemicals used in the process are carried over and are present in the final product. The main impurities encountered in illicit heroin samples are listed in Table 3.1.

Some heroin manufacturing impurities	
1. Meconin	18. N-acetylnorlaudanoline
2. Hydrocotamine	19. Papaveraldine
3. 3,6-dimethoxy-4,5-epoxyphenanthrene	20. Desbaine
4. Thebaol	21. 4,8-diacetoxy-3-methoxy-8-[2-(N-methylacetamido)]-ethylphenanthrene
5. O <sup>6</sup> -acetylthebaol	22. Narcotine
6. 4,6-diacetoxy-3-methoxyphenanthrene	23. 4-acetoxy-3,6-dimethoxy-8-[2-(N-methylacetamido)]-ethylphenanthrene
7. (E)-3-[2-(2-N-methylacetamido)ethyl]-4,5-methylenedioxy-6-methoxyphenyl] acrylic acid	24. N-acetylnornarcotine
8. Papaverine	25. 4,6-diacetoxy-3-methoxy-8-[2-(N-methylacetamido)]-ethylphenanthrene
9. O <sup>6</sup> ,N-diacetyl- $\alpha$ -norcodimethine	26. (E)-N-acetylanhydronornarceine
10. O <sup>6</sup> ,N-diacetyl- $\alpha$ -normorphimethine	27. (1R,9S)-1-acetoxy-N-acetyl-1,9-dihydroanhydronornarceine
11. O <sup>6</sup> ,N-diacetylnorcodeine	28. (Z)-N-acetylanhydronornarceine
12. N-acetylnormorphine	29. (1R,9R)-1-acetoxy-N-acetyl-1,9-dihydroanhydronornarceine
13. O <sup>3</sup> ,O <sup>6</sup> ,N-triacetyl- $\alpha$ -normorphimethine	30. O <sup>2</sup> -monoacetylmorphine
14. O <sup>6</sup> ,N-diacetylnormorphine	31. O <sup>6</sup> -monoacetylmorphine
15. 4-acetoxy-3,6-dimethoxy-5-[2-(N-methylacetamido)]ethylphenanthrene	32. 4-acetoxy-3,6-dimethoxyphenanthrene
16. O <sup>3</sup> ,O <sup>6</sup> ,N-triacetylnormorphine	33. N,O <sup>6</sup> -diacetylnorcodeine
17. 4,6-diacetoxy-3-methoxy-5-[2-(N-methylacetamido)]ethylphenanthrene	

Table 3.1. Impurities detected in illicit heroin exhibits [5]

Relating heroin samples to common origins on the basis of the impurities present was reported for narcotine and norlaudanosiine-related manufacturing impurities using a one-step extraction procedure and capillary gas chromatography [59]. Acidic and neutral impurities in illicit heroin, analysed by capillary electrophoresis, were also reported [60]. Using reversed-phase HPLC, O<sup>6</sup>-monoacetylmorphine, acetylcodeine, noscapine, and papaverine [23] were also detected and identified. Huizer [57] provided a study of the formation of O<sup>3</sup>-monoacetylmorphine during heroin production and during its hydrolysis. Also in another study [58] quantitative and qualitative determinations of some naturally occurring opium alkaloids were used to draw conclusions about the common origin of different samples.

### Cocaine

Cocaine was first isolated from coca leaves by Albert Niemann who gave it the name cocaine in around 1860. Coca leaves grow in two main regions, the Far East (Indonesia, Taiwan, and Sri Lanka) and South America (Colombia, Peru, and Bolivia). The latter is the source of most cocaine in the world. The Far Eastern leaves have low content of cocaine and are not known to be involved in the illicit market [5]. Cocaine is also produced from total synthesis. However, the majority cocaine found in the illicit market is not from this source because the synthesis requires a degree of expertise not easily found in clandestine laboratories.

### CLANDESTINE COCAINE PROCESSING

The flow chart below (Figure 3.8) describes three different stages of clandestine cocaine processing, *pasta lab*, *base lab*, and *crystal lab* as they are called by the clandestine community [6]:

The start is in the pasta lab where 100 to 150 kg of dry leaves are soaked in water. A strong base is then added to force the nitrogenous alkaloids into an organic phase (gasoline or kerosene) resulting in an alkaloid mixture which consist of cocaine, cis & trans-cinnamoylcocaine, tropine, tropacocaine, hygrine, cuscohygrine, ecgonine, benzoylecgonine, methylecgonine, and stereoisomers of truxillines, truxinic and truxillic acid, methyl benzoate, benzoic acid, cinnamic acid, and soluble organic plant waxes. After an addition of mineral acid (such as sulfuric or hydrochloric acid), the organic solution of alkaloids is precipitated as alkaloid salts.

The crude cocaine salt produced in the pasta lab is passed to the base lab or directly to the crystal lab. In the base lab a further purification is performed by potassium permanganate oxidation of cis & trans-cinnamoylcocaine. An overoxidation usually result in the formation of N-formyl cocaine which means reduced profits. This is why the base lab stage is sometimes omitted.

In the final stage (crystal lab) crude cocaine is converted to cocaine hydrochloride by dissolving it in a solvent such as ether followed by the addition of hydrochloric acid. These solvents are sometime detected in cocaine hydrochloride samples and contribute another parameter for cocaine comparison and profiling.

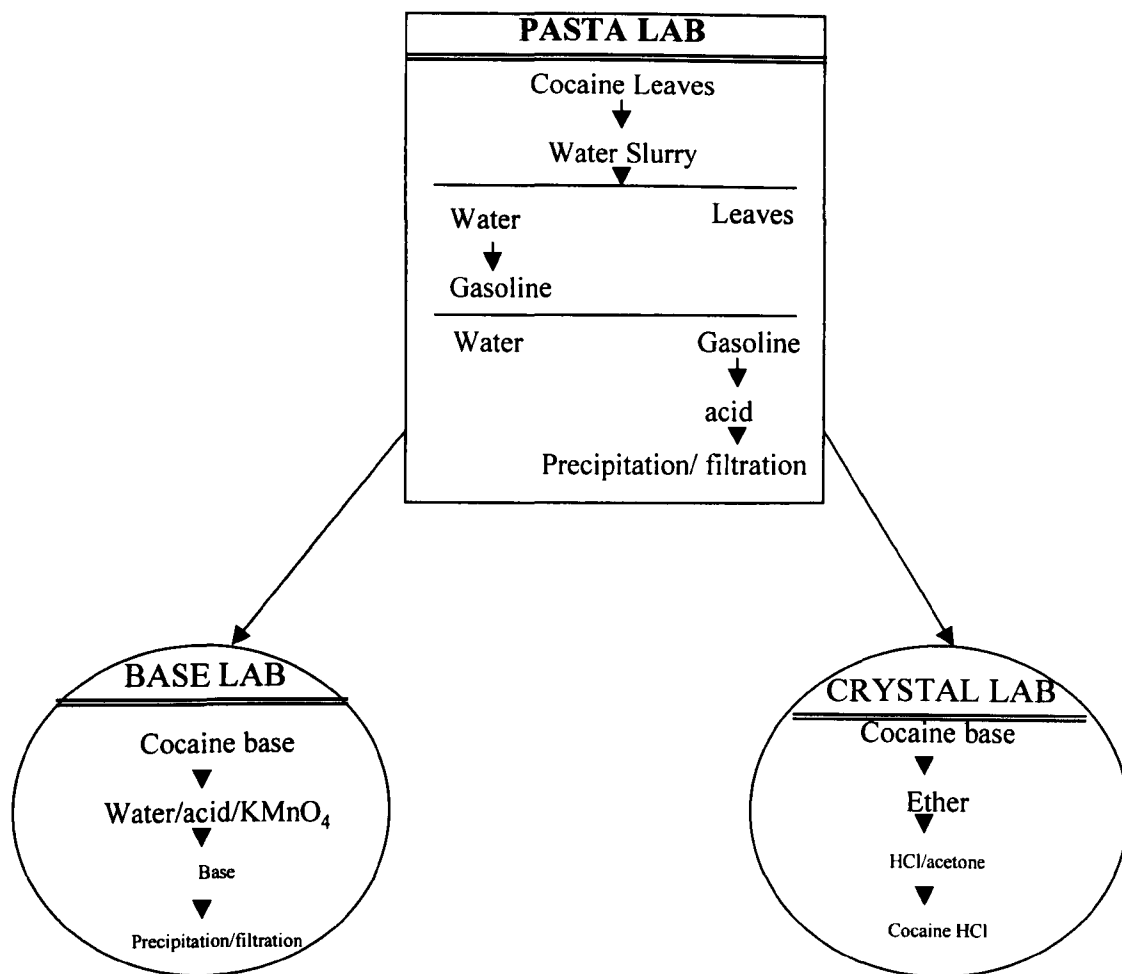


Figure 3.8. Cocaine production process.

Impurities in illicit cocaine samples were also detected and results were used to relate samples. Comparison of illicit cocaine samples by determination of the main coca alkaloids cocaine, cis-cinnamoylmethylecgonine, and trans- cinnamoylmethylecgonine using two HPLC systems [24] was reported. The identification of N-formyl cocaine [61], and norcocaine, N-benzoylnorecgonine methyl ester, and N-formyl norcocaine [62], which originate from using potassium permanganate to oxidise impurities in the cocaine processing procedure, was used to characterise illicit cocaine samples. The absence of N-formyl cocaine indicates that no potassium permanganate was employed [61]. The identification of the common minor alkaloids of *E. coca* in illicit cocaine samples means that the cocaine is natural [64]. Baugh et al [65] provided an excellent review of

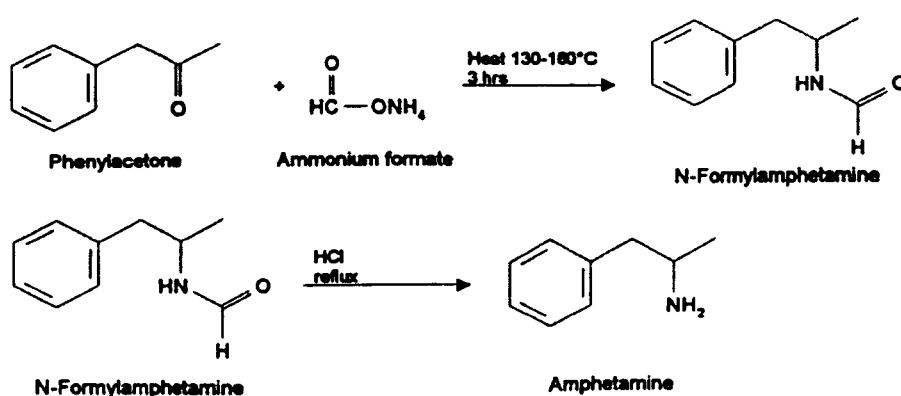
impurities encountered in illicit cocaine samples. Table 3.2 gives a list of major impurities in cocaine samples.

impurities in illicit cocaine	
1. l-cocaine	10. l-norcocaine
2. ecgonine	11. N-formylnorcocaine
3. ecgonidine	12. N-benzylnorecgonine
4. benzoylecgonine	13. cis-cinnamic acid
5. trans-cinnamoylcocaine	14. cis-methyl cinnamate
6. cis-cinnamoylcocaine	15. benzoic acid
7. truxiline	16. methyl benzoate
8. truxilic acid	17. hygrine
9. tropacocaine	18. cuscohygrine

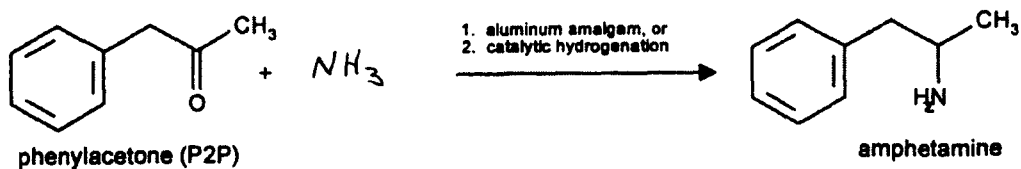
Table 3.2. Compounds associated with cocaine exhibits from natural sources

### Amphetamine

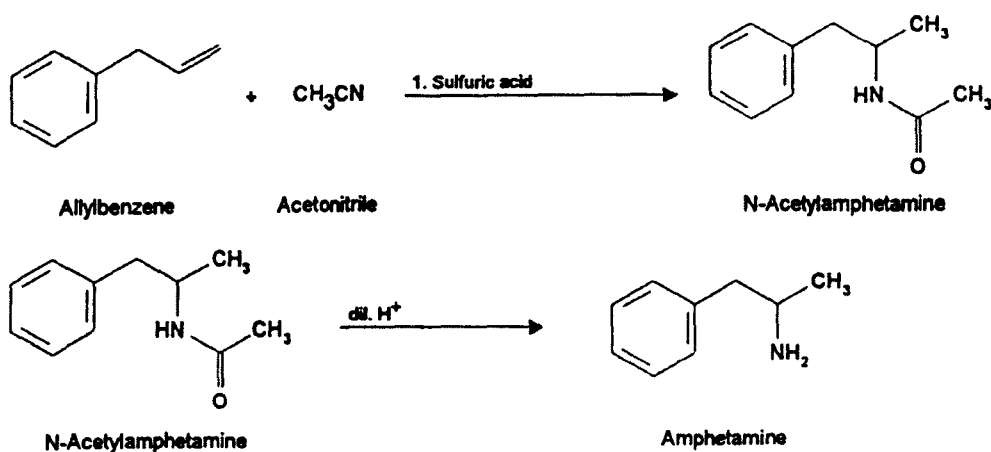
The Leuckart amphetamine synthesis has been the most popular method for the production of amphetamine in the U.K., U.S.A. and The Netherlands and other European countries [40, 41, 47]. Other methods such as the reductive amination of benzyl methyl ketone and the allylbenzene route have also been used [47] (Figure 3.9)



#### 1. Leuckart reaction



## 2. Reductive amination of P-2-P



## 3. Allylbenzene route

Figure 3.9. Routes of amphetamine synthesis.

The detection of 4-methyl-5-phenylpyrimidine indicates that the sample was synthesised by the Leuckart method [40, 44]. Isolation by repeated TLC using various solvent mixtures and identification by mass spectrometry and  $^1\text{H}$  and  $^{13}\text{C}$  NMR of two pyrimidines, five pyridines, and one pyridone in illicit amphetamine was reported [42]. Knowledge of these impurities helps avoiding interference by the by-products in analytical technique [43]. Figure 3.10 shows a typical Leuckart amphetamine impurity profile. Reviews of the most common synthetic routes for

manufacturing amphetamine and impurities identified have also been published [38, 39, 47].

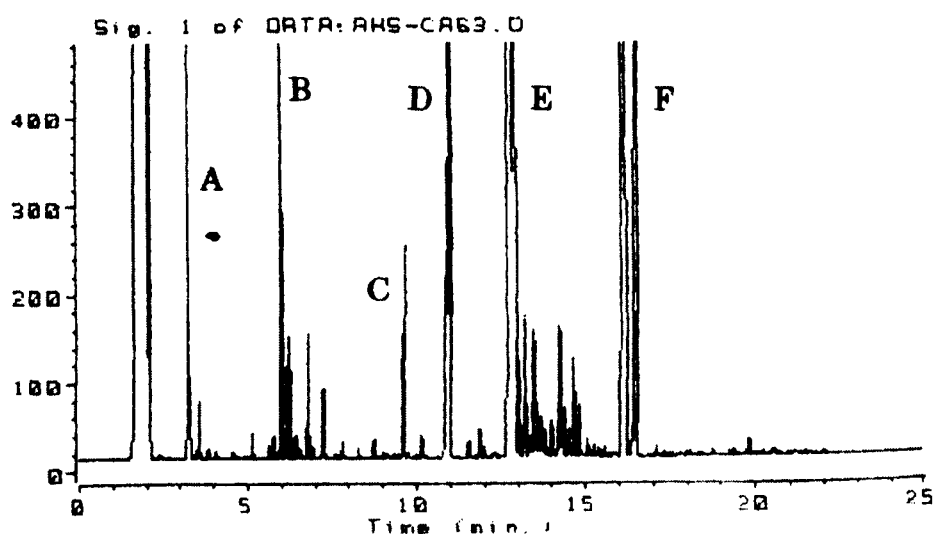
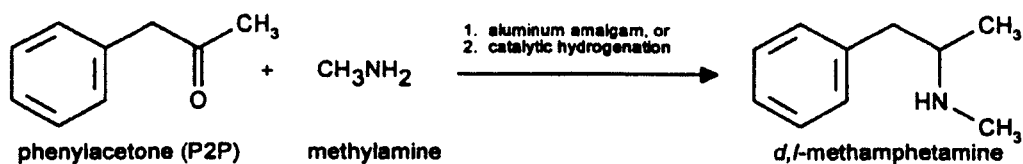


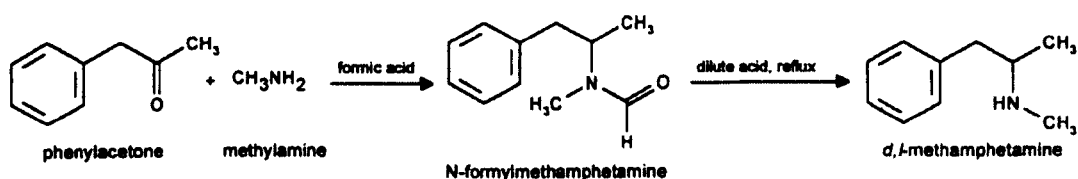
Figure 3.10. Leuckart amphetamine impurity profile. A, amphetamine; B, 4-methyl-5-phenylpyrimidine; C, N-benzylamphetamine; D, di( $\beta$ -phenylisopropyl)amine; E, N-methyl-di( $\beta$ -phenylisopropyl)amine; F, N,N-di( $\beta$ -phenylisopropyl)formamide [66]

### Methamphetamine

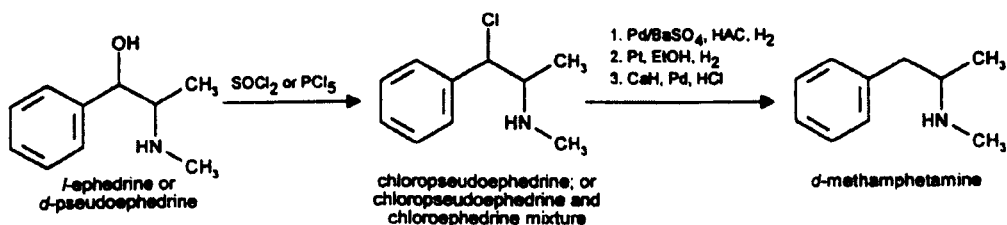
Methamphetamine is synthesised by various routes, e.g., reductive amination of benzyl methyl ketone, Leuckart reaction [47], and from ephedrine [45] as illustrated in Figure 3.11. Other routes are the allylbenzene route [49] and the phenylacetic acid route [48].



### 1. Reductive amination of P-2-P



### 2. Leuckart reaction



### 3. Ephedrine route

Figure 3.11. Synthesis of methamphetamine from different routes

Many impurities were detected as route-specific impurities such as iodoephedrine, 1,2-dimethyl-3-phenylaziridine from the ephedrine route [45], 1-phenyl-2-bromopropane, an intermediate from the allylbenzene route [49], and N-formylmethamphetamine as a route specific impurity from the Leuckart reaction [47].



Determination of route specific impurities has led to the conclusion that the methamphetamine abused in Japan is prepared from ephedrine [46].

Several dozen analogs of amphetamine and methamphetamine came out in the 1960s and became more widely abused in the early 1980s. They were called designer drugs. Designer drugs are produced in clandestine laboratories to mimic the psychoactive effects of controlled drugs. Among the most widely known designer drugs is MDMA.

### MDMA

MDMA, called “Adam,” “Ecstasy,” and other names in the street, is a synthetic drug. Its chemical structure (3,4-methylenedioxymethamphetamine) is similar to two other synthetic drugs, MDA and methamphetamine, which are known to cause brain damage. It was first synthesized in 1914, rediscovered and became popular as a recreational drug in early 1980s. MDMA is an analogue of MDA and it is related to both amphetamine and mescaline. Although it is often referred to as a psychedelic drug, it also possesses stimulant properties. As a free base MDMA is a white, musty smelling oil with a searing taste, insoluble in water but soluble in most organic solvents[67].

Clandestine production of 3,4-methylenedioxymethamphetamine, MDMA was discussed in detail [56]. Many different synthetic routes such as the reductive amination route, the Leuckart method, the bromopropane route, the Ritter reaction, the nitropropene route and the cinnamic acid route were reviewed. Safrole, isosafrole, piperonal, and 3,4-methylenedioxy phenyl-2-propanone are the main precursors used. MDMA is always illicitly manufactured [51, 54] and because of the lack of quality control in clandestine laboratories, impurities arising from

precursor chemicals, intermediates and other impurities are also produced and will be present in the final product. In a review [51], mass spectral data of impurities in MDMA samples synthesised by the reductive amination route, the Leuckart reaction, the nitropropene, and the bromopropane route were reported. Impurities in illicit MDMA samples were also isolated by TLC and detected by mass spectrometry and  $^1\text{H-NMR}$  [54]. Renton et al [52] provided data for precursors, intermediates and reaction by-products in illicit MDMA. They used these results for establishing the route of synthesis in items received in casework. Verweij reported [50] the structural elucidation of impurities in illicit MDMA prepared by the low-pressure reductive amination of 3,4-methylenedioxy phenyl propanone with methylamine. The presence of the intermediate imine (1,2-methylenedioxy-4-2-N-methylimino propyl benzene) provided information about the synthetic route. Chapter 6 provides a comprehensive overview of the synthesis used in the manufacture of MDMA and impurities detected and identified.

#### *3.3.2.1.2. Comparison and classification of impurity profiles (signatures)*

A second different method of comparative analysis is the detailed examination of co-extracted constituents of natural plant materials as well as impurities in synthetic drugs originating from synthesis, such as precursor chemicals, reaction by-products, intermediates and others. The results of this analysis often in the form of chromatograms, are called chemical signature, impurity profile, or chemical fingerprints. Such analysis can provide vital information concerning batch or source relation comparison, grouping drug exhibits into classes or determination of geographic origin of seized drugs for investigative purposes, court testimony, and drug intelligence. Cluster analysis of impurity profiles of drug exhibits allows the classification of exhibits into smaller groups (Figure 3.12), hence providing important information to drug intelligence. The importance of source of supply

identification and origin of seized drugs determination was recognised at an international drug expert group meeting [68]. Drug signature profiling has been defined [69-71] in the literature.

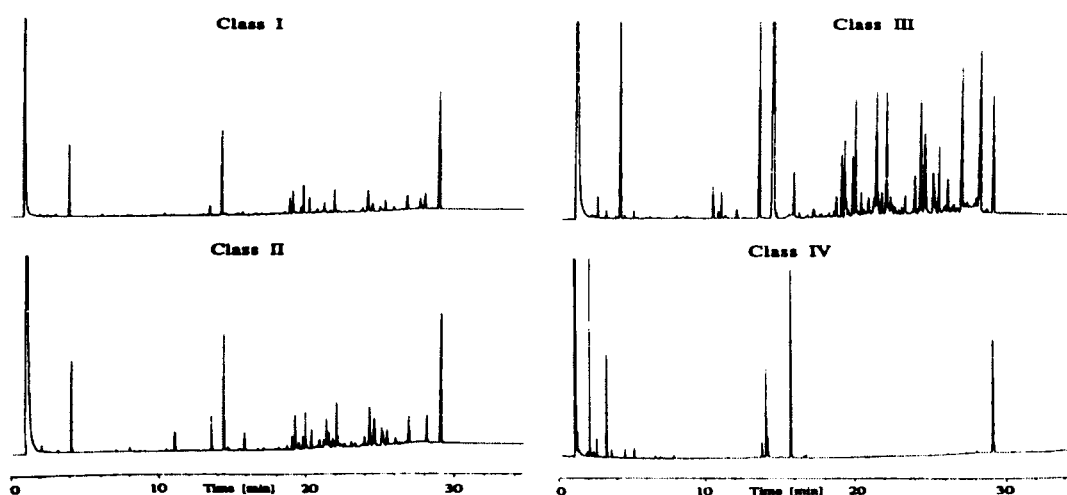


Figure 3.12. Grouping heroin exhibits into classes (smaller groups). [72]

The same expert group meeting concluded that it was feasible to use chemical characterisation of heroin for the purpose of identifying source of supply and trafficking trends [68]. Many workers developed methods for heroin impurity profiling using gas chromatography [72-76] and using a capillary-to-capillary column-switching system [77]. HPLC was also used to profile acidic and neutral impurities in heroin samples [78] and basic impurities using an ultraviolet and a fluorimetric detector simultaneously [79]. Sample preparations using a robotic automated system and derivatisation with trimethylsilylation (TMA) [72], involving extraction of impurities from acidic solution by toluene [76], were reported. The advantage of using toluene [80] is that acidic and neutral extracts contain smaller amounts of heroin and other amines.

In a recent paper [81] new cocaine profiling methodology was reported. The authors aim was to achieve a chromatographic profiling methods specifically comparable to human fingerprinting and DNA profiling techniques. Impurity profiles for sample/sample comparison in a court case has been described [17]. Gas chromatography with flame ionisation detector has been widely used but nitrogen-phosphorus [82], and electron capture [18] detection have also been used. An HPLC method for cocaine profiling has also been described [83]. Cocaine samples comparison using computer programs and different statistical approaches are found in the literature [81, 82, 84-86]. Some used a pattern recognition technique of comparing the Euclidian distances between samples [85]. Others developed [86] neural network pattern recognition software which was found to be superior to conventional statistical methods such as correlation, maximum likelihood, etc. and distance functions classifications, for example, nearest neighbour, clustering, etc.

The first synthetic drug to undergo comparative analytical studies was amphetamine [87]. In that study and in others [88] impurity profiles (chromatograms) were compared visually. A visual method was used at first for comparison of impurity profiles, then and because of increasing numbers of samples profiled it became more difficult to perform comparison and classification of samples. The use of computer programs based on statistical analysis such as, quotient method [89] for comparison on batch level or in combination with principal components [90] for cluster analysis (high level classification) were discussed. Other computerised methods reported for classification and cluster analysis were base on principal component and nearest neighbour [66] and pattern recognition methods and SIMCA [91]. Comparison procedures using computerised methods will allow for consistent decision rules compared to the inevitable element of subjectivity associated with visual methods and are also faster and more economical than the visual ones [91]. Most studies on amphetamine profiling reported the use of gas chromatography with FID [37, 66, 92-94] but others have used HPLC with solid phase

extraction of impurities [95] and with automated extraction and analysis using column-switching techniques [96].

Methamphetamine is widely abused in the United States and Japan [97], and is the mostly seized drug in Australia [98]. Among the first published works for profiling methamphetamine [99] used capillary GC with flame ionisation and nitrogen phosphorus detection. In that study, acidic-extraction for the analysis of impurities was used. However, acidic extract of methamphetamine seized in Japan provided no GC peaks [46]. Methods for comparing impurity profiles included the use of Euclidian distance [97, 98, 100] cluster analysis using SAS [101], Systat for Windows and other in-house programs [102].

#### ***3.3.2.2. Measurements of isotope ratios of naturally occurring isotopes***

Characteristic isotopic composition is acquired by molecules from starting materials as they undergo synthetic and biosynthetic pathways. The isotopic composition or ratios constitute their own isotopic signature. During the synthetic process the isotopic signature can either evolve with or without intentional modification. Therefore, organic substances provide specific isotopic signature originating from a natural or synthetic source allowing for precise determination of origin [102]. On this basis, measurements of the isotopic ratios of isolative and plant drugs such as cocaine and cannabis respectively, can be done to establish origin authentication. Carbon isotope ratios provide information regarding the botanical origin while oxygen and hydrogen isotope ratios give information on environmental conditions under which the plant was cultivated [102]. In the case of drugs of semi- synthetic origin where the isotopic signature is effected by the synthesis process, the isotope ratio is linked to both the natural (geographic) origin and to the synthesis process as the case with manufacture of heroin from natural opium or morphine via acetylation

[103]. With synthetic drugs such as amphetamine, the isotopic ratio is linked to the chemicals used in the synthesis and will allow for establishing batch relation of exhibits.

Measurement of isotope ratios can give vital information for tracing trafficking routes. Early reports on isotope ratio determination include the work done by Liu et al [104] who tried to obtain isotopic information regarding the geographical origin of cannabis by measurements of carbon isotope ratios from both leaves and flowers of the plant. They concluded that cannabis grown indoors and in a metropolitan area contain lower <sup>13</sup>C content. Since heroin is a diacetylated derivative of morphine , information regarding the geographical origin are derived from the morphine moiety of the molecule while information regarding the illicit laboratory is provided by the acetyl moiety. Reports on isotope ratios determination of heroin to establish geographical origin and /or relate samples to a common source or batch has been cited [103, 105-108]

Mean <sup>13</sup>C enrichment of heroin from various geographical origins

Origin	Mean <sup>13</sup> C enrichment	95% confidence interval
Turkey <sup>a</sup>	− 33.561	− 33.822 to − 33.300
Unknown <sup>b</sup>	− 31.302	− 31.504 to − 31.100
Niger <sup>a</sup>	− 32.154	− 32.416 to − 31.893
Thailand <sup>a</sup>	− 32.173	− 32.493 to − 32.853
Pakistan <sup>a</sup>	− 32.322	− 32.583 to − 32.061
India <sup>a</sup>	− 31.568	− 31.892 to − 32.306

<sup>a</sup> n = 9. <sup>b</sup> n = 12.

Table 3.3. Mean <sup>13</sup>C enrichment of heroin from different geographical origins [106]

The Comparison of isotope ratios for heroin samples from different countries was described using GC-IRMS (Figure 3.13) [105]. Heroin  $^{13}\text{C}$  enrichments (Table 3.3) for each origin were compared using two-sample t-test. In another study [106] confiscated heroin samples were clustered into four different groups according to diacetylmorphine and acetylcodeine  $^{13}\text{C}$  enrichments. Isotopic fractionation due to morphine acetylation was determined [103]. The isotopic fractionation showed differences of the  $^{13}\text{C}$  enrichment for morphine (raw material) and heroin suggesting that utilisation of the  $^{13}\text{C}$  enrichment for morphine (raw material) and heroin suggesting that utilisation of the  $^{13}\text{C}$  analysis of heroin may be unsuitable for determination of heroin samples origin. Thus a single morphine sample acetylated with two different acetic anhydrides will probably yield two diacetylmorphine samples with two different  $^{13}\text{C}$  enrichments.

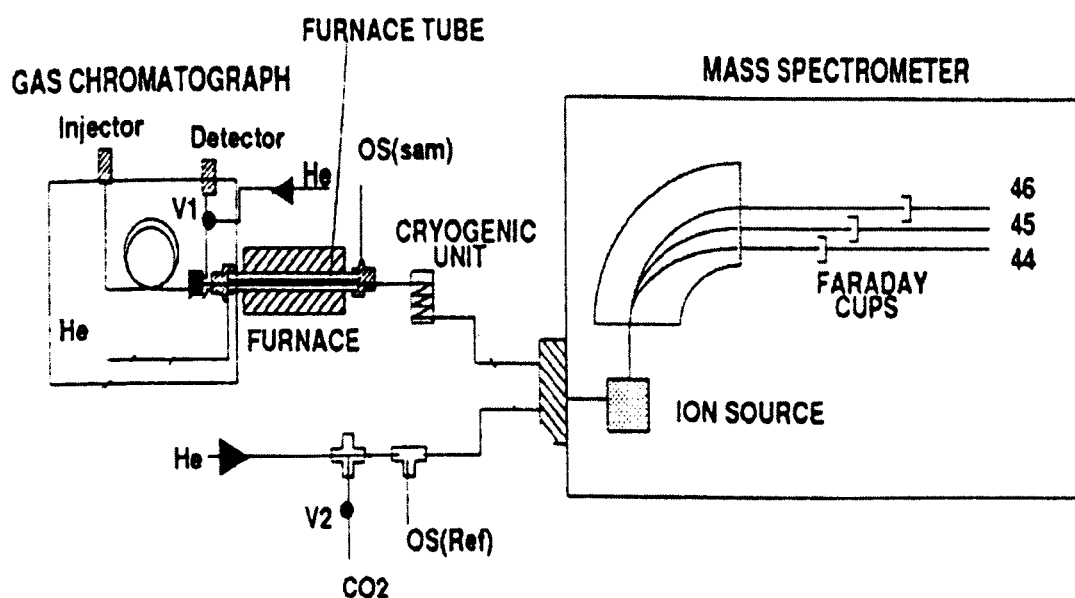


Figure 3.13. Schematic diagram of the gas chromatograph-isotope-ratio mass spectrometer [102]

In another report 3,4-methylenedioxy- methylamphetamine samples were classified into smaller groups [109] after determination of their isotope ratios by measuring  $^{13}\text{C}$  enrichment. Further discrimination was possible using  $^{15}\text{N}$  /  $^{14}\text{N}$  isotopic ratios.

### ***3.3.2.3. Differentiation and classification of drug samples using isomeric procedures***

Isomers are compounds with the same molecular formula and are classified as either enantiomers or diastereomers. Enantiomers are isomers which are mirror images of each other with identical physical and chemical properties. Diastereomers are not mirror images of one another and have similar, but not identical physical and chemical properties. Figure 3.14 shows a diagram of isomerism.

In recent years, the identification and quantitation of enantiomers of controlled substances have received a great deal of forensic attention. Because of differences in pharmacological effects and government regulatory measures, forensic science analysis of optically active drug samples face unique challenges and controversies. The development of analytical methods suitable for the differentiation and determination of optically active drugs is more than an academic exercise or useful only for clinical or pharmacokinetic studies; it fills apparent forensic analytical needs. The enantiomeric ratios may provide intelligence information on batches of illicitly produced drugs or their origin or route of synthesis [110-112]. It has been reported that certain drugs derived from a natural source usually exist as one enantiomeric form, and clandestine produced drugs, are usually a mixture of enantiomers. Thereby it is possible to classify a sample as to being of either natural or synthetic origin by isomeric determination [64].



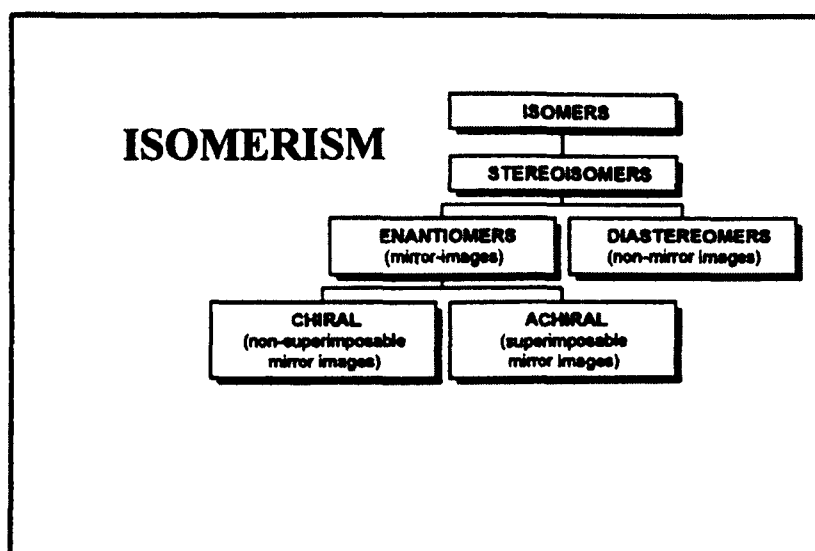


Figure 3.14. Chart of isomerism.

Furthermore, analysis of the composition of isomers (if present) may differentiate samples prepared under different synthetic conditions. For example, identification of *l*-ephedrine in optically pure *d*-methamphetamine is an indication of the synthesis of *d*-methamphetamine from *l*-ephedrine.

Several approaches have been described for enantiomeric analysis including chromatographic methods, NMR and infrared technology.

#### 3.3.2.3.1. Chromatographic methods

Gas and liquid chromatography and capillary electrophoresis have been used to separate enantiomers [110, 111, 113-117]. Separations have been achieved by the use of either chiral stationary phase or by chiral derivatising reagents.

### 3.3.2.3.1.1. *Gas chromatography, GC*

Methamphetamine enantiomers were determined using chiral and achiral capillary columns on a gas chromatograph / mass spectrometer (GC-MS) system [115]. Samples, a seized methamphetamine and a simulated illicit methamphetamine synthesis product, were derivatised with N-trifluoroacetyl-*l*-propyl chloride (*l*-TPC). The conclusion drawn was that the resolution on an achiral column is adequate for the determination of methamphetamine enantiomers and impurities, providing the enantiomeric purity of *l*-TPC is known. In another paper [110] the enantiomers of methamphetamine, ephedrine, pseudoephedrine and methcathenone were determined by gas chromatography after derivatisation with R-(+)- $\alpha$ -(trifluoromethyl)phenylacetic acid (MTPA). Casale [113] reported a method in which pseudoecgonine was detected and resolved from ecgonine in illicit cocaine samples by narrow-bore capillary gas chromatography. The detection of pseudoecgonine in illicit cocaine can be used for comparing samples from a common source. A comparative gas chromatographic determination of total isomeric truxilline manufacturing impurities / by-products in illicit cocaine samples was outlined [118]. These isomers were  $\alpha$ -,  $\beta$ -,  $\delta$ -,  $\epsilon$ -,  $\gamma$ -,  $\omega$ -,  $\zeta$ -, peri-+neo- and epi-truxilline.

### 3.3.2.3.1.2. *Liquid chromatography, HPLC*

High-performance liquid chromatography was used extensively for the enantiomeric analysis of illicit drugs [111, 114, 117, 119]. With the use of a chiral crown ether column (CROWNPACK CR (+)), *d*-amphetamine and *l*-amphetamine were separated by HPLC analysis without using any derivatising agents prior to analysis [117]. A small amount of *d*-amphetamine (0.1 %) was detected in a methamphetamine sample which gave an indication that there was a possibility of

illicit synthesis. Chiarotti et al [114] described an HPLC method to separate cocaine and pseudococaine using a chiral stationary phase (Supelcosil LC-urea) and acetonitrile as mobile phase. A chiral derivatising agent, 2, 3, 4, 6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl isothiocyanate (GITC), was successfully used [111] to simultaneously resolve R, R-(-)-pseudoephedrine, S, S-(+)-pseudoephedrine, R, S-(-)-ephedrine, S, R-(+)-ephedrine, S-(+)-methamphetamine, and R-(-)-methamphetamine using a standard achiral C-18 stationary phase with tetrahydrofuran (THF) / water (3:7) as the mobile phase.

#### 3.3.2.3.1.3. *Electrophoresis, CE*

Chiral analysis by capillary electrophoresis was successfully applied by several authors for ring-substituted amphetamines [116], methamphetamine [120, 121], amphetamine [120, 122] and cocaine [123]. Lurie [122] reported a MEKC separation of amphetamine, methamphetamine, and their hydroxyphenylamine precursors, after derivatisation to form diastereoisomers. Fanali [124] described an application for amphetamine, using cyclodextrins (CD) which have the ability to provide direct chiral separation of a vast spectrum of compounds. Tagliaro et al [116] optimised a direct chiral capillary electrophoresis method based on  $\beta$ -CD for the separation of amphetamine, methamphetamine, MDA, MDMA, and MDEA. Lurie et al [125] also separated amphetamine, methamphetamine, and other stimulants of forensic significance (including cathine, cocaine, propoxyphene and others) by using mixtures of neutral (2,6-di-O-methyl) and anionic (sulfobutyl ether)  $\beta$ -CD. The use of chiral separations of drugs offers a flexible, reproducible and robust methodology, which, could become the first choice analytical technique [124].

### 3.3.2.3.2. Nuclear Magnetic Resonance, NMR

There are four different approaches used in the NMR determination of enantiomeric composition. The first two are performed before the use of chiral lanthanide shift reagents. Derivatising enantiomers with a chiral reagent [126] is one approach and combination of enantiomers with a chiral solvent [127] to induce a chemical shift difference between corresponding enantiomeric groups is another. The third approach [128] involves the use of a chiral solvent and an achiral lanthanide shift reagent. The fourth approach employs optically active shift reagents to induce nonequivalent NMR spectra of enantiomers [129].

Enantiomeric compositions have been reported for norpseudoephedrine, norephedrine, and cathinone [130], and ephedrine, pseudoephedrine, methamphetamine, and methcathinone [110] using NMR with a chiral solvating reagent, (R)-(+)-1,1-bis-2-naphthol. A conclusion was drawn [110] that synthesis batches of methcathinone may be differentiated on the basis of the enantiomeric data obtained.

### 3.3.2.3.3. Infrared methods

Few articles are found in the literature on the use of infrared methods for the enantiomeric determination of illicit drugs. However, it has been reported that infrared method can be very a useful tool for enantiomeric determination in seized drugs [112, 131].

Identification of chiral salt forms that can precipitate from the reaction of chiral amines with chiral organic acids has been reported using Infrared spectroscopy. Distinct crystalline phases for a single enantiomer, and for a racemic mixture of the

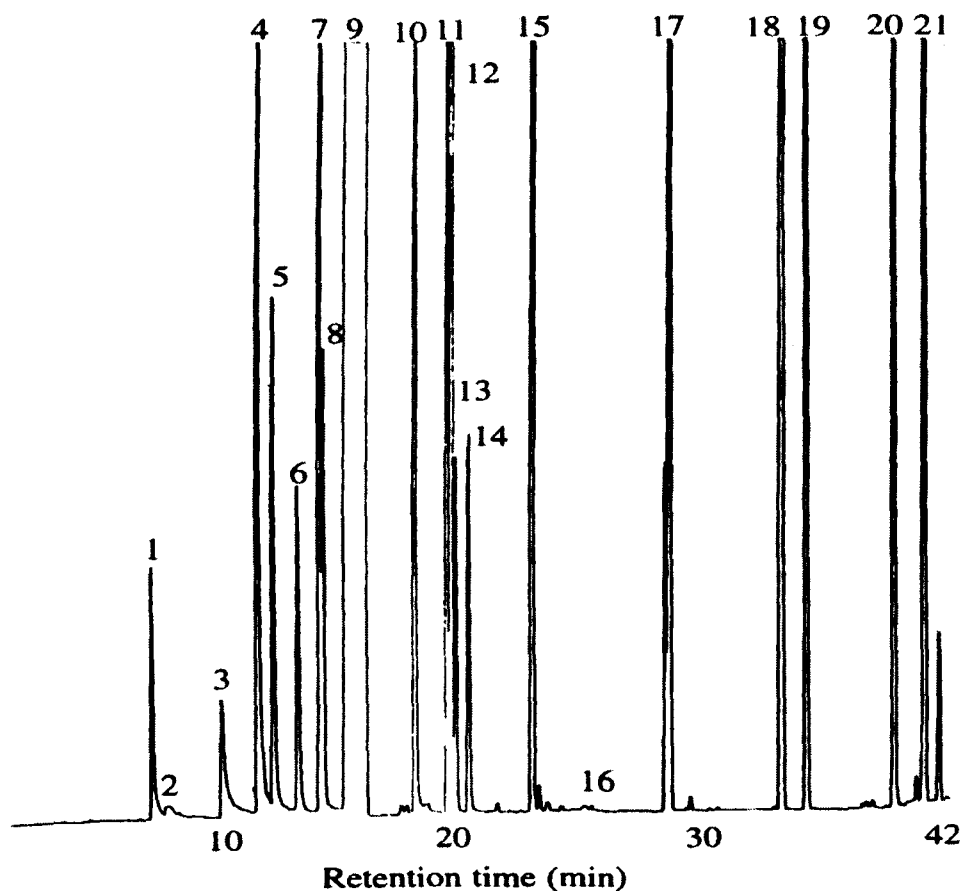
two enantiomers can be formed by many chiral substances [131], thereby, allowing clear distinction of the two samples by condensed phase infrared transmission spectrum. Chappell [112] described a method for enantiomeric determination of methamphetamine which, employs an infrared transmission spectrometry technique on solid samples dispersed within an alkali metal halide matrix.

#### ***3.3.2.4. Determination of residual solvents for comparative analysis***

Almost, all illicit amine-based drugs are manufactured and distributed as salts [10]. The manufacture process involves the use of concentrated acids such as hydrochloric acid, to provide a rapid precipitation resulting in crystal matrices containing significant quantities of trapped solvents [6]. Qualitative and quantitative analysis of these residual solvents may provide vital information for intelligence purposes including comparison of different exhibits. In addition, it is important for drug enforcement agencies to get updated information of new solvents used in illicit drug preparations to allow for more control of their availability.

The detection and identification of residual solvents in illicit heroin [132-136] and cocaine [132, 133, 137, 138] has been described using different analytical tools. Proton nuclear magnetic resonance was used to determine solvent residues in illicit cocaine samples [137]. The internal standard 1,4-diacetylbenzene was used because it exhibits high and low field singlets and also aids in the comparison of the integration values of the signals of the solvents to that of the internal standard. The five most common solvents identified were benzene, toluene, acetone, methyl ethyl ketone and ethyl ether. Two other solvents, methyl chloride and ethyl acetate, were also detected for the first time in cocaine exhibits. GC-FID was employed for the analysis of occluded solvent residues in cocaine and heroin samples [133] after elution with carbon disulphide. The occluded solvent residues were first trapped by

solid phase adsorption of headspace vapours onto activated charcoal. Up to 16 solvents were identified in the cocaine samples and 12 solvents were detected in the heroin samples (Figure 3.15). Using this information allowed for comparison of different cocaine exhibit however, relating heroin samples was more problematic.



**Figure 15. Chromatogram of solvent standards identified in cocaine and heroin samples in this study. Elution order:**  
1, acetaldehyde; 2, methanol; 3, ethanol; 4, acetone;  
5, isopropanol; 6, diethylether; 7, dichloromethane;  
8, methylacetate; 9, carbon disulphide;  
10, methylethylketone; 11, ethylacetate; 12, hexane;  
13, chloroform; 14, butanol; 15, benzene (from carbon disulphide); 16, methylisobutylketone; 17, toluene;  
18, m & p-xylene; 19, o-xylene; 20, n-decane (internal standard); 21, 1,2,3-trimethylbenzene.

Static headspace-gas chromatography-mass spectrometry (SHS-GC-MS) [132] was utilised to identify and quantitate residual solvents in cocaine and heroin samples.

Aqueous sodium sulfate (22 %) was used for solubilisation of the crystals and ensured liberation of the trapped solvents. Solvents detected are listed in table 3.4.

Cocaine HCl (% of Samples) <sup>a</sup>	Heroin HCl (% of Samples) <sup>b</sup>
Methyl Ethyl Ketone (65%)	Ethyl Acetate (81%)
Hexanes (61%)	Acetone (57%)
Toluene (59%)	Ethyl Ether (34%)
Benzene (55%)	Methyl Ethyl Ketone (25%)
Acetone (52%)	Toluene (25%)
Methylene Chloride (41%)	Xylenes (19%)
Xylenes (31%)	Ethanol (10%)
Ethyl Ether (31%)	Isopropanol (5%)
Cyclohexane (27%)	Hexanes (3%)
Ethyl Acetate (23%)	Methyl Acetate (3%)

<sup>a</sup>Number of samples = 75.

<sup>b</sup>Number of samples = 826.

Table 3.4. Encountered occluded solvents in illicit cocaine HCl and heroin HCl. [132]

### 3.3.2.5. Active drug(s) and cutting agents content to compare exhibits

Several reports appeared in the literature describing various methods for comparison of drug exhibits or predicting their country of origin based on the concentration and ratios of selected alkaloids and adulterants present in seized substances [10, 52, 139-143]. Determination of the source of supply or origin of illicit heroin samples was established [142] using a method based on the content of morphine and codeine and acetyl products and the ratio between them. The author noticed that the theoretical ratio of heroin to acetycodeine increased by two-fold at each stage during the opium-morphine-heroin process. Opium samples of known

origin showed significant variation in the heroin to acetylcodeine ratio, thereby allowing the author to have distinct composition profiles of the alkaloids for each geographical area. This information made it possible to compare heroin samples of known origin with unknown seized heroin samples and also determine the place of origin. King [10] concluded that it was possible to distinguish Turkish heroin from other South West Asian types on the basis of the acetylcodeine / noscapine ratio. He also elaborated on the importance of the drug content of a tablet / capsule or the purity of an illicit powder to support a comparative analysis to link two or more samples. In another report [141] the detection of arsenic in opium and strychnine in opium and heroin samples was found as an approach for the possible identification of their source in the local market and their origins of import.

Recently, the use of statistical analysis using available computer software have been outlined [139, 140]. The first report [139] utilised a multivariate statistical analysis of the concentration of selected alkaloids and adulterants in seized material. Table 3.5 lists the alkaloids and adulterants included in the study. The other report [140] described a method for numerical comparison of street samples based on Fisher's linear discriminant analysis following analysis of the opiate, papaverine and noscapine content of the samples.

Alkaloids	Adulterants
6-Acetylcodeine	Caffeine
6-Acetylmorphine	Methaqualone
Diamorphine	Paracetamol
Noscapine/Narcotine	Phenobarbitone
Papaverine	

Table 3.5. Component of heroin used for the statistical analysis [139]



### ***3.3.2.6. Drug profiling using infrared spectrometry***

Infrared spectroscopy and in particular fourier transform infrared, FTIR, can serve as another tool for comparing drug seizures. Each exhibit has its own characteristic spectrum, and comparison between the different spectra gives a gross estimate of the similarity or dissimilarity between the different exhibits [36, 144-146].

FTIR has some discriminating capability to classify powders into a large number of groups, and consequently can be used for gross comparison of ecstasy exhibits. FTIR has some advantages over chromatographic methods such as reduced sample handling, faster analysis time, no need for impurities extraction and concentration procedure, and lack of volatility, solubility, thermal stability or column limitation.

Early works on comparison of illicit drug samples [144] using infrared technologies reported development of a spectrum compensating technique. In this method a wedge disk for one of the components in the drug mixture is prepared. The component spectrum is then subtracted from the mixture spectrum. In another study on comparison of heroin seizure [36], 419 heroin exhibits were compared among them and were clustered into sub groups based on similarities of spectra. The method of classification utilised an algorithm for Euclidean distance and library search facilities. Figure 3.16 shows a library search result. Visual examination of spectra was used as a confirmation to the library search results.

Classification of some common drug groups, including amphetamine, barbiturates and opiates using a multivariate analysis of infrared spectra was also reported [146]. Cluster analysis, principal component analysis, and nonmetric multidimensional scaling elements of a computer program were used to structure taxonomic distances between drugs. A procedure was developed [145] to separate carbohydrate diluents

from active drugs in order to identify them by FTIR. Once determined, diluents were useful for comparing different drug exhibits.

Rashed et al [147] reported a method for profiling ecstasy tablets through the use of fourier transform infrared spectroscopy (FTIR). Around 47 ecstasy tablets plus standards of MDMA, glucose, lactose, and caffeine were used for the study. Samples prepared as KBr disks were analysed directly without any extraction procedures or other sample preparations. Library search facilities were used to compare spectra using an algorithm of correlation coefficient. The library search provided best fits or closest matches from the library spectra to the unknown spectrum. A higher number means a closer match (1 being the highest and 0 being the lowest).

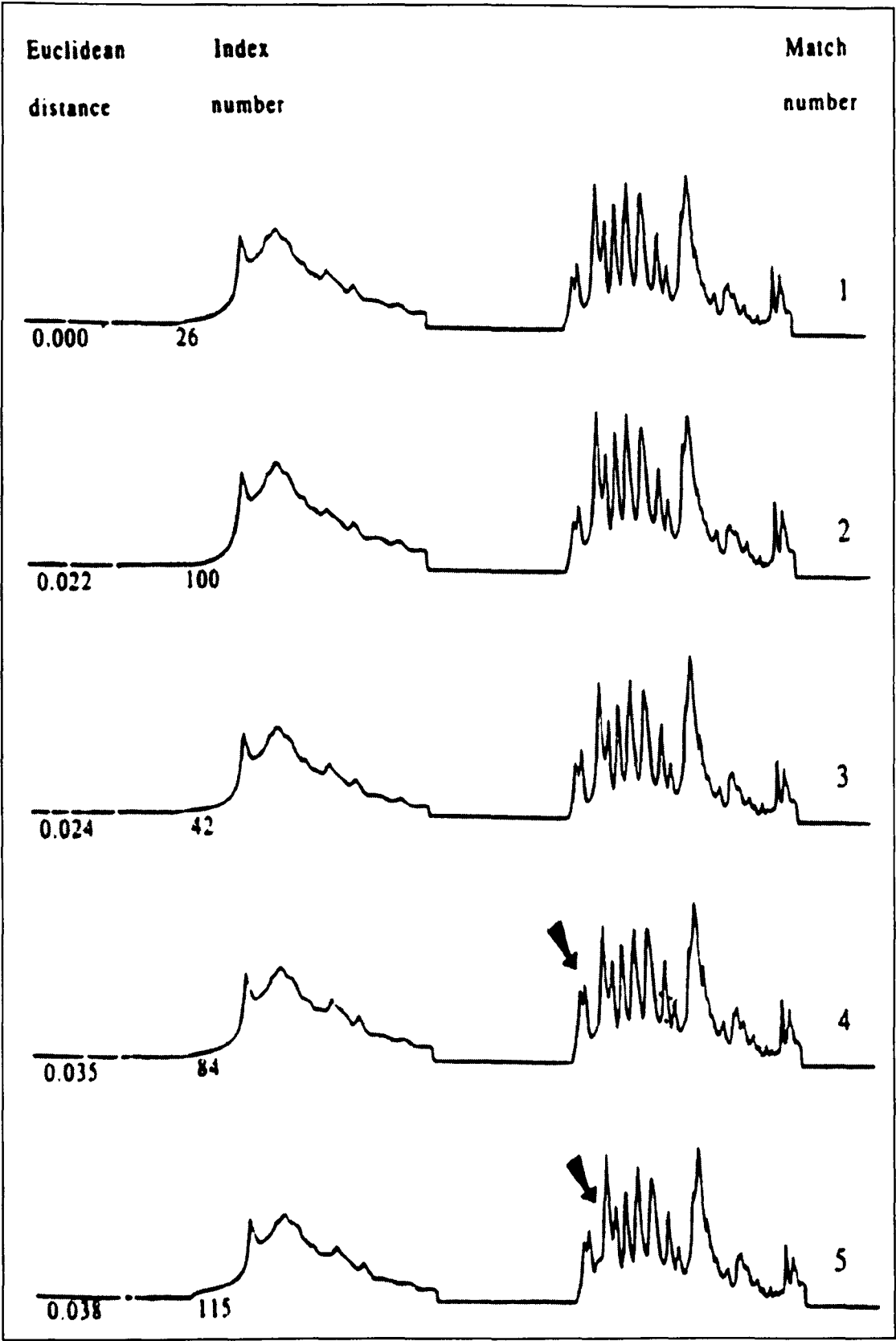


Figure 3.16. FTIR library search of a powder exhibit [36]

### 3.4. DISCUSSION

Impurity profiling procedures have dominated the illicit drug comparison methods. Two main approaches have been reported, (i) identification of trace impurities for establishing synthetic routes and identification of precursor chemicals used in synthesis and (ii) comparing impurity profiles (chemical signatures) to establish drug origin, grouping, and sample-to-sample comparison. Impurities formed during the manufacturing process can lead to a danger to the public health. The unintended production of 1-methyl-4-phenyl-1, 2,5,6-tetrahydropyridine, MPTP in the process of manufacturing 1-methyl-4-phenyl-4-propionoxypiperidine, MPPP, a heroin substitute, has been linked to symptoms of Parkinson's disease [5]. Lead poisoning in methamphetamine samples was also discussed [138].

Drug profiling usually consists of three main steps: extraction and concentration of trace impurities from the drug exhibit, analysis of extracts (chromatography) and data interpretation using computers and statistical analysis. Harmonisation of analytical condition in profiling analysis is an important issue, which should include selection and quantification of marker impurities for comparison procedures, synthesis of these impurities, and finally determination of impurity peaks that are characteristic to the synthetic route. Profiling using more than a single analytical technique with regard to instrumentation would provide better results. To combat continuous development in clandestine manufacturing of illicit drugs, identification of new synthesis routes and new precursors and chemicals should be regarded as a priority in profiling programs. It is worth noting that profiling generally is a costly procedure that requires dedicated analytical systems and well-trained experienced analysts.

### 3.5. FUTURE DEVELOPMENT

Notable advances in impurity profiling analysis have been established since the early days of these programs. Liquid/liquid extraction procedures are used in laboratories engaged in illicit drug profiling and GC-FID has been the method mostly used in profiling analysis. New developments sought in drug profiling should include: evaluation of new extraction techniques for drug profiling, such as SPE [148], SPME, mix phase cartridges, exploring new analytical tools, e.g. CE, NMR, IR, and finally continuing development of new statistical and computerised methods for data interpretation and comparison.

### 3.6. CONCLUSION AND AIMS OF THE STUDY

This chapter reviewed published papers on the area of drug profiling. Methods reported on this subject include: physical analysis, impurity profiling procedures, measurements of isotopic ratios, use of infrared technology for drug comparisons, elemental analysis and determination of diastereoisomers and enantiomers to differentiate samples. Other methods were also discussed such as analysis of solvents in drug exhibits, comparison of drug samples using statistical procedures to calculate the main constituents of an exhibit including adulterant, diluents and the active drug(s). These methods provide vital information to the forensic chemist in addition to the usual conclusion that the exhibit contains a controlled substance or not. It allows for comparative analysis between two or more samples, determination of the geographic origin of drug exhibits, estimation of the clandestine manufacturing and illicit drug trafficking and distribution trends and it provides information on precursor chemicals used in illicit drug synthesis which will allow for their control.

Among the main objectives of this study was the development and optimisation of a new extraction procedure such as solid phase extraction, for impurities in seized ecstasy tablets. Another area of research was the use of infrared technology for profiling ecstasy tablets since only chromatographic methods have been studied. Identification of impurities in ecstasy tablets confiscated within the UK was also described. Synthesis of MDMA using different synthetic routes was performed to acquire authentic samples representing impurities normally encountered in street samples. These authentic samples were analysed and their mass spectra and retention indices were used to identify impurities in actual street samples.

## **4. SOLID PHASE EXTRACTION FOR ECSTASY PROFILING**

### **4.1. INTRODUCTION**

Illicit ecstasy tablets produced in clandestine laboratories usually contain various impurities such as reaction by-products, intermediates and contaminants within reagents, which accumulate as a chemical reaction proceeds because of the lack of quality control in these laboratories. These impurities represent a very small portion of the total weight of the illicit tablet. Therefore, dissolving the tablet in an aqueous medium or in an organic solvent and directly performing an analysis would not yield satisfactory results.

The instrumental analysis of impurities found in ecstasy tablets usually requires a preliminary extraction process to extract, isolate, and concentrate these impurities from the total tablet content. In the process, interfering materials are removed, and the required substances are concentrated into a solvent that is suitable for introduction into the instrument. The impurities found in illicit tablets are extracted, after crushing the tablet and dissolving the fine homogeneous powder in deionised water or buffer solution which forms an aqueous matrix, into an organic solvent. Although lengthy and time-consuming, these procedures are of paramount importance to remove the principal component(s), diluents and adulterants, and concentrate impurities.

Liquid-liquid extraction (LLE) of impurities from the bulk drug has been the method for sample preparation for quite some time. It is an isolation and separation method based on the distribution between two immiscible solvents that are in close

contact with one another [149]. Many problems are associated with this method including lengthy handling time and the need to concentrate the sample after extraction [95].

As a consequence of the above-mentioned problems sample preparation techniques using a liquid-solid approach or as it is widely called solid-phase extraction (SPE) have been developed and used extensively. SPE is a sample preparation technique that uses disposable extraction cartridges containing bonded sorbents, to selectively and efficiently extract specific compounds from complex mixtures. SPE is different than LLE, which often results in variable sample recovery in that it is based on specific molecular interactions for more reproducible results [150]. The main advantages of SPE over LLE are summarised below: [151-154].

1. Speed.
2. Selectivity (The variety of bonded chemical moieties provides the selectivity needed to extract a particular compound from potential interferences in a complex matrix) and versatility (readily adaptable to automation).
3. Efficiency (no cross contamination of phases and no emulsions problem).
4. Reduction of solvent use (reduce the cost of hazardous waste disposal).
5. Reduction of labour-intensive procedures.
6. SPE is 5-fold less costly than LLE.

Several other reports provided comparisons of LLE and SPE. Rood [155], compared deposition of sample residues from urine extracts by LLE and SPE methods for drugs of abuse and found that samples extracted by the liquid-liquid



method contaminated the chromatographic system to a greater extent and much faster than those extracted by SPE method.

Solid-phase extraction (SPE) procedures have been used with various types of samples, from biological fluids to environmental pollutants. Many articles on this type of work are published in the literature, but only one paper has been cited on the use of SPE for the extraction of impurities present in illicit drugs [95]. In this project special emphasis will be given to the use of SPE for the extraction of impurities in illicit (ecstasy) tablets. This chapter emphasises optimisation of profiling procedure for ecstasy tablets which includes sample preparation steps and evaluate a comparison of LLE and SPE for extracting impurities from ecstasy tablets for profiling purposes.

## **4.2. REVIEW**

### **4.2.1. Solid-Phase Extraction (SPE)**

Solid-phase extraction (SPE) is an approach in which solid-phase adsorbents are used as the media for retaining compounds of interest, followed by selective elution. The aim of SPE is to isolate the relevant compounds from a sample matrix and to concentrate them. SPE is a physical extraction process that involves a solid phase and liquid phase [156]. The most widely used SPE materials are silica or bonded silica gel, in which the end-standing silanol groups have been derivated with an organic moieties that may consist of alkyl chains with or without a variety of functional groups, such as -OH, -C<sub>6</sub>H<sub>5</sub>, -NH<sub>2</sub>, -CN, -SO<sub>3</sub>H, and -COOH [157].

The extraction and elution of the drug are interdependent on the drug of interest, the sorbent in the extraction column, and the solvent used in the elution step. The drug of interest interact chemically and/or physically, i.e. based on polarity or ion exchange, with both the sorbent and the solvent used to extract and elute the drug. Therefore optimising the chemical and physical properties of the functional groups of the analyte (drug) during the solid-phase extraction is essential to enhance efficient separation [151]. Each step of the extraction procedure must be optimised carefully.

Among the factors that may affect the behaviour of the relevant drugs during the extraction procedure include the selection of a suitable sorbent, the pH of the sample and the extraction system, the clean-up step, the properties and volume of the eluent, and the flow rate of the sample and the eluent passing through column [157].

The choice of sorbents is often based on how optimally it can extract the compound of interest by retaining the compound and allowing the impurities to pass through the column, by concentrating the retained compound after passing large sample volumes through the column, or by retaining and thus removing interfering impurities from the sample matrix.

#### **4.2.2. Bonded-phase partition chromatography (BPC)**

The sorbent in BPC is covalently bonded to a rigid silica gel support, and that allow it to be used with either negative or positive pressure. The resin will neither swell nor shrink; thus, reequilibration does not enter into consideration, hence widely varying pH will not destroy the resin in the short time it takes to perform an extraction. The result of these combined advantages is an extremely versatile and

powerful sample preparation technique [151]. Bonded-phase partition chromatography columns are classified as:

#### **4.2.2.1. Normal phase (Polar):**

These are used for the extraction of polar or hydrogen bonding compounds from a nonpolar, nonaqueous matrix. Here, the polar groups present on both the bonded phase sorbent and the drug sample are partitioned [158]. For maximum extraction efficiency, high solvent polarity and high solubility of the analyte must be considered [151].

Normal phase sorbents are not usually used for extraction of aqueous solutions, which results in few applications with regard to aqueous drug matrices.

#### **4.2.2.2. Reversed Phase (Nonpolar):**

Nonpolar columns extract nonpolar compounds from polar sample matrix. Here the extraction mechanism is based on retention of the compound of interest by hydrophobic interaction with the column sorbent [156, 158, 159]. When eluting nonpolar compounds from a sample solution, a combination of both low solvent polarity and high solvent solubility of the drug of interest is of optimal consideration [151].

Reversed phase columns are versatile and are used for almost all classes of drugs of abuse. They only require a small amount of organic solvent as an eluent.

#### 4.2.2.3. Ion Exchange:

The ionic character of the drug, which is always the opposite of the ionic charge of the functional groups of the resin, is the force behind binding the drug to an ion exchange column resin. Ion exchange chromatography SPE columns are classified as:

- *Negatively charged groups (anionic)*: Anion resins are used to extract analytes present in the sample matrix in an anionic form.
- *Positively charged groups (cationic)*: Cation resins are usually employed in the extraction of analytes present in the sample matrix in cationic form.

These groups can be cationic or anionic depending on the pH of the solvent surrounding them. Hence for good retention of an isolate on a sorbent, two conditions must be met [156]:

- The pH of the matrix/solvent must be at values that allow for both isolate and sorbent to be charged.
- High concentrations of strongly competing ionic species of the same charge as the isolate must not be present in the matrix/solvent.

A solid-phase extraction procedure usually involves five main steps: column pre-conditioning, sample application, column wash, column drying (if necessary), and drug elution.

### **4.2.3. Extraction Procedure**

#### **4.2.3.1. Column Preconditioning (Solvation)**

The column has to be pre-treated with suitable solvent(s) for high reproducible recovery. The dried sorbents in bonded silica gel extraction columns are packed, hence the active sites are not available to interact with analytes and proper column conditioning is required before sample application. The sorbent must be preconditioned with suitable organic solvent, for example, methanol followed by water or buffer wash (non-polar and multiple interaction phases) or hexane (polar phase). The organic solvent is used in this step to solvate the bonded functional groups for opening the hydrocarbon chains, and to remove organic residues from the sorbent that may interact with the analyte or interfere in the detection stage. Water or buffer, of which the pH, ionic strength, and polarity have been adjusted, is added afterwards to remove excess organic solvent to prepare the SPE column to receive an aqueous sample [157]. The main task at this stage is to keep the sorbent solvated before sample application.

#### **4.2.3.2. Sample Application**

Once the column is preconditioned, the sample is transferred onto the SPE column, and drawn it by applying a gentle vacuum. It is important to note here that a slow flow rate is necessary to ensure excellent retention of the analytes [160, 161].

#### **4.2.3.3. Column Wash**

Retention of relevant compounds is usually accompanied by retention of other endogenous components. Thus a column wash with water or an appropriate solvent is required to remove any interfering substances. Careful selection of the wash

solvent is required in order to avoid any substantial loss of relevant compounds during the wash process. Finding a solvent with the ability to clean the column without losses of the relevant compounds is difficult. Therefore a compromise must be reached between acceptable recoveries of a wide range of different substances and adequate removal of matrix compounds. The first step should be dedicated to an acceptable recovery of the relevant substances followed by cleaning up optimising step [157]. Sometimes pH alteration of the extraction system is introduced at this stage to allow for selective elution as the behaviour of most of drugs is dependent on the pKa and the pH of the extraction system [160-162].

#### **4.2.3.4. Column Drying**

Column drying is not usually necessary when water miscible organic solvent or buffer solution is used as an eluent and the final analysis is carried out on reversed-phase HPLC. But if a water immiscible solvent is used as an eluent and the final analysis is to be performed with GC, then a drying step is necessary to remove all traces of water from the SPE column prior to the elution step. If water present it may cause difficulties with sample elution, further steps such as derivatisation or damage the GC column. There are two ways to remove residual water, either by applying airflow through the sorbent or by adding a suitable organic solvent, or both [160].

#### **4.2.3.5. Elution of relevant substances**

Performing elution of the relevant substances is completed by passing a suitable eluent strong enough to ensure complete elution of the drugs through the SPE column. The eluent should be selective so that the interfering substances are retained on the column.

There are certain factors to be considered before eluent selection, such as, the polarity ( $P'$ ), the solvent selectivity, and the eluotropic strength ( $E$ ) of the elution solvent [163, 164]. Solvent strength and solvent selectivity are not the same. Snyder [163] stated that solvent strength is its polarity or ability to preferentially dissolve more polar compounds, while the solvent selectivity is its ability to selectively dissolve a particular compound when polarities of compounds are similar. According to Snyder, solvents are classified into eight selectivity groups (I-VIII) depending on their proton donor, proton acceptor, and dipole interactor characteristics. However, Chen et al [160] proved that the  $P'$  value is not too critical. Thus when selecting the best eluent system that have similar  $P'$  values but with different selectivities, both  $P'$  and the selectivity of the solvent should be considered. For example, ethyl acetate and isopropanol have the same  $P'$  value but belong to different selectivity groups.

Among other factors that have noticeable effect on the process of developing an extraction procedure are:

*Eluent Volume:* It is important to realise that using large volumes of the eluent will cause dilution of the extract [165]. Increasing the eluent volume will also result in prolonged extraction period, causing loss of the more volatile drugs and elute more interfering substances [160].

*Flow Rate of the Eluent:* In order to allow an adequate interaction of the relevant substances with the stationary phase and mobile phase, the flow rate should be controlled. Slow flow rates (0.5-2 ml/min) have been tested and showed improved recovery [149, 162, 166-169].

Solid-phase extraction (SPE) has now taken its place in the analytical laboratory as a fast, convenient and efficient method to prepare a clean sample for chromatography and spectroscopy. Although the cartridge has been traditionally the main format, which was used for SPE sample preparation, recently, new formats have proved to be more suited to the type of sample being prepared. There are four major extraction techniques used prior to analysis presently: Soxhlet (SOX), liquid-liquid (L-L), solid phase (SPE), and supercritical fluid (SFE). Of the analysts performing sample preparation, 46% use L-L, 36.4% use SPE, 23% use SOX, and 3% use SFE [154]. This was a result of a survey conducted in 1991. Since then, conventional and automated solid phase extraction have been used on wider range of applications.

## **4.3. EXPERIMENTAL**

### **4.3.1. Chemicals**

1. Column conditioning solvents: methanol & water both HPLC grade.
2. Buffer solution: Phosphate buffer at pH 4-10
3. Eluting solvents: Isopropanol, ethyl acetate, isooctane, hexane, and dichloromethane.

### **4.3.2. Apparatus**

1. Solid phase extraction columns:
  - a. Bond Elute: Certify (130 and 300 mg), C18 (200 and 500 mg)
  - b. Isolute: C18 (200 and 500 mg), C8 (200 and 500 mg)
2. Vacume Manifold: VAC-ELUT (Varian).



### 4.3.3. Optimising SPE Procedure

The optimisation of the SPE procedure was carried out in three main steps

1. Examination of the best extraction pH
2. Evaluation of eluting solvents used for extracting impurities
3. Assessment of different SPE columns

#### 4.3.3.1. *Examination of the best extraction pH*

One ml of phosphate buffer was added to 50 mg of fine homogeneous ecstasy powder, the solution was then mixed and shaken for 30 min, centrifuged for 5 min. and the supernatant was taken off. A SPE column (Bond Elut Certify 130 mg) was conditioned with 10 ml of deionised water (to remove water-soluble impurities due to manufacture of the cartridge), 10 ml methanol (to solvate the column and remove other impurities), and 10 ml deionised water (to remove excess solvation solvent). The sample (supernatant) was then applied to the column at 1-2 ml/min. The column was then washed with 10 ml deionised water (to remove sugars and other unwanted water-soluble compounds) and dried under maximum vacuum (15 psi) for 5 min. The sample was subsequently washed with 0.7 ml isopropanol (to remove any remaining water and reduce lipophilic bonding between the analytes and the sorbent surface) followed by 1 ml ethyl acetate with 2 % ammonia (to elute freed analytes by eliminating ion-exchange interactions). The collected sample was evaporated in a nitrogen stream to about 100  $\mu$ l. 1 $\mu$ l of the sample was injected in the GC-FID. The pH of the phosphate buffer ranged from 5 to 10. The optimum pH was determined using visual comparison of the chromatograms.

#### **4.3.3.2. *Evaluation of eluting solvents for the one with most impurities extracted***

The same extraction procedure was repeated under the optimum pH determined above using four different eluting solvents, ethyl acetate, dichloromethane, hexane, and isooctane. An internal standard, triethylamine, was added before sample introduction to the gas chromatograph. The relative peak areas were calculated and bar charts were drawn for the main peaks to determine the best eluting solvent.

#### **4.3.3.3. *Assessment of different SPE columns***

Once the optimum extraction pH and the best eluting solvent were determined an assessment of different SPE columns using the same extraction procedure as above was performed. The columns used for the assessment study were, Bond Elut C18 200 mg, C18 500mg, Certify 300mg, Certify 130mg and Isolute C8 200mg, C8 500mg, C18 200mg, C18 500mg. An internal standard, triethylamine, was added before sample introduction to the gas chromatograph. The relative peak areas were calculated and a bar chart was drawn for the main peaks to determine the performance of each type of SPE column. Throughout all the extraction procedures performed above, the same ecstasy sample was used to assure that any variations were due to different extraction procedure parameters not due to different samples.

#### **4.3.3.4. *Blank Extracts***

The same extraction procedure was used with blank extracts to ensure impurities other than those present in the tablets were absent.

#### **4.3.3.5. *Stability of Extracts during Storage***

Extracts were stored at 4°C in the dark, and were re-analyzed over a period of 4 weeks to check their stability

#### **4.3.3.6. *Application to Real Samples***

Three ecstasy samples seized from different individuals at different locations were analysed with optimised conditions.

#### **4.3.3.7. *Gas Chromatography***

The GC was an Hewlett-Packard 5890series II with flame ionization detector. One µl of sample was injected with an injection port temperature of 280°C. Gas chromatography was carried out using a fused silica capillary column (HP5, 30 m x 0.32 mm i.d., 0.25 µm film thickness) programmed from an initial temperature of 80°C (1 min) at 35°C/min to 180°C (held for 18 min) then at 50°C/min to 300°C (held for 2 min).

#### **4.3.4. Solid phase extraction (SPE) Vs Liquid-liquid Extraction (LLE)**

A comparison of SPE and LLE to evaluate and justify the use of SPE as an alternative extraction procedure to LLE. Ten portions, 50 mg each, of a ground and homogenised ecstasy sample, were separated into two groups each consisting of five portions. One group was subjected to LLE while the other to SPE. Both groups were analysed by GC-FID.

#### **4.3.4.1. Solid phase extraction (SPE)**

SPE procedures were carried out using Varian Bond Elute C18 (200 mg) columns. The tablets were crushed and 50 mg of the homogeneous powder were dissolved in 1ml phosphate buffer at pH 9. The solution was then mixed and shaken for 30 min., centrifuged for 5 min. and the supernatant was removed. The SPE column was conditioned with 10 ml of deionised water (to remove water-soluble impurities due to manufacture of the cartridge), 10 ml methanol (to solvate the column and remove other impurities), and 10 ml deionised water (to remove excess solvation solvent). The sample was then applied to the column at 1-2 ml/min. The column was then washed with 10 ml deionised water (to remove sugars and other unwanted water-soluble compounds) and dried under maximum vacuum (15 psi) for 5 min. The sample was subsequently washed with 0.7 ml isopropanol (to remove any remaining water and reduce lipophilic bonding between the analytes and the sorbent surface) followed by 1 ml ethyl acetate with 2 % ammonia (to elute freed analytes by eliminating ion-exchange interactions). The collected sample was evaporated in a nitrogen stream to about 100 µl. This is the developed SPE procedure from section 4.3.3.

#### **4.3.4.2. Liquid-liquid Extraction (LLE)**

A volume of 1ml of phosphate buffer at pH 9 was added to 50 mg of the same homogeneous powder used for the SPE procedure. The solution was then mixed for 1 min. and shaken for 30 min. Another volume of 1ml of ethyl acetate was added to the solution and shaken again for 30 min. The sample was then centrifuged for 5 min. at 3000 rpm and the organic layer was removed and evaporated in a nitrogen stream to about 100 µl. At this stage 100µl of triethylamine, internal standard, was added. Only 1µl of the sample was injected in the GC-FID.

#### **4.3.4.3 Blank Extracts**

Two blank solutions were extracted by LLE and SPE using the same buffer and same solvent used throughout the whole work to insure that impurities other than those present in ecstasy samples are not present.

#### **4.3.4.4. Application to Real Samples**

Twelve different samples of seized ecstasy tablets were analysed using solid phase extraction, GC-FID. The extraction procedure was similar to the SPE procedure mentioned above as were the GC-FID.

### **4.4. RESULTS AND DISCUSSION**

The optimisation procedure carried out on a sequence to allow the use of the optimum determined parameter for the next step of the optimisation procedure.

#### **4.4.1. Examination of the best extraction pH**

Six different pH values (5-10) were examined using the same eluting solvent (ethyl acetate) and the same SPE columns (Bond Elut Certify 130mg). The SPE procedures were run for all pH range on portions of the same homogeneous ecstasy powder using the same. Figure 4.1a and 4.1b illustrate the superiority of extracting with pH 9 over other pH values.

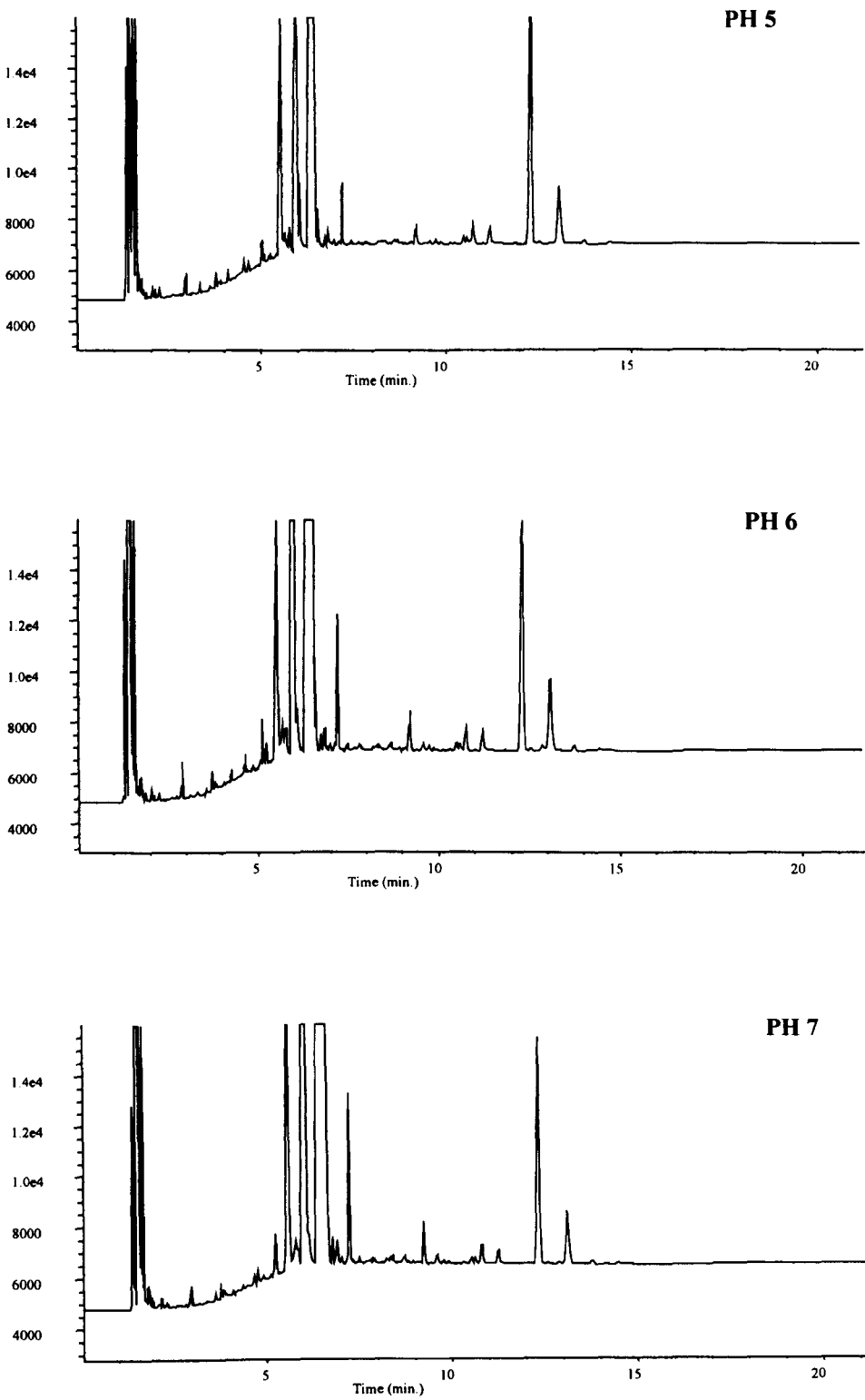


Figure 4.1a. Impurity profile of sample 001 at pH 5, 6, and 7 respectively.

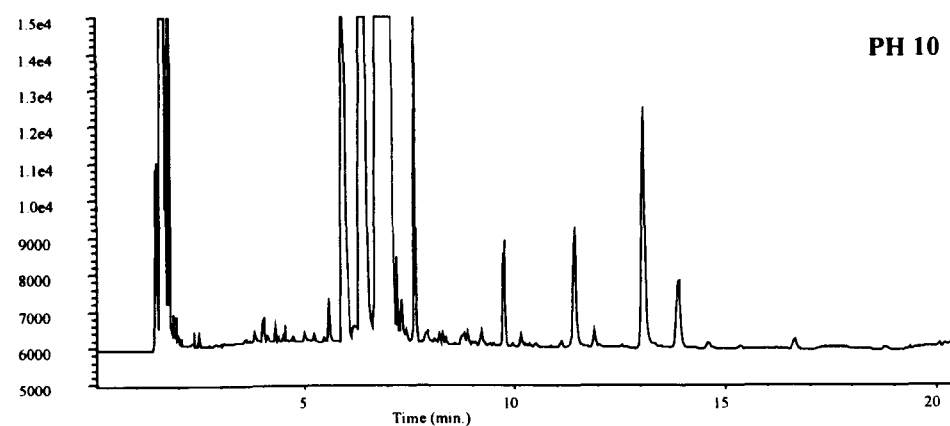
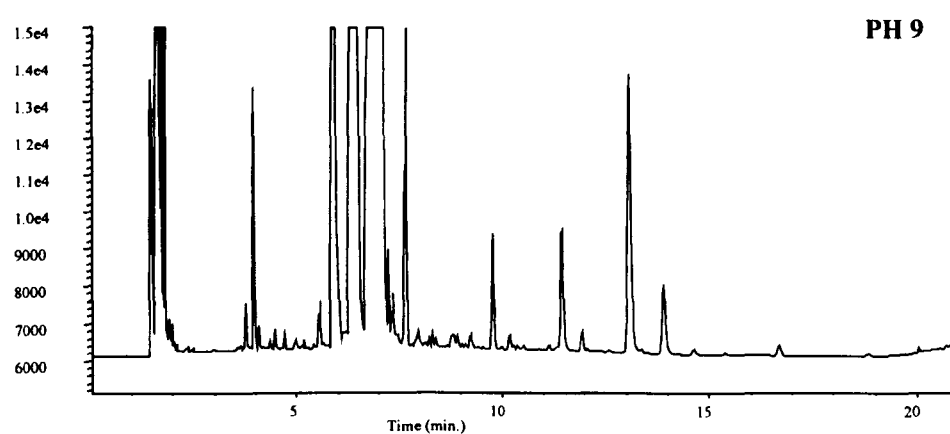
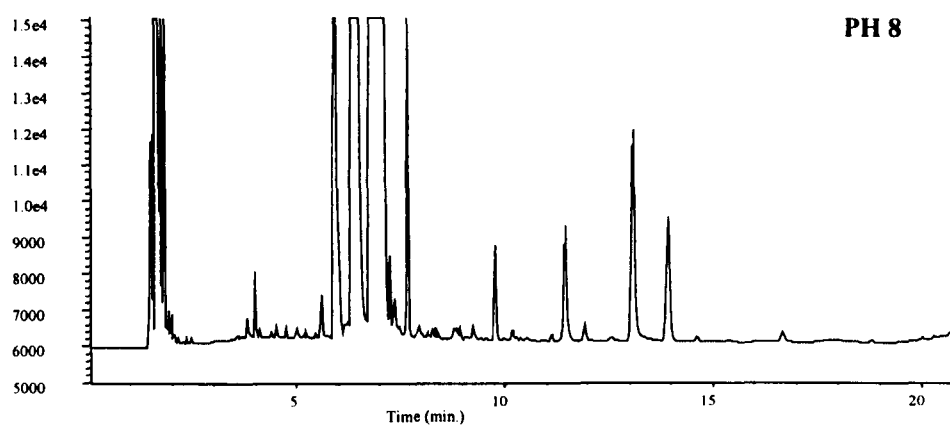


Figure 4.1b. Impurity profile of sample 001 at pH 8, 9, and 10 respectively.

The optimum pH at 9 yielded better impurity profile with a greater number of impurity peaks present allowing for a better chemical signature comparison.

#### **4.4.2. Evaluation of eluting solvents for the one with most impurities extracted**

In the evaluation of the eluting solvents procedure four solvents were used; ethyl acetate, hexane, dichloromethane and isooctane using the optimised pH 9 and Bond Elut certify 130 mg. Ethyl acetate yielded the best impurity profile providing impurities with higher relative peak areas as illustrated in figure 4.2. Although all four eluting solvents examined yielded acceptable profiles, ethyl acetate produced a profile for all the ten studied impurity peaks with reasonable relative peak area. Therefore ethyl acetate was the solvent of choice for the elution step.



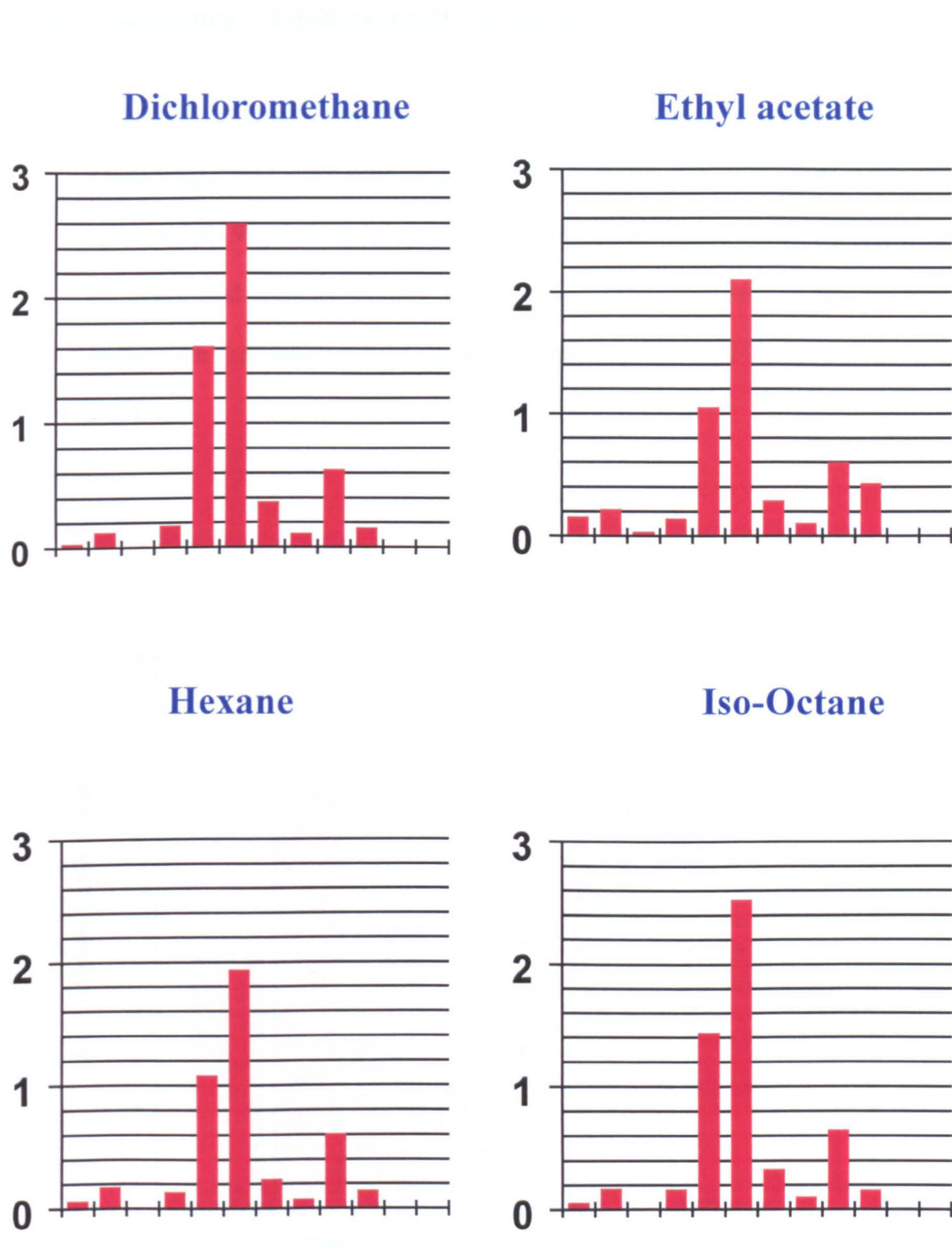


Figure 4.2. Bar charts demonstrate the extraction power of different elution solvents, mean relative peak areas (y-axis) Vs peak number (x-axis).

#### 4.4.3. Assessment of different SPE columns

Eight SPE columns from two manufacturers (Varian and International Sorbent Technology, IST) were evaluated. All SPE columns were examined using the optimised pH 9 and the optimised eluting solvent, ethyl acetate. After evaluation of the determined impurity profiles, Varian C18 (200mg) provided the best impurity profile with the highest relative peaks areas as it is illustrated in figures 4.3, 4.4a and 4.4b.

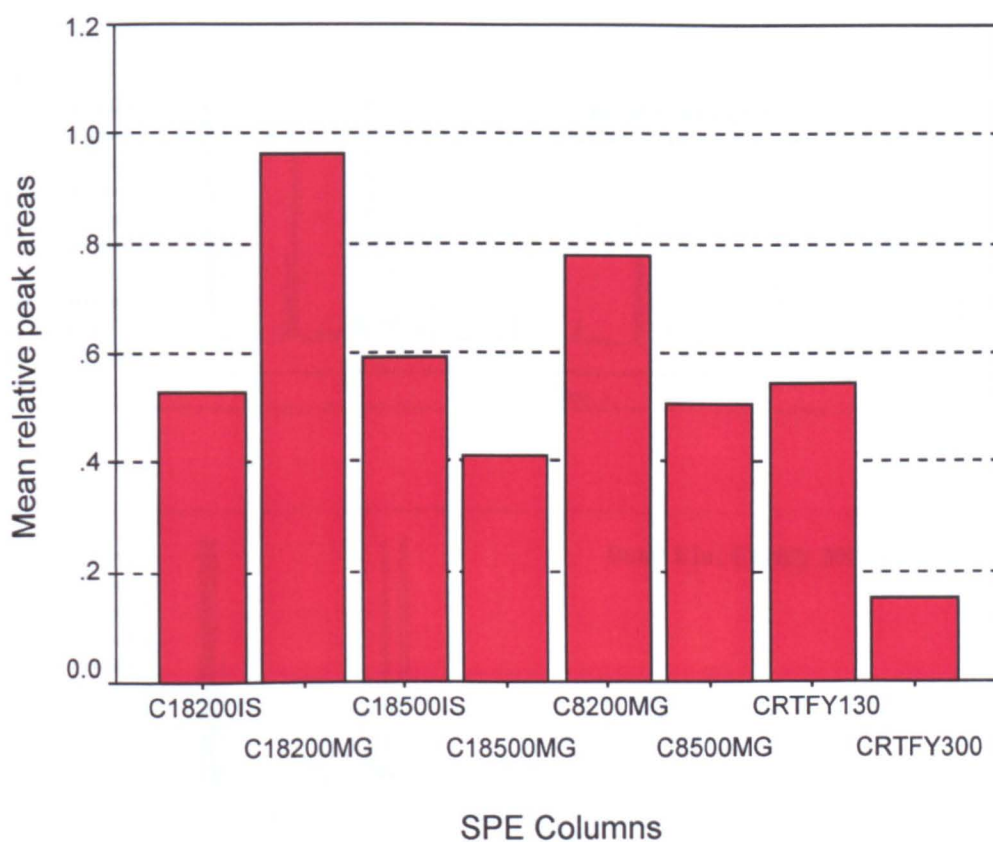


Figure 4.3. Means of relative peak areas Vs SPE columns

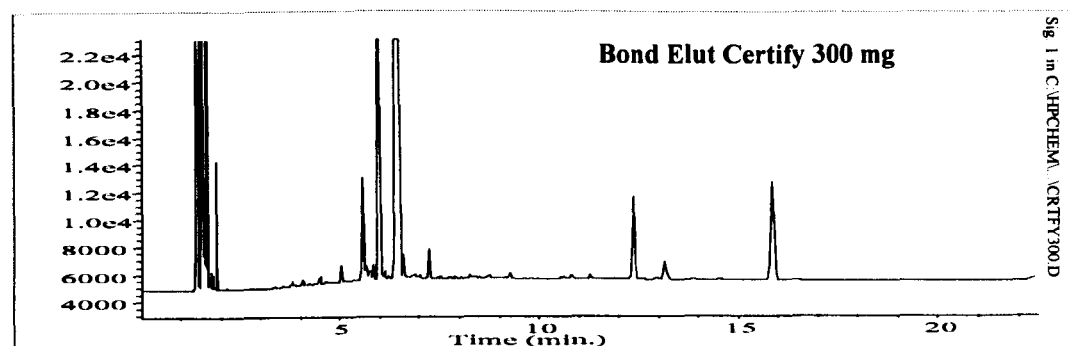
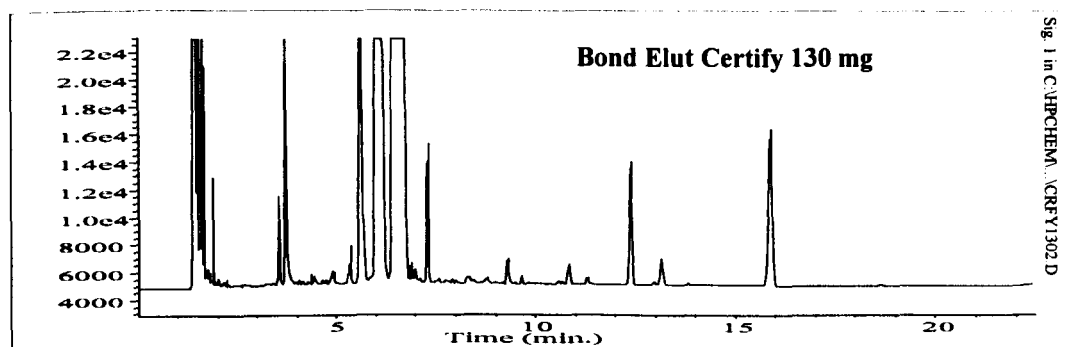
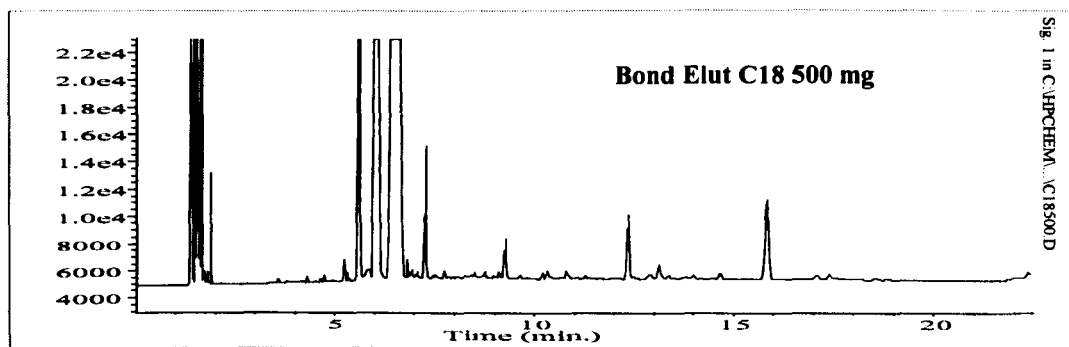
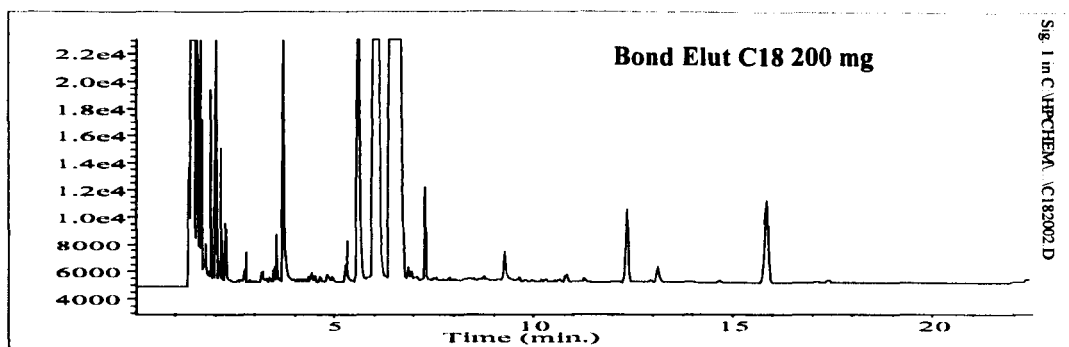


Figure 4.4a. Impurity profiles of sample 001 extracted on Bond Elut columns.

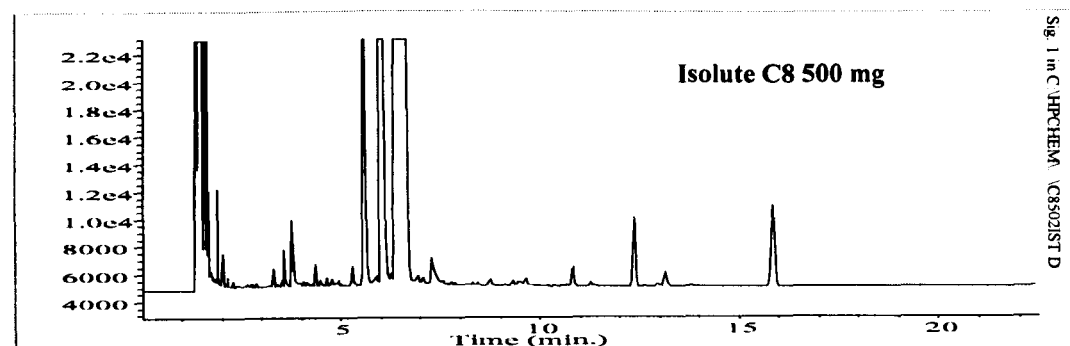
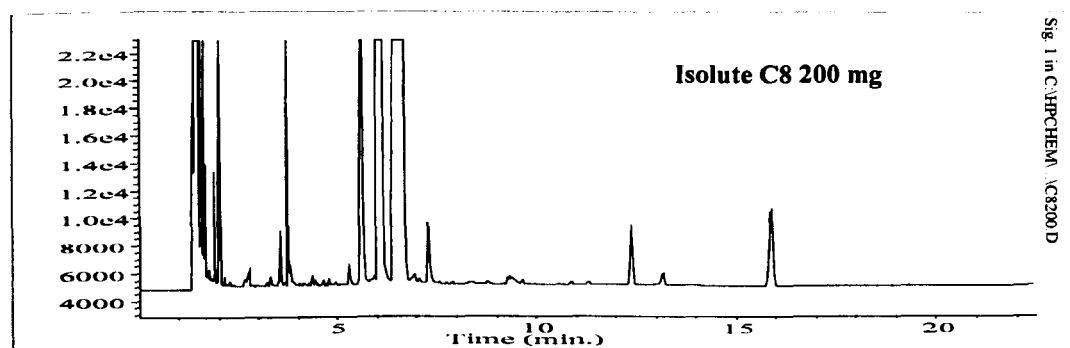
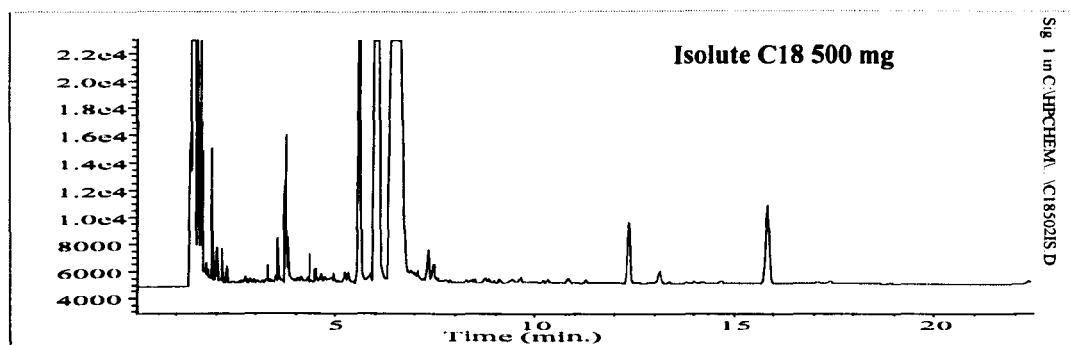
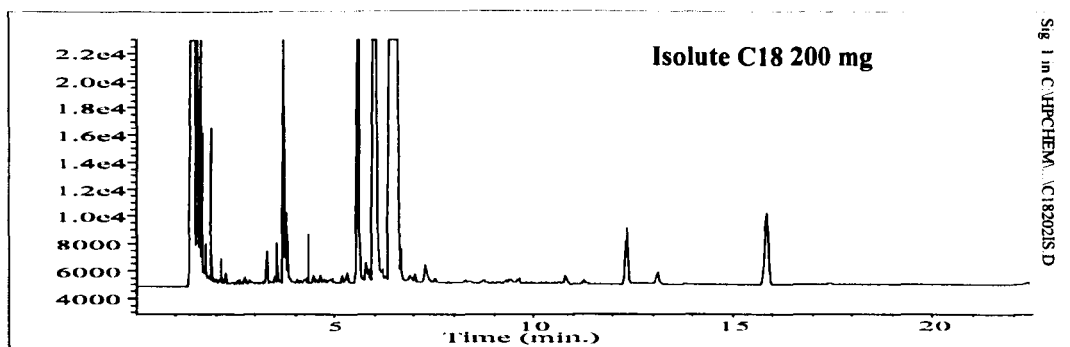


Figure 4.4b. Impurity profiles of sample 001 extracted on Isolute columns.

#### 4.4.4. Blank Extracts

A blank sample consisting of only a buffer solution at pH 9 was extracted with the optimised eluting solvent, ethyl acetate and by the optimised SPE column, Varian Bond Elut C18 200mg. This step was necessary to be certain that all the peaks used for the evaluation were not generated from any source other than the homogenised ecstasy powder. Figure 4.5 demonstrates a chromatogram of a blank sample extracted with the conditions mentioned above.

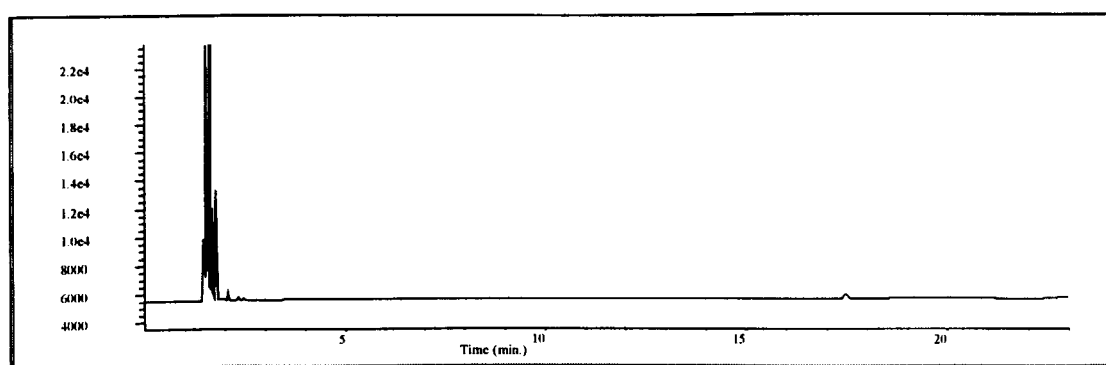


Figure 4.5. Chromatogram of a blank extracted with the optimised parameters

#### 4.4.5. Stability of Extracts during Storage

A demonstration of the stability of extracts during storage was examined for an ecstasy sample extracted with optimised procedure. The extract was analysed by GC-FID four times over a period of four weeks, meanwhile stored in the dark at 4°C for the whole period and was taken out briefly for the analysis. Figure 4.6 illustrates the stability study of the extracts profile over the four weeks period.

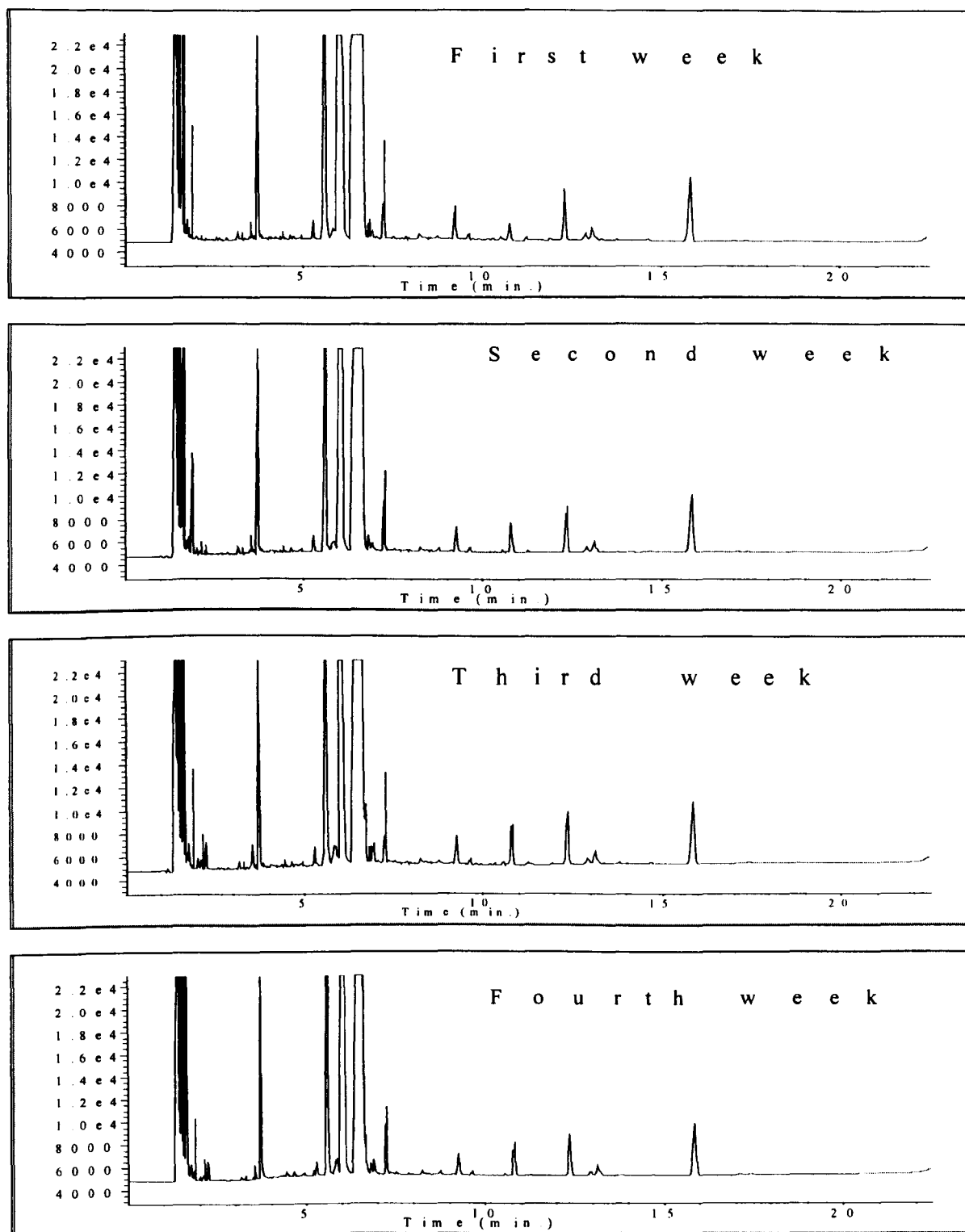


Figure 4.6. Impurity profiles of ecstasy sample demonstrating stability of extracts over a period of four weeks

After four weeks of storage of extracts in the dark at 4°C, the impurity profiles showed no major changes and were still valuable for comparison purposes.

#### 4.4.6. Liquid-liquid Extraction (LLE) Vs Solid Phase Extraction (SPE)

Five portion of the same ecstasy sample extracted by LLE and analysed by GC-FID. The principal peaks of chromatograms representing impurities of each portion were numbered and the means of their relative peak areas were calculated. The same procedure was performed for the other five portions extracted by SPE.

Extraction Method	Peak No.	n	Mean	S.D.
SPE	1	5	1.617	0.842
	2	5	3.276	0.222
	7	5	0.5178	0.0396
LLE	1	5	0.628	0.140
	2	5	0.968	0.110
	7	5	0.4016	0.0398

Table 4.1. Mean and standard deviation of the relative peak areas of peaks 1, 2, and 7.

To evaluate the data collected from the various chromatograms of ecstasy sample extracted by SPE and LLE, statistical analysis was performed to compare the means of important impurity peaks present in the chromatograms. The means of seven major impurity peaks were used for the statistical analysis and three of those means (peak number 1, 2, and 7) as illustrated in table 4.1 were further analysed by running a significant test. The two-sample t-test was used to assess if the differences in the means of the relative peak areas of the selected peaks were significantly different for each extraction method. Comparison was performed for the means of relative peak areas of peaks number 1, 2, and 7 of each extraction

method and the  $p$  value was calculated by the use of computer software (Minitab). The  $p$  value of each t-test as shown in table 4.2 proved that there is significant difference between the evaluated means. Since the means of relative peak area for samples extracted by SPE were larger than those extracted by LLE and that the  $p$  value showed significant difference in those means it is safe to conclude that SPE provided a better extraction yield of impurities in ecstasy samples.

Two sample t-test for peak 1
95% C.I. for LLE 1 – SPE 1: (-1.859, -0.12) T-Test LLE 1 = SPE 1 (not =): T= -2.57 P=0.030 DF= 9
Two sample t-test for peak 2
95% C.I. for LLE 2 – SPE 2: (-2.564, -2.053) T-Test LLE2 = SPE 2 (not =): T= -20.82 P=0.001 DF= 9
Two sample t-test for peak 7
95% C.I. for LLE 7 – SPE 7: (-0.176, -0.057) T-Test LLE 7 = SPE 7 (not =): T= -4.63 P=0.0025 DF= 9

Table 4.2. Two sample t-test results of peaks 1, 2, and 7 for both extraction methods SPE and LLE

Another illustration of the differences in the mean of relative peak area of peaks 1, 2, and 7 is shown in figure 4.7 where SPE yielded better peak recovery than LLE.



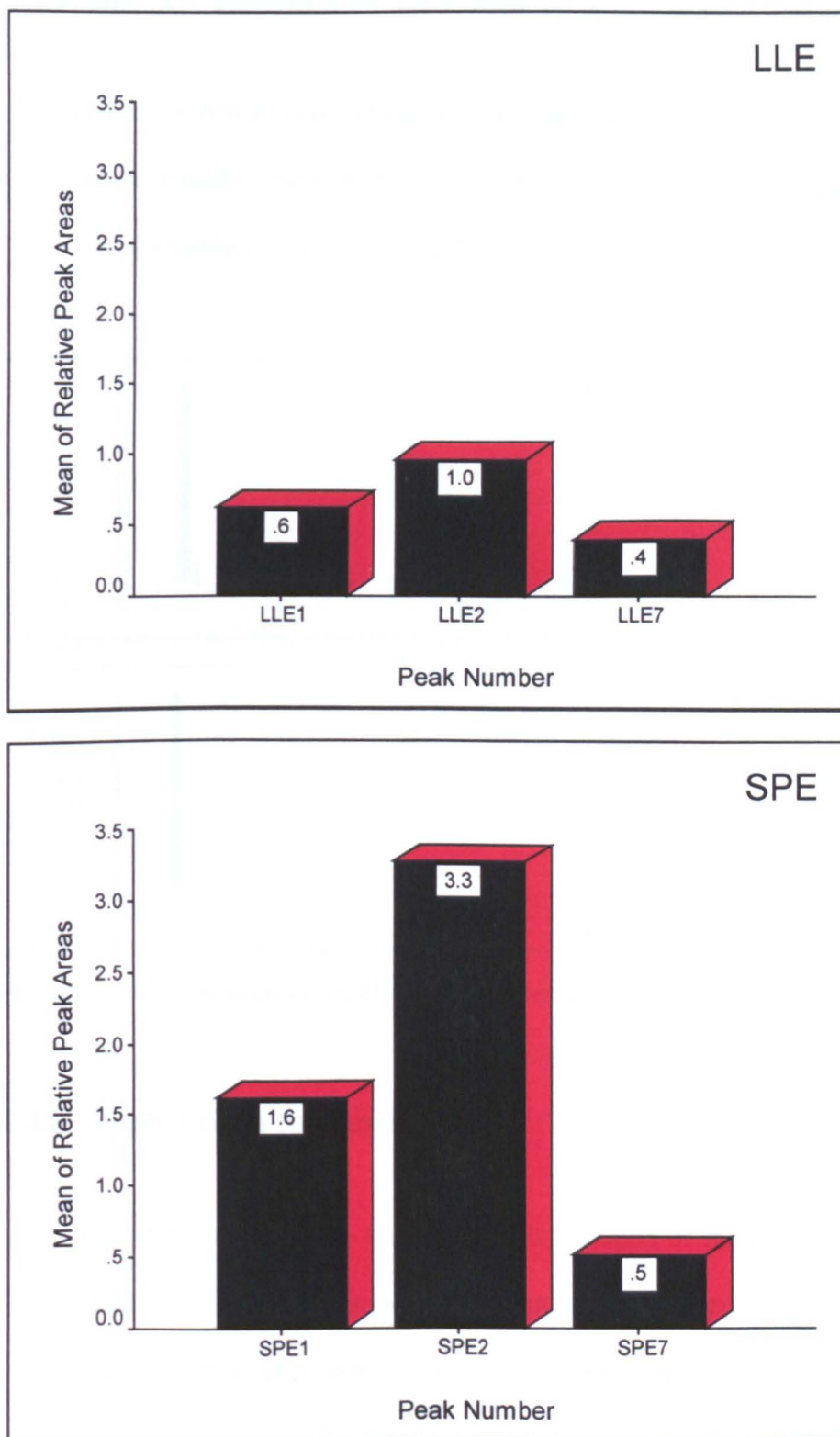


Figure 4.7. Comparison of means of relative peak areas for three different peaks of an ecstasy sample extracted by LLE and SPE.

#### 4.4.7. Blank Extraction by LLE and SPE

Blank extracts showed no major peaks especially at the retention time as the ecstasy impurity peaks were present. Figure 4.8 demonstrates chromatograms of two blank samples extracted by LLE and SPE.

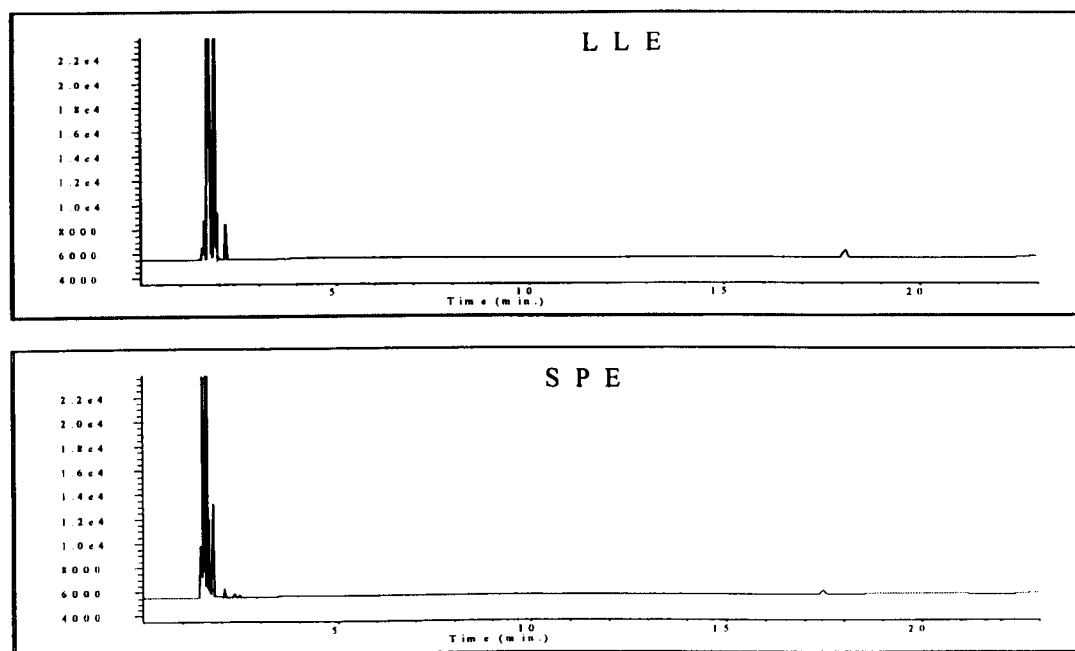


Figure 4.8. Chromatograms of blank sample extracted by LLE and SPE .

#### 4.4.8. Applications to seized samples

The magnitude of inter- and intra-batch variation of impurity profiles governs the establishment of a “common source”. Intra-batch variation should be less than inter batch-variation since samples belonging to the same batch expected to show more similarities than samples belonging to different batches.

##### 4.4.8.1 Comparison of different seizures (Inter-sample variation)

Ecstasy samples from 12 different seizures (packages) were extracted by solid phase extraction and analysed by GC-FID for the purpose of establishing

commonality. Figure 4.9 shows a comparison of impurity profiles of three ecstasy samples from three different seizures.

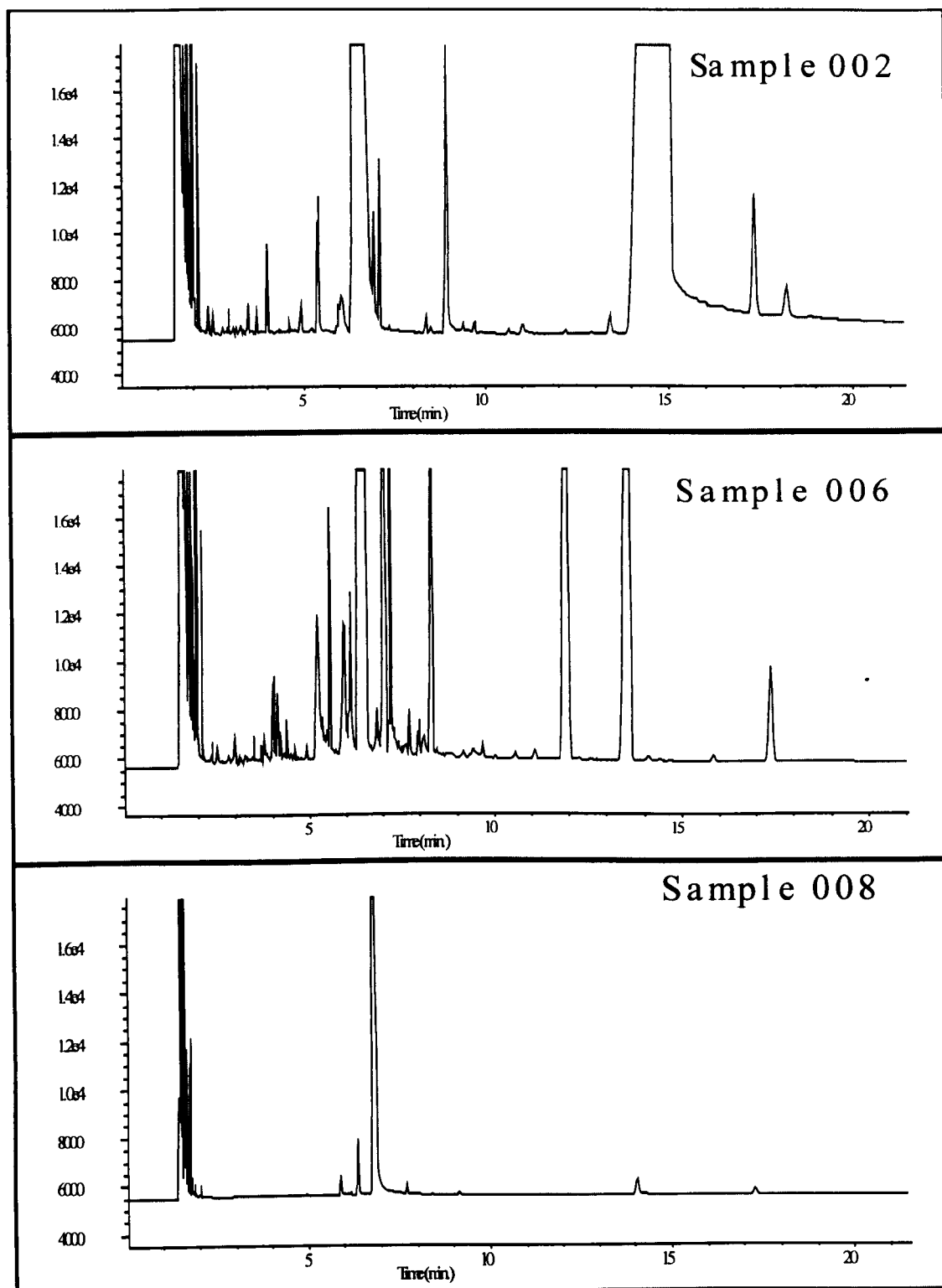


Figure 4.9. Impurity profiles of three different samples 002, 006, and 008 from three different seizures

The above impurity profiles allowed for visual comparison of three different ecstasy samples. It is clear that they contain great variations and consequently do not belong to a common batch (source).

4.4.8.2 Comparison of tablets from same seizure (Intra-sample variation)

Impurity profiles of two randomly chosen ecstasy tablets from the same seizure (package) were obtained using solid phase extraction and GC-FID. The generated impurity profiles showed similarities indicating that both samples belong to the same batch. Figure 4.10 demonstrate an overlay comparison of their impurity profiles showing little variation.

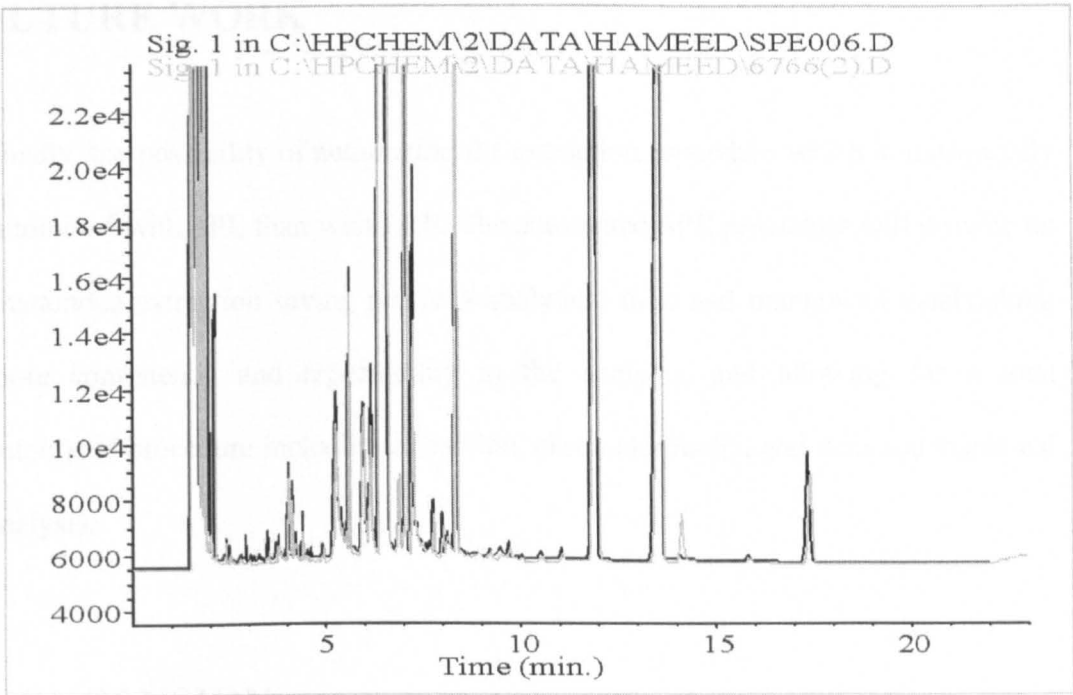


Figure 4.10. Impurity profiles of two samples from the same seizures

The advantages of using solid phase extraction for profiling of illicit drugs are numerous but most among those are efficiency, absence of contamination of phases and absence of emulsions problem, as found with LLE, due to the presence of fatty acids in ecstasy tablets. Secondly, profiling can be very difficult with a samples of high purity since the impurity profile is dependent on impurity abundance in the sample. An excellent extraction of impurities in high purity drug exhibits by LLE requires repeated extractions and the use of large volume of solvents, which can cause loss of analytes and consequently poor impurity profiles. SPE can provide excellent impurity profiles with superior selectivity, high recovery and reduced organic solvent consumption.

## **4.5. FUTURE WORK**

Finally, the possibility of automating the extraction procedure which is more easily automated with SPE than with LLE. The automated SPE procedure will provide an unattended extraction saving precious analytical time and manpower, establishing more consistency and repeatability in the analysis, and allowing for a total automated procedure including extraction, chromatography, and data and statistical analysis.

## **4.6. CONCLUSION**

The optimisation of the solid phase extraction (SPE) procedure for the purpose of profiling impurities found in ecstasy tablets manufactured in clandestine laboratories was examined. The determined optimum parameters for the SPE procedure were as follows:

1. Buffers with pH 9 were found to be the optimum for extracting impurities of ecstasy tablets.
2. The eluting solvent with most impurities extracted was ethyl acetate.
3. Among all the eight SPE columns evaluated, Varian Bond Elut C18 200mg produced the best impurity profile allowing for easy comparison of profiles of real samples.

Solid phase extraction of impurities in ecstasy tablets proved to be more efficient than the traditional liquid-liquid extraction. SPE provided impurity peaks with higher intensities than did LLE and SPE has a shorter extraction time. Street samples of ecstasy were analysed for profiling purposes using SPE procedure. Samples from different seizures (packages) showed little variations and great similarities indicating common batch origin while those from different packages showed great variations indicating different batch origin.

## **5. ECSTASY PROFILING BY INFRARED SPECTROSCOPY**

### **5.1. INTRODUCTION**

In previous chapters, chromatographic instrumentation was used to perform impurity profiling of ecstasy tablets, in particular gas chromatography with flame ionisation detection. Chromatographic methods are powerful tools for comparing drug seizures, but they do have their inherent limitations so a combination of at least two methods is more acceptable. Infrared spectroscopy and in particular fourier transform infrared, FTIR, can serve as another tool for comparing drug seizures. Each exhibit has its own characteristic spectrum, and comparison between the different spectra gives a gross estimate of the similarity or dissimilarity between the different exhibits. FTIR has some discriminating capability to classify powders into a large number of groups, and consequently can be used for gross comparison of ecstasy exhibits.

FTIR has some advantages over chromatographic methods such as reduced sample handling, faster analysis time, no need for impurities extraction and concentration procedure, and lack of volatility, solubility, thermal stability or column limitation. However, it has some major disadvantages including low sensitivity and inability to detect trace components.

This chapter deals with the subject of ecstasy profiling (that is, attempt to carry out gross comparisons between ecstasy seizures) through the use of FTIR as a screening tool in drug profiling procedures.

## 5.2. FOURIER TRANSFORM INFRARED, FTIR

In a dispersive instrument a dispersing element, such as a grating or prism, produces a narrow band of infrared wavelengths. The sample absorption occurring for each of these bands is compared with that occurring for a reference beam, by continuously varying the wavelength of the chosen band, one obtains a spectrum. With an FTIR, all of the radiation from the source is sampled [170]. Based on an interferogram that is produced, a mathematical dispersion is computed, which produces a spectrum. Fourier transform infrared instruments measure light absorbed or emitted from a sample. The measurements provide valuable chemical composition information. Fourier transform infrared spectrometers record the interaction of IR radiation with experimental samples, measuring the frequencies at which the sample absorbs the radiation and the intensities of the absorption. Determining these frequencies allows identification of the samples chemical make up, since chemical function groups are known to absorb light at specific frequencies [171].

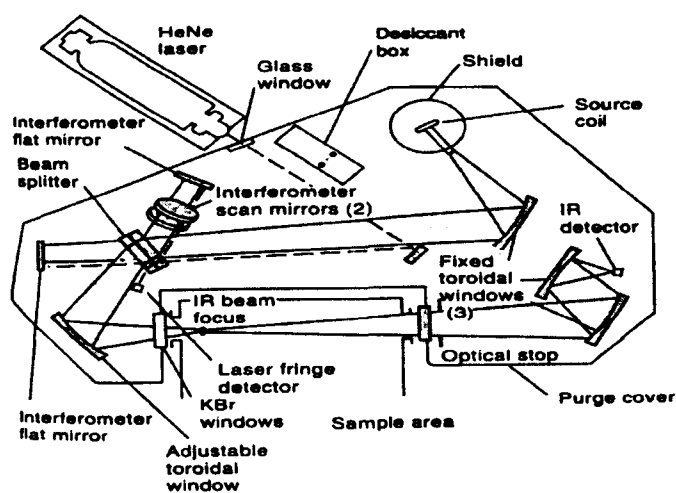


Figure 5.1. A single-beam FTIR spectrometer [170]



The key components of a FTIR system are the source, the interferometer, and the detector (Figure 5.1). The interferometer provides a means for the spectrometer to measure all optical frequencies simultaneously. The interferometer modulates the intensity of individual frequencies of radiation before the detector picks up the signal. The basic components of an interferometer include a beam splitter, a fixed mirror and a moveable mirror (figure 5.2). Half of the infrared light is reflected from the beam splitter to the fixed mirror and back to the detector, while the other half of the light is passed through to the moveable mirror, and then back to the beam splitter, where the beam is again split, and half of the light from the reflected beam is passed to the detector. Using a polychromatic light source, the moveable mirror is moved so that the two light beams interact constructively. The detector records the sum of the cosine waves of the polychromatic light, and hence data for all wavelengths to be considered is collected at once, improving the speed of the instrument [171, 172].

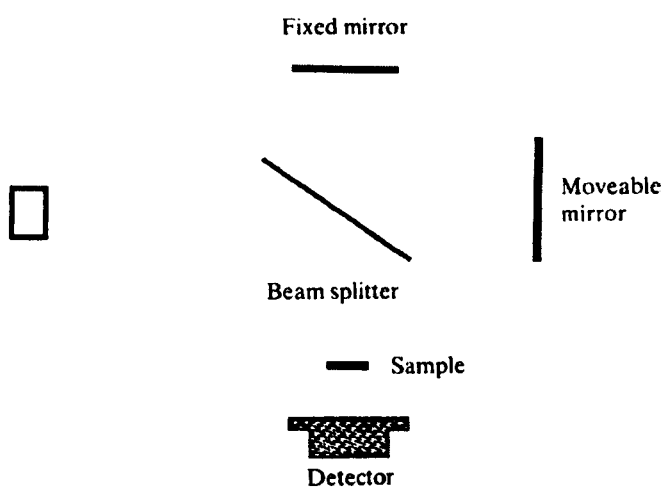


Figure 5.2. An interferometer [173]

The product of an interferomete (scan) is called an interferogram, which is a plot of intensity versus mirror position. Using a mathematical process called Fourier Transform (FT), the system computer converts the interferogram into a spectrum.

FTIR instruments have many advantages over the conventional dispersive instruments such as; quick scanning time, higher throughput energy since FTIR instruments have no slits to isolate a band of wavelengths as in dispersive instruments, better signal to noise ratio which result in improved spectrum quality, possibility of mathematical manipulation of the data, and the possible coupling of FTIR to microscopy and chromatography [173].

### **5.3. SAMPLING TECHNIQUES**

There are various ways of presenting samples for FTIR analysis: as gaseous, liquid and solid sampling.

#### **5.3.1. Gaseous Sample**

Because of the low volatility of drugs of abuse, measurements of the gaseous infrared spectrum of drug samples is of little importance to the forensic scientist. There are only few compounds such as nitrous oxide that are suitable for gaseous phase FTIR spectroscopy

#### **5.3.2. Liquid Sample**

Liquids are analysed in pure form or in solutions. Analysis of liquids may be the easiest to handle since they may be poured into cells made of alkali metal halides such as sodium chloride or potassium bromide and the IR spectrum determined

directly. However, cell etching occurs with time because of water content in samples and interference patterns becomes more noisy.

### **5.3.3. Solid Sample**

Three main methods are used for solid samples, the nujol mulls, thin films and pressed pellets.

#### **5.3.3.1. *Pressed Pellets***

The most commonly used, but basic, method of analysing solid samples is the traditional KBr disk. Also known generically as the halide disc technique, the system was developed over 40 years ago and has remained unchanged since. The sample is first ground into a fine powder. It is then mixed with KBr and the grinding process continued. A mortar and pestle is normally used to grind the materials, but in laboratories where many discs are made each day an electrical mill is used. A capsule is used to contain the sample during the milling process. Materials of capsule construction include polystyrene, stainless steel and agate. The finely ground materials are then removed from the mill and placed in a KBr die to complete the process. Die sizes range from 1mm diameter for micro samples up to the traditional 13mm diameter disc. Although the designs of dies differ widely, their basic function is to sandwich the mixture between two polished surfaces and press the KBr and sample in to a disc. High pressure is applied, either by means of levers or a hydraulic press, to coalesce the sample into a transparent or semi-transparent disc. A vacuum line, connected to the die, removes entrained air and, to a limited extent, entrained moisture. As entrained moisture can cause the finished disk to be opaque the KBr powder should be kept dry by means of a desiccator or a heated oven. Moist samples are not suited to this technique, as they will cloud the disc.

Such samples should be dried before analysis. The prepared disc is mounted in the beam of the spectrometer, so that it may be analysed. Careful attention to the sampling methods, disk making techniques and water content of the sample and KBr powder is still considered to be at the heart of making a quality disc.

#### **5.3.3.2. *Nujol Mulls***

The sample is usually ground to a fine paste in a liquid hydrocarbon such as nujol. This method is rapid and easy, but it is unsuitable for quantitative analysis because it is difficult to prepare mulls with accurately known concentrations of analyte. The other main disadvantage of this method is that it requires a large amount of sample.

#### **5.3.3.3. *Thin Films***

Thin films are used to obtain a spectrum free from a dispersing medium. A small amount of the drug is dissolved in a volatile, water free solvent and a portion of this solution is then placed on a salt plate where the solvent left to evaporate. The compound is then analysed on FTIR as a thin film.

### **5.4. DATA ANALYSIS**

The use of computers in data collection and manipulation has led to an increase in IR spectral libraries. Collection of data in digital form allows a spectrum to be manipulated, stored and retrieved. Most manufacturers of infrared instruments now offer computer search systems to assist the analyst in identifying compounds from stored infrared spectral data. The position and relative magnitude of peaks in the spectrum of the analyte are determined and stored in memory to give a peak profile, which can then be compared with profiles of pure compounds stored. The computer

then matches profiles and prints a list of compounds having spectra similar to that of the analyte.

A number of factors that effect data interpretation should be taken into account when performing a computerised library search. Has the spectrum under consideration been prepared in the same conditions as the library spectrum? If not then small shifts in wavenumbers at which absorbances occur may happen [173]. A good solution to this problem is to create an in-house library of spectra of most drugs encountered by the analyst under the same conditions. Another factor is that databases and libraries are only means of providing possibilities for the identity of the compound with a measure of degree of fit of the spectrum obtained with that in the library. Hence, While library searching allows the analyst to gain some idea of the identity of a drug, the spectrum should always be compared to that obtained for an authentic sample of the compound, obtained under identical conditions.

## **5.5. APPLICATIONS**

Various applications of IR spectroscopy for drugs analysis are cited in the literature, from screening unknown exhibits for identification of controlled substances to comparison of different seizures for intelligence purposes.

### **5.5.1. Samples Screening and Qualitative Analysis**

Infrared spectroscopy can be valuable tool for screening samples for the purpose of identifying a controled substance. Screening with FTIR in particular is convenient because of both its speed and its ability to use accessories requiring minimal sample preparation. Many illicit drugs clandestinely manufactured often contain adulterants

and diluents such as sugars, starch or inorganic substances (for example, talc or sodium bicarbonate). Conventional drugs screening methods, such as colour tests, gas chromatography, or ultraviolet spectroscopy, do not normally detect these substances. Infrared spectroscopy screening is possible for these substances because they have strong absorption in the infrared region.

### **5.5.2. Comparisons of Drug Seizures**

As mentioned at the beginning of this chapter, infrared spectroscopy can be used as an add-on tool for the characterisation and comparison of seized drugs for intelligence purposes by direct/rigorous comparison of two exhibits or classifying seizures into groups by gross comparison.

Many workers reported the use of infrared spectroscopy for 1) identification of impurities in seized drugs to establish the method of synthesis [53, 174], 2) direct comparison of samples [36], and 3) identification of major excipients in illicit drugs for comparative studies [144, 145].

## **5.6. EXPERIMENTAL**

### **5.6.1. Materials**

Forty-seven ecstasy samples from twenty-four seizures were used in the experiment. The Forensic Science Service, Metropolitan laboratory, London, provided street samples of ecstasy tablets. Standards of MDMA, lactose, glucose and caffeine were obtained from Sigma.

### 5.6.2. Sample Preparation and Instrumental Analysis

Samples were prepared using KBr disks. Around 1 mg of sample was ground by pestle and mortar into a fine powder. Then about 300 mg of oven dried KBr were added and ground again to a fine homogeneous powder. The finely ground sample was then removed and placed in a 13mm KBr die to sandwich the mixture between two polished surfaces and press the KBr and sample into a disc. High pressure (12000kg) was then applied by means of a lever to coalesce the sample into a transparent or semi-transparent disc. Vacuum was applied to the die to remove entrained air, and to a limited extent, entrained moisture. As entrained moisture can cause the finished disc to be opaque. The prepared disc was then mounted in the beam of the spectrometer for analysis. Infrared spectra were obtained using a Jasco FTIR-410 Spectrometer (Jasco UK Ltd., Essex, UK).

### 5.6.3. Spectra Comparison

An in-house library was created which contained spectra for standards and 47 ecstasy samples. Once spectra for standards and all 47 ecstasy samples were obtained, comparisons of spectra using a library search facility from a commercially available spectral program called Spectra Manager SSP-400 search program (Jasco UK Ltd., Essex, UK) were performed for the purpose of establishing similarity or dissimilarity between the different samples. The library search was done using an algorithm for correlation coefficient for comparison purposes. This method conducts a search by comparing the spectrum waveform of an unknown sample with the spectral waveforms of library spectra. The library search provides best fits or closest matches from the library spectra to the unknown spectrum. A higher number means a closer match (1 being the highest and 0 being the lowest). The

comparison procedure was based on two main elements, 1) the library search results (best fit) and the visual comparison of spectra using an overlay diagram.

#### **5.6.4. Repeatability of Analysis**

To test the repeatability of spectra a single sample was analysed as above ten times using the same procedure and parameters including sample preparation as a disc and analysis on FTIR. Statistical analysis was then performed to calculate relative standard deviation (RSD).

#### **5.6.5. Comparison of tablets from different seizures**

Tablets from different seizures chosen randomly were analysed for the purpose of establishing common source. Each tablet was ground, extracted, and analysed separately to avoid cross contamination. The result spectra were compared by the use of library search facilities.

### **5.7. RESULTS AND DISCUSSION**

The aim of comparative analysis is the comparison of confiscated drugs for investigative purposes and court testimony. Drug intelligence involves two main streams with regard to comparative analysis or drug profiling. The first includes court cases such as sample/sample comparison and relating a user to a dealer. The second task for drug intelligence involves establishing relationships between seizures and providing information on the networking of samples, which maybe of use in investigating major trafficking operations. In such operations the laboratory may have to analyse many samples. FTIR analysis can be utilised for such



operations as an elimination tool because of its sheer simplicity and short analysis time.

5.7.1. Repeatability of Analysis

Repeatability is a vital factor in drug profiling procedures since little variations in data (spectra or chromatograms) can effect the interpretation of the comparison process. After analysis of sample number (M01) for ten times, reproducible spectra were obtained and the calculation of percentage relative standard deviation (RSD) showed excellent repeatability of results. The procedure included running a library search on ten different spectra of sample (M01) and using the fit factors (library scores) from the search results to calculate percentage RSD. Table 1 illustrates the statistical results for the repeatability study.

Column Statistics	Results
Mean	0.98
Standard Deviation	.0098
Minimum	0.974
Maximum	1
Count	10
RSD (%)	1.003

Table 5.1. Statistical results for the repeatability study.

### 5.7.2. Comparison of tablets from different seizures

Comparison of spectra of samples from different seizures yielding different physical features such as colour and logos showed correlation of their spectra as seen in the example given in Figure 5.3 which shows an overlay comparison of two samples from different seizures. This technique can be used as a visual means of comparing spectra of similar tablets from different seizures or comparing two different exhibits to establish similarity or dissimilarity.

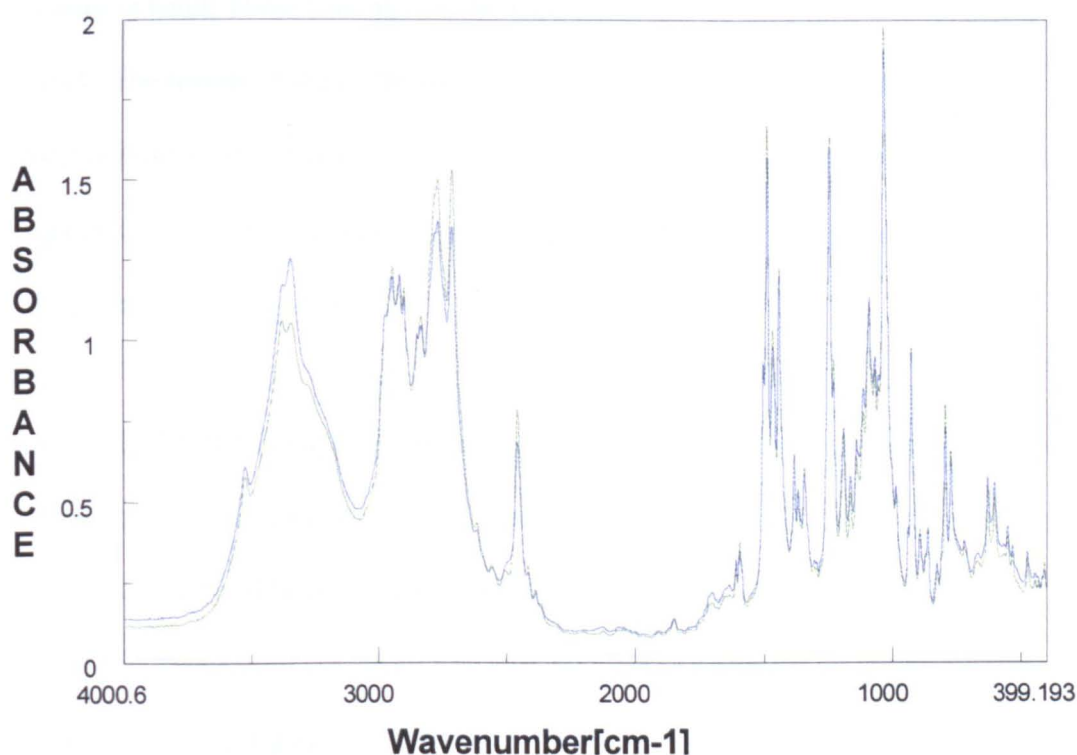


Figure 5.3. An overlay of spectra of sample 001 and sample 026

The above method is useful when only small amounts of exhibits are compared. In the case of large amounts of seizures or in the case of building a database with many spectra included and samples to be related or grouped into smaller clusters, then use of computer software is faster and more useful. Visual comparison is still handy as a confirmation to the library search results and for court testimony

The above method is useful when only small numbers of samples are compared. In the case of large number of samples or in the case of building a database with many spectra included and samples to be related or grouped into smaller clusters, then use of computer software is faster and more useful. Visual comparison is still handy as a confirmation to the library search results and for court testimony presentation. In Table 5.2 an example is given of a library search result where a spectrum of an unknown (confiscated ecstasy tablet) was compared with a database of spectra of street ecstasy tablets. Figure 5.4 illustrates the library results in terms of spectra. Samples 013, 026 and the unknown showed similarities indicating their relation to a common batch. These findings can be used positively to save time and laboratory work. The results of the FTIR analysis may be used to eliminate many samples before proceeding to more sensitive procedures such as chromatography. FTIR results can also provide a gross estimation of the different groups or profiles of drugs under investigation.

Although FTIR technique is not sensitive enough to detect minute amounts of compounds such as manufacturing impurities, which are essential to the profiling process, it can still be a useful tool when its results are combined with other analytical procedures including physical analysis and microscopy and information from police investigations.

Order of Fit	Score	Sample Name
1	0.977	013
2	0.971	026
3	0.900	032
4	0.789	010
5	0.769	014
6	0.761	002

Table 5.2. Library search results of sample 002 against library database for the closest match

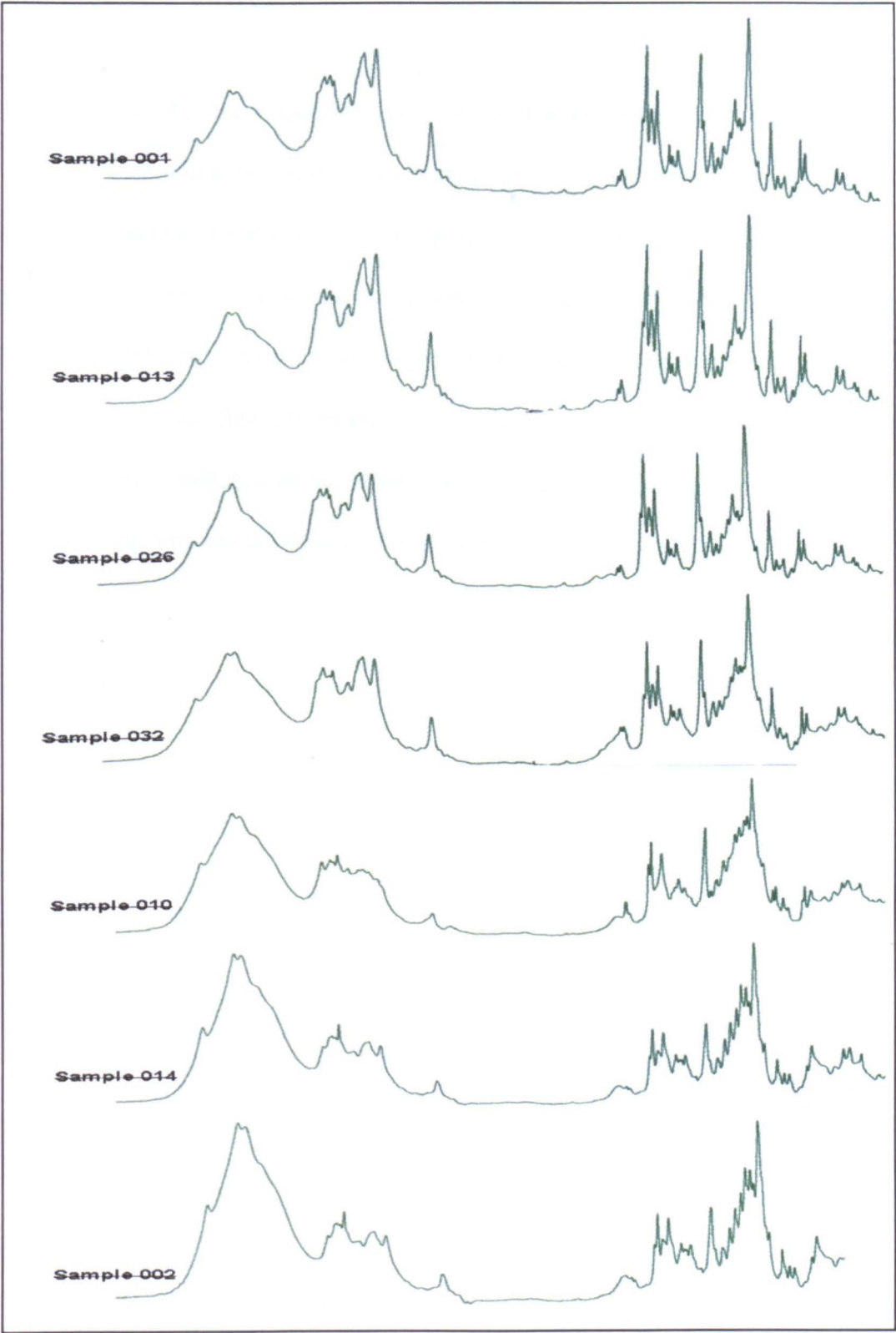


Figure 5.4. Library search results of matching an ecstasy exhibit (sample 001) with a database of other ecstasy samples

## 5.8. CONCLUSION

FTIR can be a very useful tool for comparative analysis of confiscated drugs for its sheer simplicity with regard to sample preparation and instrumental procedure, short analysis time and excellent repeatability of results. The library search facility provided a way to compare two samples and relate a sample to a group of samples from different seizures analysed previously. Comparative studies on confiscated ecstasy tablets through the use of FTIR when combined with a study of the physical features of tablets such as colour, size, logo, and microscopic analysis are possible and help provide information for the drug investigation process.

## 6. SYNTHESIS OF MDMA

### 6.1. INTRODUCTION

The various N-substituted derivatives of 3,4-methylenedioxyamphetamine, MDA have been popular drugs of abuse since the early eighties. The N-methyl derivative, 3,4-methylenedioxymethylamphetamine (MDMA, Ecstasy, or XTC) is perhaps the most widely abused drug of this series. MDMA has been known and patented since 1914.

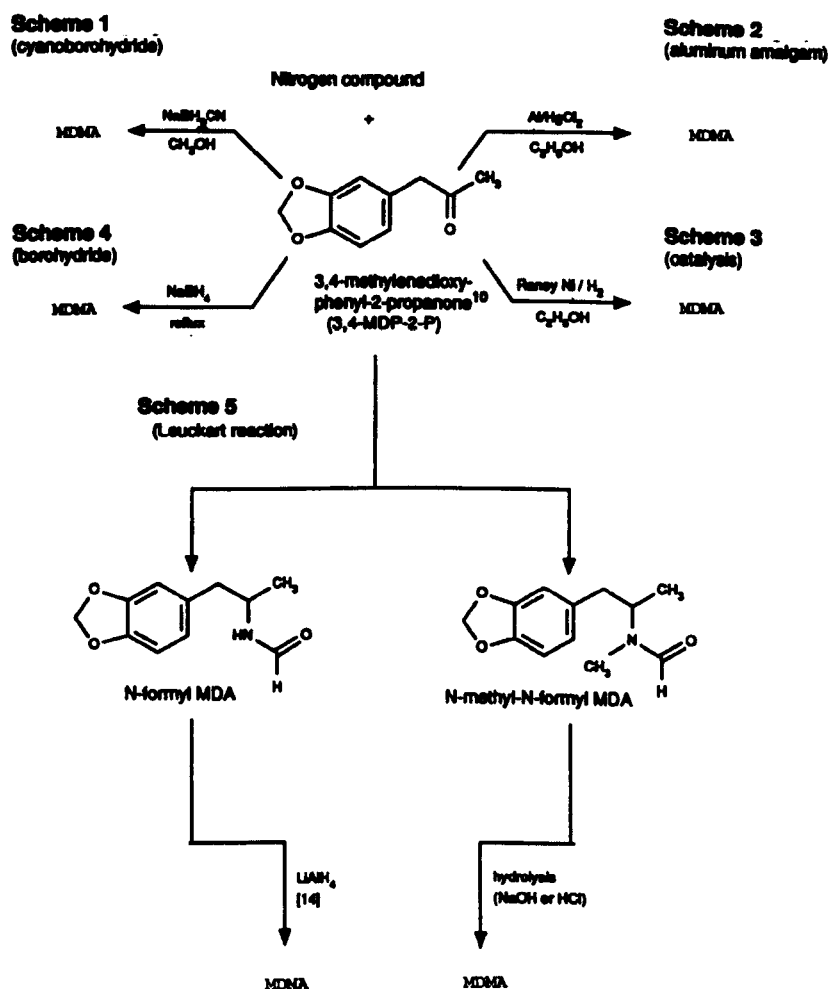
### 6.2. SYNTHETIC ROUTES

Because there is no therapeutic application of MDMA its synthesis is carried out exclusively in clandestine laboratories. A variety of methods have been reported for the synthesis of MDMA [56].

There are five precursors that are most often used for the manufacture of MDMA, including safrole, isosafrole, piperonal, beta-nitroisosafrole, and piperonyl methyl ketone (PMK) or as it also called 3,4-methylenedioxy-phenyl-2-propanone (3,4-MDP-2-P). The first three are available commercially but require a drug-purchasing license. However, safrole can be extracted from the sassafras plant native to the United States. Safrole is then easily isomerized into isosafrole when heated with NaOH or KOH. The isosafrole is then oxidised into PMK. Safrole and isosafrole can be used either directly via the bromopropane route to synthesise MDMA or used to prepare piperonylacetone which is not commercially available any more.

Piperonal in general is used in the preparation of beta-nitroisosafrrole, which is also not available in the licit market.

The most common routes for the production of MDMA are from the precursor MDP-2-P (PMK) as seen in Figure 6.1.





2. Aluminum Amalgam
3. Sodium Borohydride
4. Raney Nickel Catalysis
5. Leuckart Reaction via N-formyl-MDA
6. Leuckart Reaction via N-methyl-N-formyl-MDA

The sodium cyanoborohydride method is probably the one most attractive to clandestine chemists. It requires no knowledge of chemistry, has a wide applicability, offers little chance of failure, produces good yields, does not require expensive chemical apparatus or glassware and uses currently available and easily synthesised precursors. The aluminium amalgam synthesis is often used but has a slightly higher risk of failure and is not as versatile. The sodium borohydride requires harsher conditions for the chemicals (i.e. reflux) than sodium cyanoborohydride or aluminium amalgam and produces lower yields. The Raney Ni synthesis is more dangerous and requires special equipment to be done right (although this scheme is used in a significant number of clandestine labs). The Leuckart reaction is a 2-step process with lower yields and requires chemical apparatus [56].

There are also other synthetic methods which proceed directly from safrole. The first method, bromination route, uses HBr to produce 3,4-methylenedioxyphenyl-2-bromopropane which is then converted into MDA or MDMA. This route is attractive to clandestine laboratories for its sheer simplicity and that it requires no specialised chemical equipment or reagents at all. The second method is the nitropropane route, which uses piperonal to synthesise 3,4-methylenedioxyamphetamine, MDA. MDA is then converted to MDMA [175], which makes this method unlikely to be used by clandestine laboratory [56]. Figure 6.2 illustrates a summary of the different synthetic routes utilised in clandestine laboratories for manufacturing MDMA.

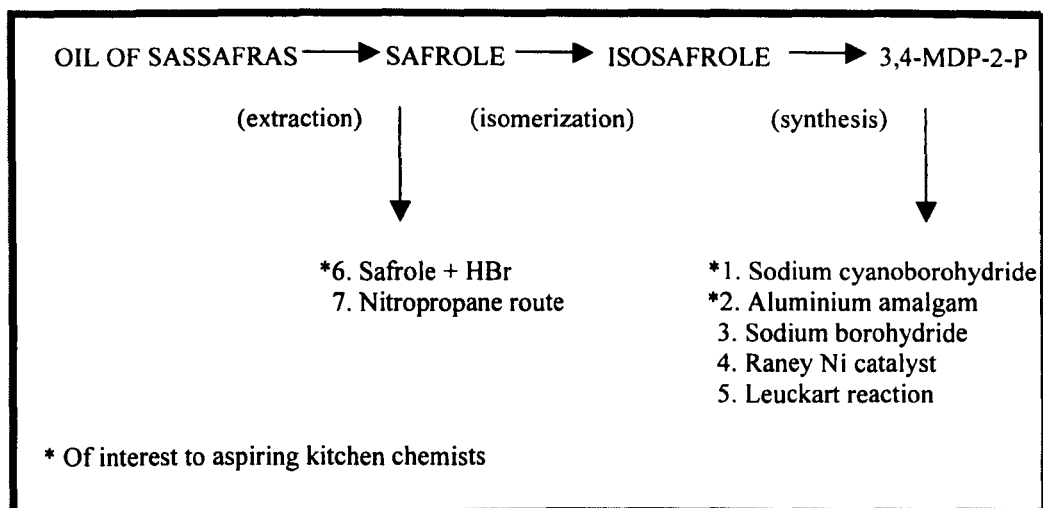


Figure 6.2. Summary of the different synthetic routes used to manufacture MDMA

- The sodium cyanoborohydride method is the most preferred method
- The aluminium amalgam route is as useful as cyanoborohydride, but may have a slightly higher risk of failure.
- The bromination route is attractive due to its sheer simplicity

Schemes 1-4 use PMK as the starting precursor, which can be synthesised from Safrole or isosafrole.

In this study only the reductive amination routes ( sodium cyanoborohydride and aluminium amalgam), the Leuckart reactoin, the bromopropene and the nitropropane routes have been used for the synthesis of MDMA.

## 6.3. EXPERIMENTAL

### 6.3.1. Synthesis of 3,4-methylenedioxyphenyl-2-propanone (PMK)

Different ways to synthesis this ketone from safrole [176], isosafrole [53, 177, 178] and piperonal [179] have been reported. Figure 6.3 illustrates different methods used to manufacture PMK.

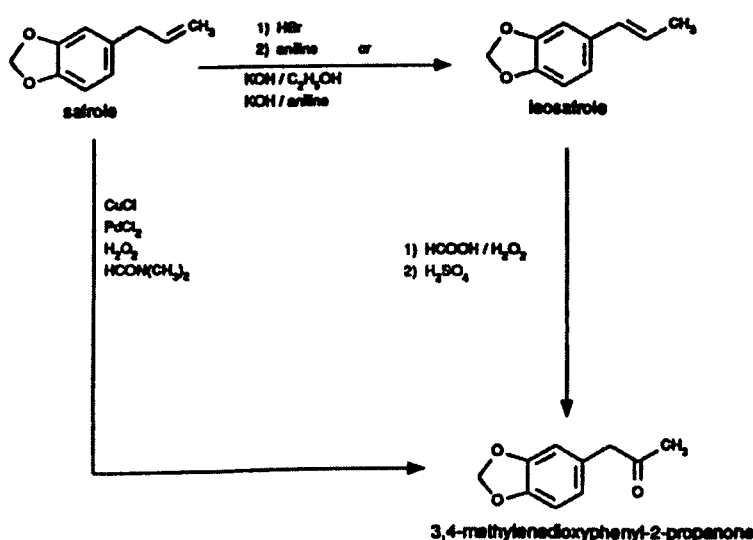


Figure 6.3. Different routes to the synthesis of 3,4-methylenedioxy-2-propanone (MDP-2-P)

In this study the precursor ketone needed for synthesis of MDMA via the reductive amination routes and Leuckart reaction was synthesised as follows:

To a well stirred, cooled mixture of 34g of 30% hydrogen peroxide in 150g 80% formic acid there was added, dropwise, a solution of 32.4g isosafrole in 120ml acetone at a rate that kept the reaction mixture from exceeding 40° C. This required a bit over 1 hour, and external cooling was used as necessary. Stirring was

continued for 16 hours, and care was taken that the slow exothermic reaction did not cause excess heating. An external bath with running water worked well. During this time the solution progressed from an orange colour to a deep red. All volatile components were removed under vacuum which yielded some 60g of a very deep residue. This was dissolved in 60ml of methanol, treated with 360ml of 15% sulphuric acid, and heated for 3 hours on a steam bath. After cooling the mixture was extracted with 3x75ml diethyl ether or benzene. It is recommended that the pooled extracts are washed first with water and then with dilute sodium hydroxide. Then the solvent is removed under vacuum to afford 20.6g of 3,4-methylenedioxyphenylacetone. The final residue may be distilled at 2.0mm/108-112° C or at about 160° C at the water pump.

### **6.3.2. Synthesis of MDMA**

#### **6.3.2.1. Reductive amination routes**

Reductive amination routes have been perhaps the most frequently used method [56] in the manufacture of MDMA from PMK. They consist of a single-step technique, which requires little knowledge of chemistry, as the case with the sodium cyanoborohydride route, and can be described as low-pressure reductive amination method. As mentioned above there are four different procedures to synthesise MDMA via the reductive amination route. They are the sodium cyanoborohydride, aluminum amalgam, sodium borohydride, and Raney nickel catalysis.

### 6.3.2.1.1. Scheme 1: Aluminium amalgamation [50, 179, 180]

#### Procedure:

H<sub>2</sub>O (140 ml) containing 0.1 g mercuric chloride was added to 4 g of thin aluminium foil cut in cm squares (in a wide mouth Erlenmeyer flask). Amalgamation was allowed to proceed until there was the evolution of fine bubbles, the formation of a light grey precipitate, and the appearance of occasional silvery spots on the surface of the aluminium. Then H<sub>2</sub>O was removed by decantation, and the aluminium was washed with 2x 140 ml of fresh H<sub>2</sub>O. Then the residual H<sub>2</sub>O from final washing was removed as thoroughly as possible by shaking followed by addition, in succession and with swirling, of 4 ml aqueous methylamine, 10 ml Isopropylalcohol, 1g NaCl dissolved in 10 ml H<sub>2</sub>O and 4 ml 15% NaOH, 1 g PMK, and finally 20 ml isopropylalcohol. Figure 6.4 shows the reaction scheme for the aluminium amalgamation route for the manufacturing of MDMA from PMK.



Figure 6.4. Aluminium amalgamation route.

### 6.3.2.1.2. Scheme 2: Cyanoborohydride [181, 182]

#### Procedure

A solution consisting of 6 mMol PMK, 60 mMol aqueous methylamine and sodium cyanoborohydride (15 mMol) in methanol (15 ml) was stirred at room temperature for 24 h. The reaction mixture was then evaporated to dryness under reduced pressure and the residue suspended in dichloromethane (50ml). The dichloromethane suspension was then extracted with 3 N HCl (2x 50 ml) and the combined acid extracts made basic (pH 12) with sodium hydroxide. The basic aqueous suspension was then extracted with dichloromethane (2x 40 ml) and the combined organic extracts dried over anhydrous sodium sulphate. Filtration followed by evaporating the filtrate solvent which gave the product in its base form. The base was then treated with ethereal HCl to give the amine hydrochloride form which was then isolated by filtration and recrystallised from mixtures of anhydrous ether and absolute ethanol. Figure 6.5 shows the reaction scheme for the sodium cyanoborohydride route for the manufacturing of MDMA from PMK.



Figure 6.5. Cyanoborohydride route

### 6.3.2.2. The Leuckart reaction [52, 53, 183, 184]

Two different routes involving the Leuckart reaction were used (Figure 6.6). Route (1) starts with formic acid and N-methylformamide in addition to PMK and yielded the intermediate N-formylMDMA. Route (2) starts with formamide and PMK and yielded the intermediate N-formylMDA.

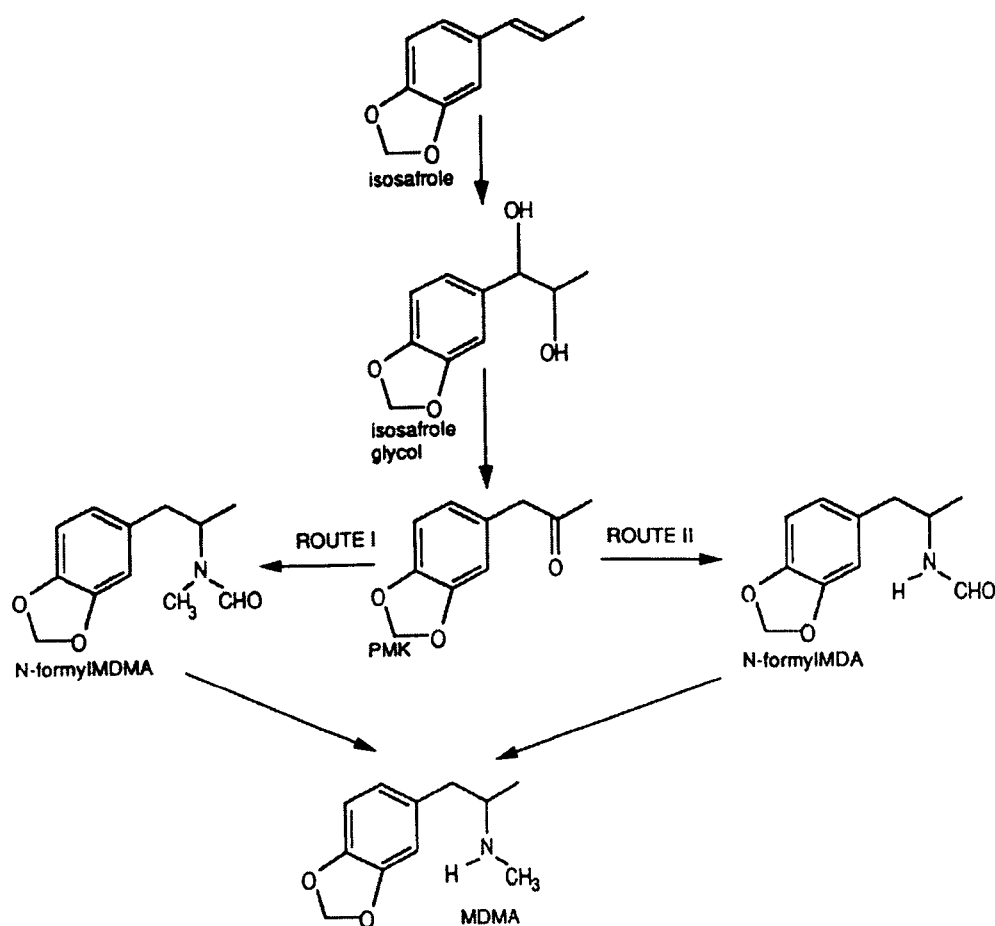


Figure 6.6. Route 1 and 2 for synthesis of MDMA via the Leuckart reaction

Route 1:

A mixture of formic acid (7 ml), N-methylformamide (0.8g), and PMK (1g) were refluxed at 150-170°C for 7 h with additional formic acid (0.4g) added periodically. N-formylMDMA was obtained after cooling. Then concentrated HCl (30 ml) was added to this intermediate and refluxed for another 3 h. The reaction mixture was then made basic with sodium hydroxide and the crude MDMA extracted into diethyl ether. The organic solvent volume was decreased and the remaining residue was treated with hydrogen chloride gas to yield a gelatinous brown precipitate of impure MDMA hydrochloride. The crude salt, dissolved in boiling methanol, was added to chilled acetone to form a crystalline product.

Route 2:

Formamide (7 ml) and PMK (1 g) were refluxed for 5 h. The solution was then made basic and extracted with diethyl ether. The ethereal solution was first washed with dilute sulphuric acid, rinsed with water and finally dried over anhydrous sodium sulphate. The diethyl ether volume was reduced to yield a solution of N-formyl-MDA. This product was then added drop-wise to lithium aluminium hydride and refluxed for 3 h. Excess lithium aluminium hydride was decomposed by the addition of ethyl acetate and then water and the resulting mixture was filtered and the precipitate washed with diethyl ether. The washings and the filtrate were combined and extracted with dilute sulphuric acid. The aqueous solution was then made alkaline with dilute sodium hydroxide and extracted with diethyl ether. The solvent was then evaporated leaving oil of crude MDMA.



### 6.3.2.3. The bromopropane route [55, 67, 182]

This is a two step procedure including first reacting safrole with hydrobromic acid to give 3,4-methylenedioxyphenyl-2-bromopropane, and then taking this material and reacting it with methylamine to yield MDMA (Figure 6.7). This procedure has the advantages of not being at all sensitive to batch size, nor is it likely to "run away" and produce a tarry mess. It has the advantage of using cheap, simple, and easily available chemicals. The sole disadvantage of this method is the need to do the final reaction with methylamine inside a sealed pipe. This is because the reaction must be done in the temperature range of 120-140 C, and the only way to reach this temperature is to seal the reactants up inside of a bomb. This is not particularly dangerous, and is quite safe if some simple precautions are taken. The first stage of the conversion, the reaction with hydrobromic acid, is quite simple, and usually produces high yield of the brominated product. Figure 6.7 illustrates the reaction process for the bromopropane route.

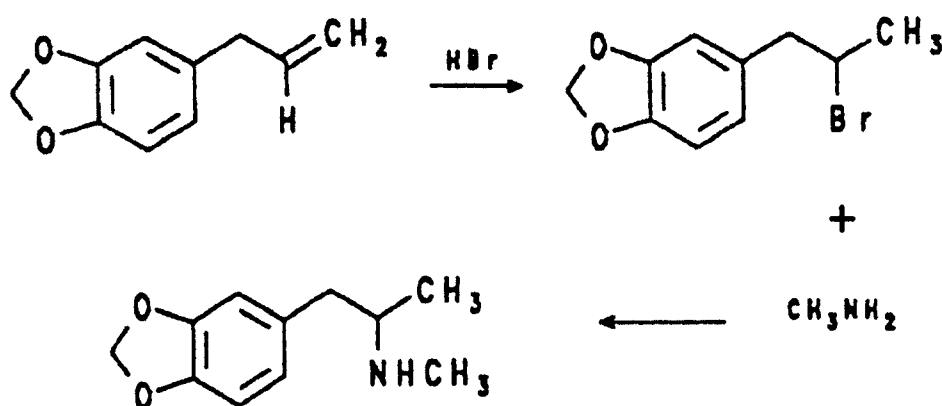


Figure 6.7. The bromopropane route

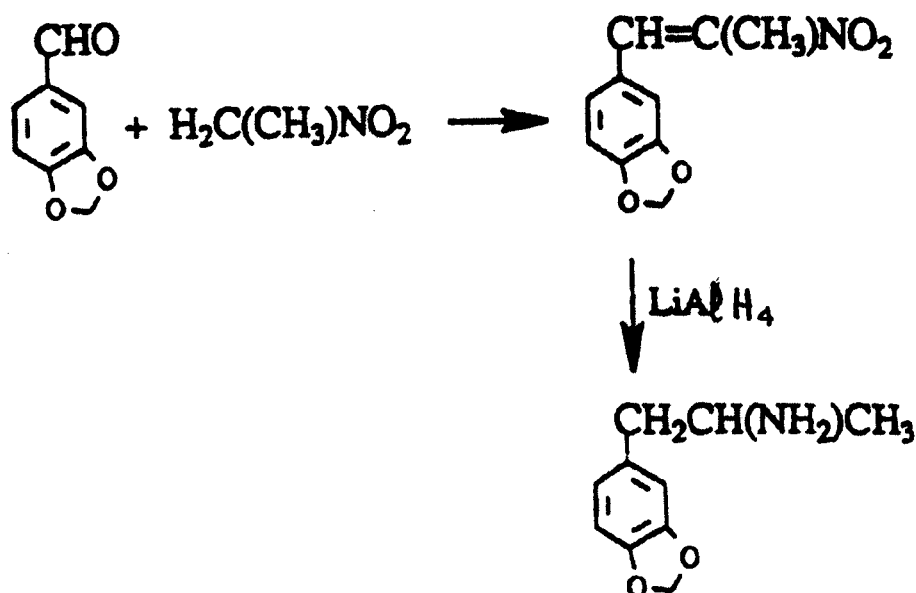
**Procedure:**

Sample of safrole (5 g) was added to 25 ml of 48% hydrobromic acid and were stirred at room temperature for 7 days. The reactions were then quenched with the addition of crushed ice (25 ml) and extracted with ether (2x40 ml). The ether extracts were evaporated to dryness under reduced pressure producing oil of bromoisosafrole.

This product was dissolved in 150 ml of methanol containing 40% aqueous methylamine (40 ml) and stirred at room temperature for 4 days. The reaction mixture was then evaporated to dryness and the resultant oil dissolved in 10 % HCl (50 ml). The aqueous acidic solution was washed with two portions of (40 ml) ether and then made basic (pH 12) by the addition of NaOH. The aqueous base solution was extracted with two portions of (50 ml) ether and the combined ether extracts evaporated to dryness under reduced pressure resulting in MDMA oil.

#### 6.3.2.4. The nitropropene route

A substantial amount of literature has been devoted to the synthesis and reduction of nitro alkanes [185-189] (Figure 6.8 ).



Nitropropene route

Figure 6.8. Diagram of the nitropropene route

**Procedure:**

Nitroethane (7 ml) followed by 7 g cyclohexylamine were added to a solution of 7 g piperonal in 40 ml glacial acetic acid. The mixture was held at steam-bath temperature for 6 h, and cooled overnight at 10°C. Bright yellow crystals were formed. They were removed by filtration, recrystallised from acetic acid and then oven dried to yield 5 g of 1-(3,4-methylenedioxyphenyl)-2-nitropropene. This product (3 g) was then added, dropwise, in tetrahydrofuran, solution (2 g lithium aluminium hydride in 40 ml anhydrous tetrahydrofuran) and the reaction mixture was maintained at reflux for 36 h. After being brought back to room temperature, the excess hydride was destroyed with 15 ml IPA, followed by 15 ml of 15 % NaOH. Additional water was added to complete the conversion of the aluminium salts to a loose, white solid. This was removed by filtration, and washed with additional tetrahydrofuran. The combined filtrate and washes were stripped of solvent under vacuum, and the residue dissolved in dilute sulphuric acid. Washing with 3x 75 ml dichloromethane removed much of the colour, and the aqueous phase was made basic and re-extracted with 3x75 ml dichloromethane. Removal of the solvent yielded 2.1 g of amber oil. It was then dissolved in 60 ml of IPA, neutralised with concentrated HCl, and diluted with 120 ml of anhydrous Et<sub>2</sub>O. Crystal which formed spontaneously were removed by filtration, washed with Et<sub>2</sub>O and oven dried to yield MDA.

**6.3.3. Extraction of intermediates and reaction by-products**

Solid Phase Extraction was the method of choice for extracting intermediates and reaction by-products from the finished products. This procedure was used to extract and concentrate intermediates and reaction by-products for their analysis by gas chromatography-mass spectrometry [148].

About 50 mg of synthesised MDMA were added to 1 ml phosphate buffer at pH 9. The suspension was then mixed on a rolling extractor for 30 min., centrifuged for 5 min. and the supernatant was taken off. A period of 30 min. was required for complete equilibrium to be achieved. SPE procedures were carried out using Varian Bond Elut C18 (200 mg) columns. The SPE column was conditioned with 10 ml of deionised water (to remove water-soluble impurities due to manufacture of the cartridge), 10 ml methanol (to solvate the column and remove other impurities), and 10 ml deionised water (to remove excess solvation solvent). The sample was then applied to the column at 1-2 ml/min. The column was then washed with 10 ml deionised water (to remove sugars and other unwanted water-soluble compounds) and dried under maximum vacuum (15 psi) for 5 min. The cartridge was subsequently eluted with 0.7 ml isopropanol (to remove any remaining water and recover neutral analytes retained only by lipophilic interaction with the sorbent surface) followed by 1 ml ethyl acetate with 2 % ammonia (to elute basic analytes retained by ion-exchange interactions). These two fractions were combined and evaporated in a nitrogen stream to about 100 µl. Care was taken not to evaporate to dryness to avoid evaporating the amphetamine impurities present in MDMA samples.

#### **6.3.4. Instrumental analysis**

##### ***6.3.4.1. Gas chromatography-mass spectrometry***

Gas chromatography was carried out using a Hewlett-Packard 5890 Series II instrument fitted with a mass spectrometry detector and equipped with a fused silica capillary column (HP5, 30 m x 0.32 mm i.d., 0.25 µm film thickness). Samples (volume 1 µl) were injected with an injection port temperature of 270°C at a linear velocity of 30 cm/sec. The column oven temperature was programmed from an

initial temperature of 80°C (1 min) at 35°C/min to 180°C (held for 18 min) then at 50°C/min to 300°C (held for 2 min). The mass spectrometer was a VG Analytical 70/250 S double focusing mass spectrometer operated at a resolution of 1000 and data was recorded by a Maspec 1 data system. The MS was used in the EI mode with electron energy of 70 eV, and was tuned daily using PFK according to the manufacturer's recommendations. Mass spectra were obtained by scanning from 40 to 600 amu at 0.3 s/scan.

## 6.4. RESULTS AND DISCUSSION

All syntheses were performed in such a manner that would be as close as possible to the clandestine way of manufacturing MDMA. The process of the synthesis was controlled at each stage to allow for retrieving a sample representing that particular step. Each sample was then analysed separately on GC-MS and spectra were collected for most intermediates, side reaction products and other impurities originating from precursors and other chemicals used in the synthesis. Final products were analysed on GC-MS after solid phase extraction to extract most of the MDMA out and concentrate impurities to allow for better detection and identification.

### 6.4.1. Reductive amination route

#### 6.4.1.1. Amalgamation scheme

Figures 6.9a and 6.9b show chromatograms of the final products (MDMA and by-products) before and after solid phase extraction of the powder product respectively. The unextracted sample was dissolved in methanol prior to analysis on GC-MS.

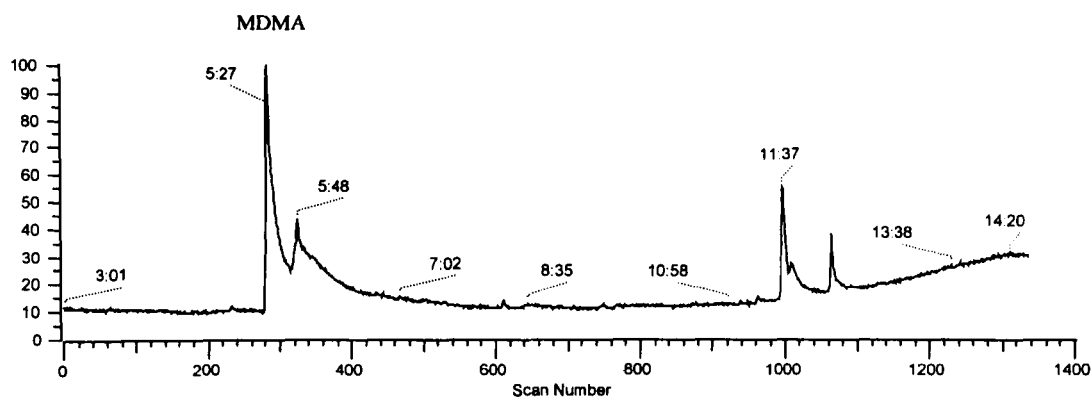


Figure 6.9a Total ion chromatogram of unextracted MDMA prepared via amalgamation.

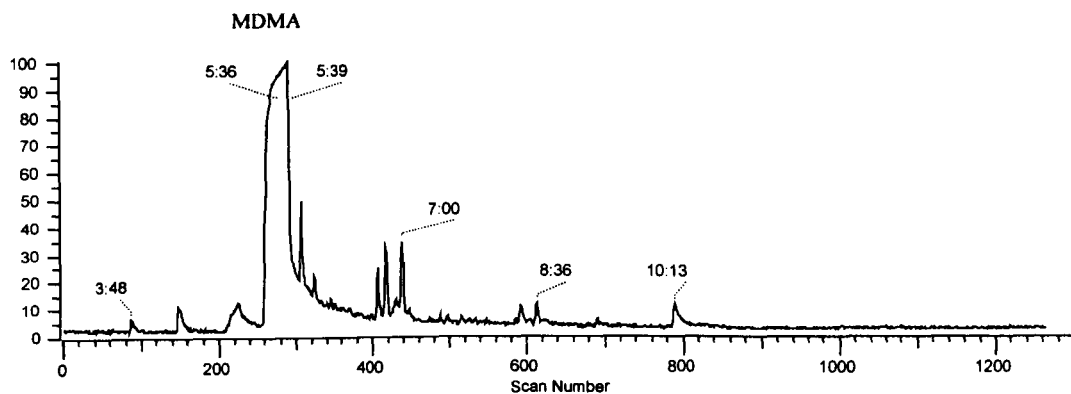


Figure 6.9b Total ion chromatogram of MDMA prepared via amalgamation after SPE.

Since this route yields the final product (MDMA) in a single step when PMK was used as the precursor, no intermediates were retrieved from this process. However, the final product had many impurities present as seen in Table 6.1.

No.	Name	Retention Indices
1	Piperonal	1293
2	Safrole	1350
3	PMK	1517
4	MDMA	1548
5	DMMDA	1593
6	N-Ethyl,N-methyl-[1,2-(methylenedioxy)-4-(2-aminopropyl)]benzene	1980

Table 6.1. Impurities found in MDMA synthesis via amalgamation process.

#### 6.4.1.2. Cyanoborohydride scheme

This route also produced the final product (MDMA) in a single step after using PMK as the precursor. Again no intermediates were retrieved from this process. However, the final product had many impurities present as seen in Table 6.2.

No.	Name	Retention Indices
1	Piperonal	1293
2	Safrole	1350
3	PMK	1517
4	MDMA	1548
5	N,N-Dimethyl-3,4-methylenedioxy-amphetamine, DMMDA	1593

Table 6.2. Impurities found in MDMA synthesis via cyanoborohydride route.

Figures 10a illustrates a chromatogram of MDMA synthesised via the cyanoborohydride route. The sample was not subjected to any extraction procedure



prior to analysis on GC-MS. In Figure 6.10 b a chromatogram is shown for the same sample after it was subjected to a solid phase extraction procedure to concentrate the impurities present in the sample.

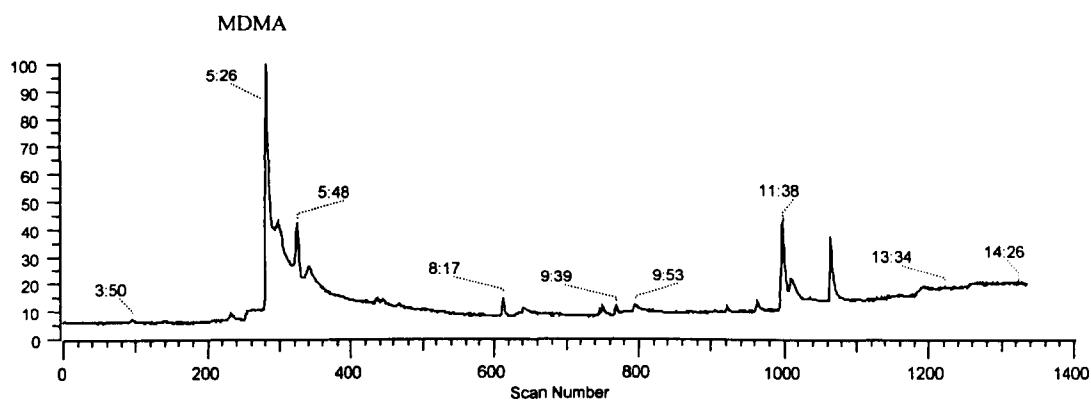


Figure 6.10a. Total ion chromatogram of unextracted MDMA prepared via cyanoborohydride route.

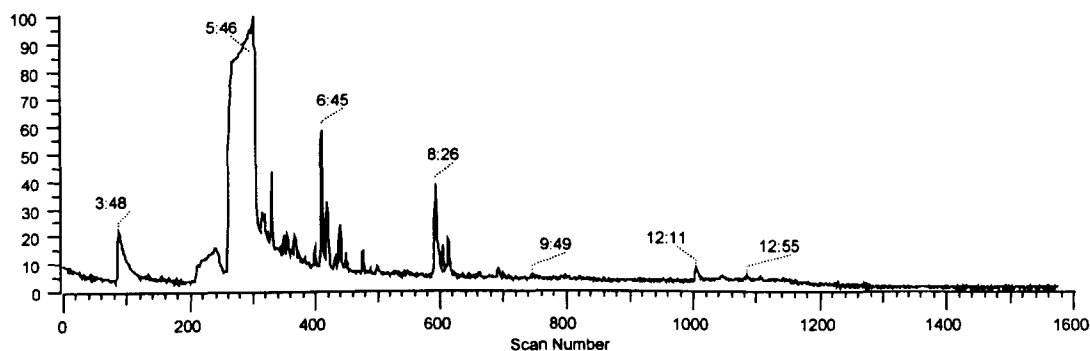


Figure 6.10b. Total ion chromatogram of MDMA prepared via cyanoborohydride route after it was subjected to SPE.

#### 6.4.2. Leuckart reaction

This route of synthesising MDMA was more problematic and difficult to perform than the reductive amination routes, the amalgamation and the cyanoborohydride.

The synthesis was performed in the first trial using 1 mg of the precursor, 3,4-

methylenedioxy phenyl-2-propanone (PMK) which was not successful in yielding MDMA as the final product. Then a larger amount (5mg) of the ketone was used which allowed for the synthesis to proceed to the final product, MDMA.

6.4.2.1. Route 1

This route did not yield MDMA even after using 5mg of the ketone as the starting precursor. However the first phase of the process was successful and produced the intermediate N-formyl MDMA - a route specific by-product- which can be used as a reference when identifying synthetic routes of seized street MDMA or ecstasy. Figure 6.11 presents a total ion chromatogram of the intermediate and Table 6.3 provides retention indices and for the intermediate and other impurities present.

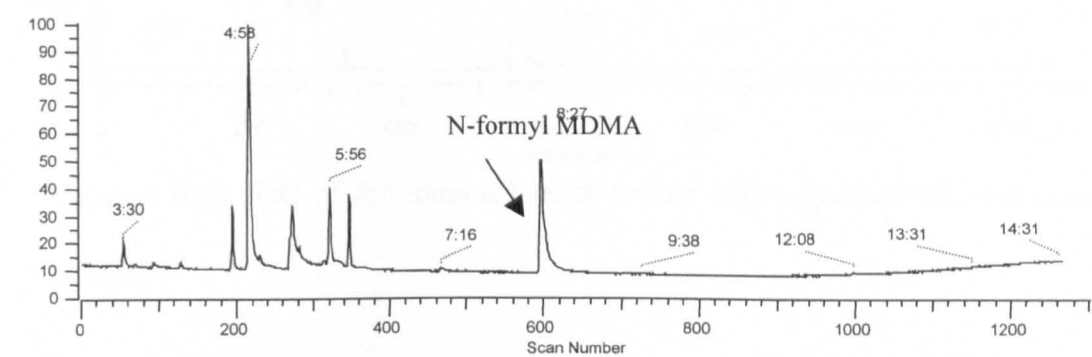


Figure 6.11. TIC of the intermediate N-formyl MDMA as generated via route 1

No.	Name	Retention Indices
1	Piperonal	1293
2	safrole	1350
3	PMK	1517
4	N-formyl MDMA	1983

Table 6.3. Impurities detected in the first reflux of Leuckart reaction-route 1.

#### 6.4.2.2. Route 2

The synthesis of MDMA via the Leuckart reaction, route 2, was more successful than route 1. MDMA was produced in the final stage with other by-products present also. Figure 6.12 illustrates a total ion chromatogram of stage one of route 2. In this chromatogram the main product was the intermediate N-formyl MDA which is a synthetic route specific impurity in street ecstasy samples manufactured via route 2 of the Leuckart reaction, in addition to a few other impurities.

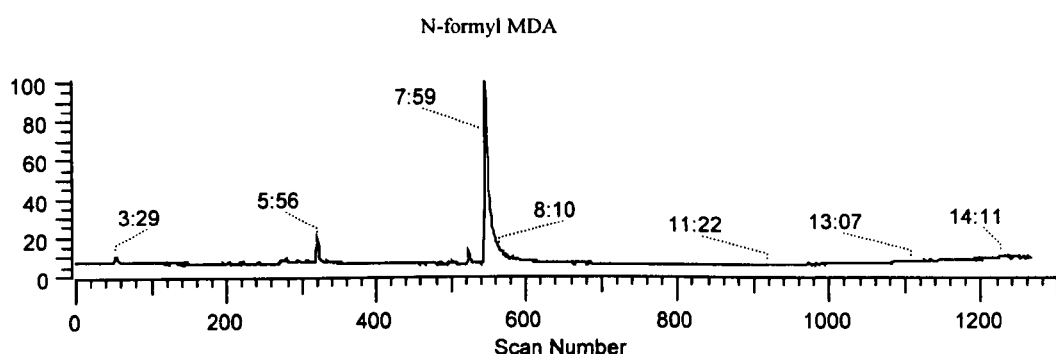


Figure 6.12. TIC of the intermediate N-formyl MDA synthesised with Leuckart reaction.

In Figure 6.13 the total ion chromatogram is shown for MDMA and other minor impurities in the final product of route 2.

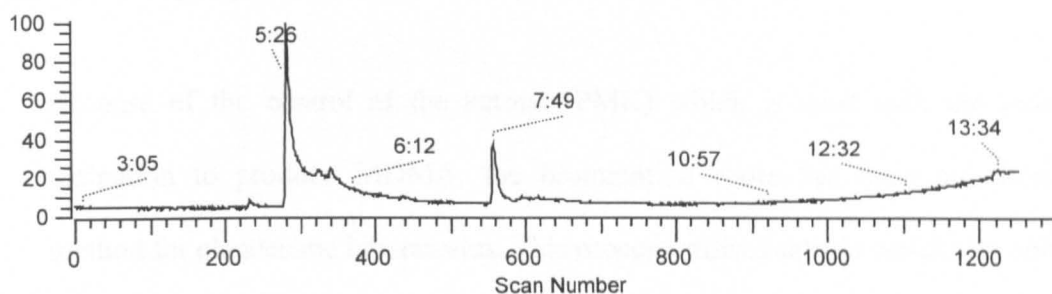


Figure 6.13. TIC of MDMA and other impurities in a product from route 2 reaction.

A list of the impurities present in the final product of Leuckart reaction-route 2 is depicted in table 6.4 including the retention indices and compounds names.

No.	Name	Retention Indices
1	Safrole	1395
2	PMK	1517
3	MDMA	1548
4	Pyrimidine	1870
5	N-formyl MDA	1920

Table 6.4. Impurities in the final product of the Leuckart reaction-route 2

### 6.4.3. Bromopropane route

Because of the control of the ketone (PMK) which is used with the reductive amination to produce MDMA, the bromination route has been an alternative method for clandestine laboratories. This process utilises safrole which can either be purchased commercially or in case where it is controlled, can be extracted from sassafras plant. The bromopropane route is a straitforward simple reaction which circumvent the need for controlled precursor chemicals. In our synthesis using this method, MDMA oil was obtained plus some other impurities illustrated in figures 6.14 and 6.15 and listed with other detailed information such as retention indices in table 6.5.

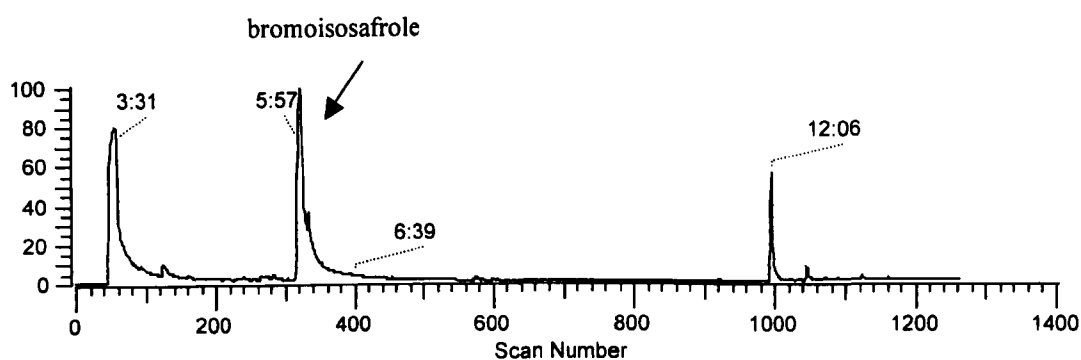


Figure 6.14. TIC of the first reflux of the bromination route.

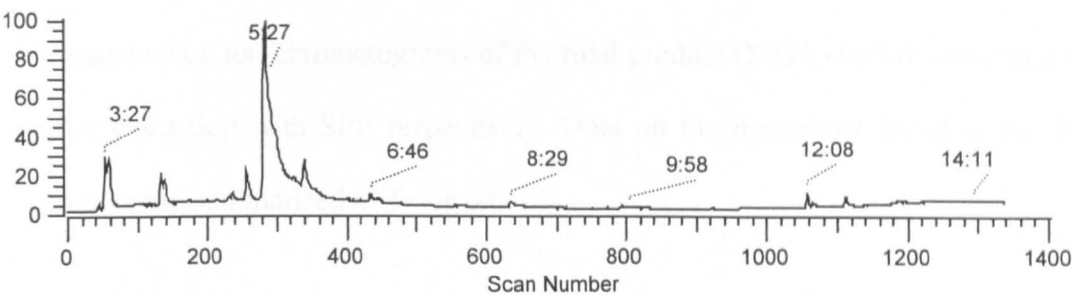


Figure 6.15. TIC of MDMA synthesised with the bromination route

No.	Name	Retention Indices
1	Safrole	1395
2	MDMA	1548
3	3,4-methylenedioxyphenylbromopropene, MDPBP	1655

Table 6.5. Impurities found in MDMA synthesised with bromination route.

6.4.4. Nitropropene route

This route yields only MDA which, can be converted with further reactions to produce MDMA. Because of this fact this route is not favourable to clandestine laboratories from economic and practicality points of view. However, for our purpose we performed the synthesis up to the MDA stage, which enabled us to obtain data on impurities normally present in such preparations. The synthesis was done in two steps; the first was to maintain the intermediate nitropropene which is a route specific impurity in street ecstasy manufactured with the nitropropene route. The second step was to produce the final product, MDA. Figure 6.16 shows a

chromatogram of the first step product (intermediate) while Figures 6.17a and 6.17b illustrate total ion chromatograms of the final product (MDA) before extraction and after extraction with SPE respectively. Data on the impurities found in the final product are summarised in Table 6.6.

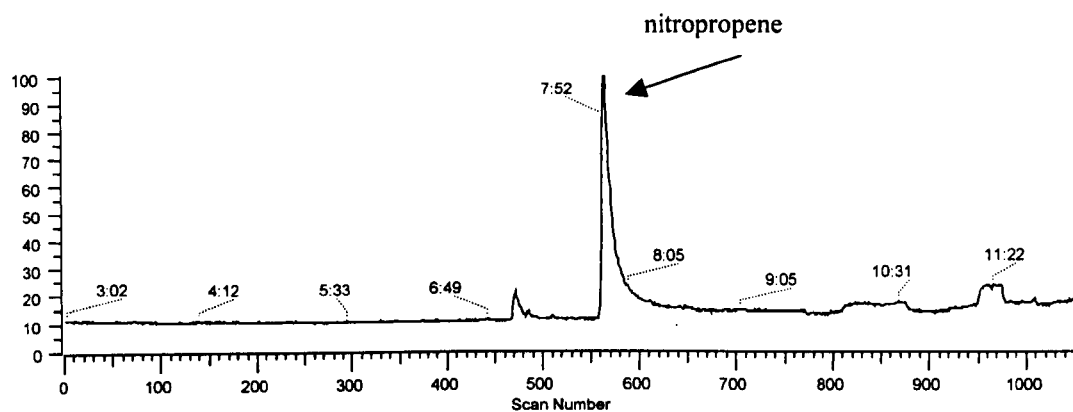


Figure 6.16. Chromatogram of the first step product which includes the intermediate 1-(3,4-methylenedioxyphenyl)-2-nitro-1-propene.

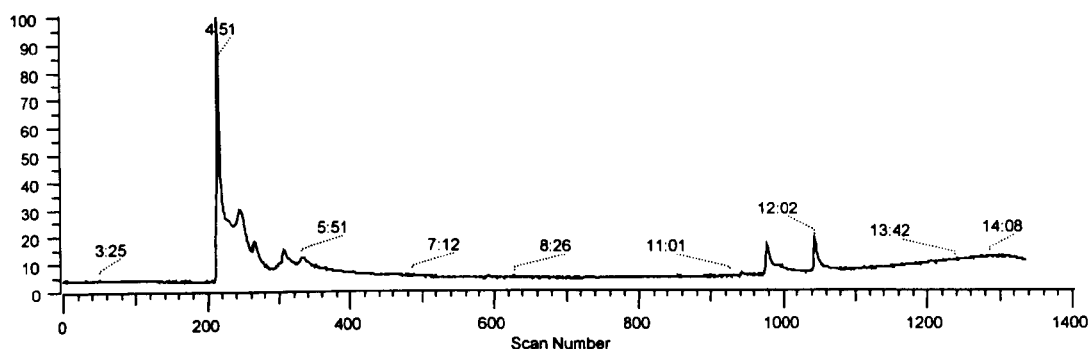


Figure 6.17a. TIC of the final product (MDA) before extraction.

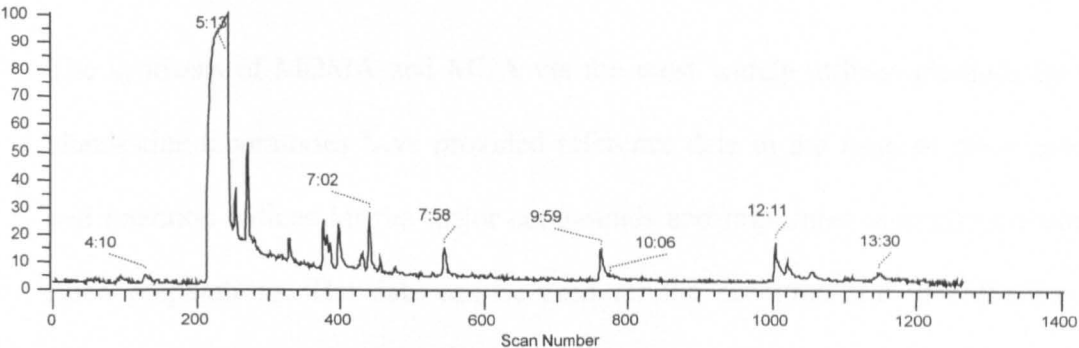


Figure 6.17a. TIC of the final product (MDA) after extraction

No.	Name	Retention Indices
1	Piperonal	1293
2	MDA	5.13
3	1-[3,4-Methylenedioxyphenyl]-2-nitro-1-propene	1875
4	N-β-3,4-methylenedioxyphenylisopropyl-3,4-methylenedioxybenzaldimine	2539

Table 6.7. Impurities found in MDA synthesised with the nitropropene route.

6.5. CONCLUSION

Synthesis of MDMA and MDA using different synthetic routes such as the reductive amination (the amalgamation and the cyanoborohydride schemes), the Leuckart reaction using two routes, the bromination route, and the nitropropene



route was performed. It was possible to obtain authentic samples of compounds found in illicit preparations including impurities.

The synthesis of MDMA and MDA via the most widely utilised methods by the clandestine laboratories have provided reference data in the form of mass spectra and retention indices for the major compounds and impurities normally present in illicit preparations. The data can be used for the detection and identification of impurities in illicit samples and allow for drawing conclusions on the synthetic routes for these samples and perhaps identify new impurities not encountered before.

Theoretical data in the form of mass spectrum or retention indices are useful. However, each analytical instrument has its own identity and therefore the results obtained can vary considerably in certain cases. From this fact it is recommended that an in-house synthesis of illicit drugs is vital and provides original samples and data using the same analytical conditions such as extraction methods, chemicals and solvents, and instrumentation.

There are some impurities that can be considered as route specific and their identification provides information as to the method of synthesis used by the clandestine laboratories for the preparation of illicit drugs. Mass spectra of some intermediates and other route specific impurities are seen in Figures 6.18a to 6.18f.

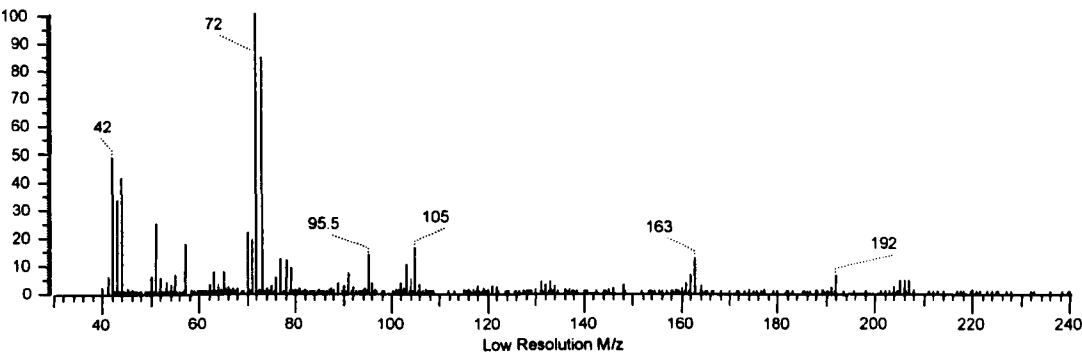


Figure 6.18a. Mass spectrum of DMMDA, a reductive amination route specific impurity.

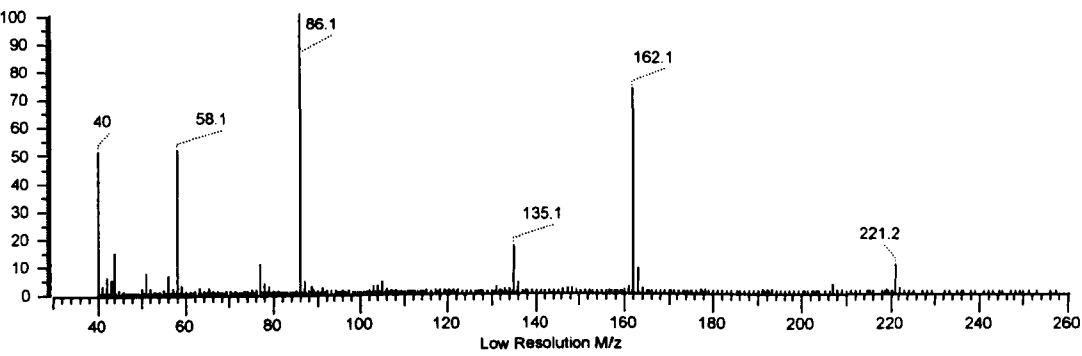


Figure 6.18b. Mass spectrum of the Leuckart intermediate N-formyl MDMA.

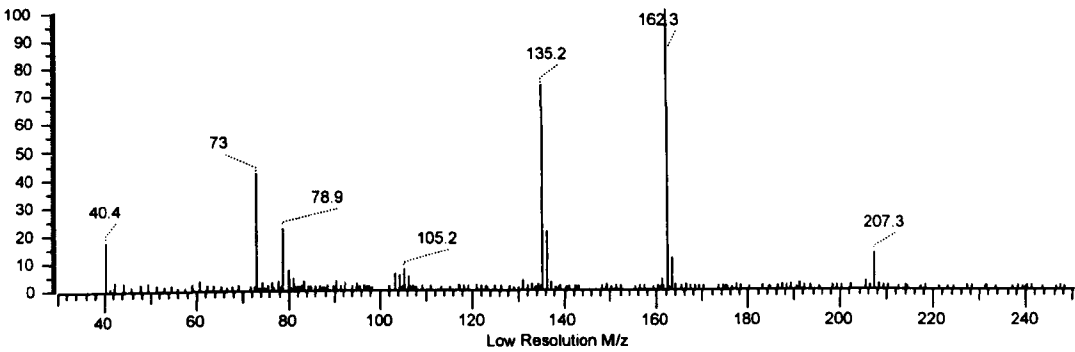


Figure 6.18c. Mass spectrum of the Leuckar intermediate N-formyl MDA.

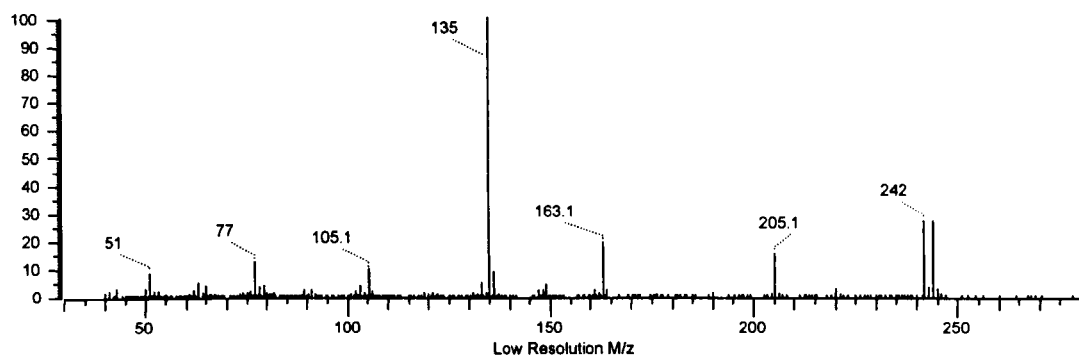


Figure 6.18d. Mass spectrum of 3,4-methylenedioxyphenyl-2-bromopropane (MDPBP), an intermediate of the bromination route.

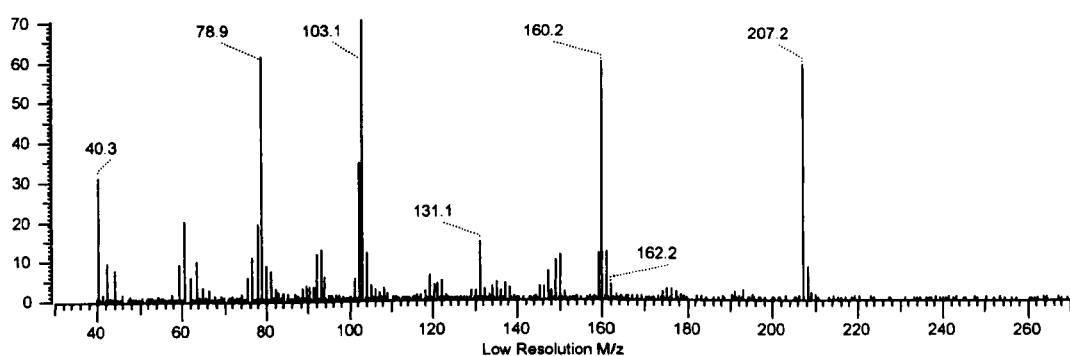


Figure 6.18e. Mass spectrum of 1-(3,4-methylenedioxyphenyl)-2-nitro-1-propene, an intermediate of the nitropropene route.

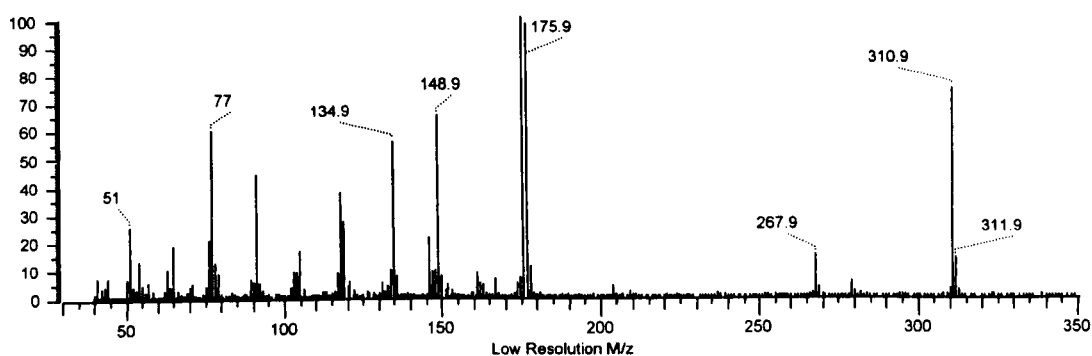


Figure 6.18f. Mass spectrum of N-β-3,4-methylenedioxyphenylisopropyl -3,4-methylenedioxybenzaldimine

## 7. IMPURITIES IN SEIZED ECSTASY TABLETS

### 7.1. INTRODUCTION

Determination and quantification of impurities or minor components present in seized drugs provide vital information. These impurities originate from precursors, natural raw materials, solvents used in the synthesis, side-reaction and intermediates. They should be distinguished from diluents and adulterants which have been added deliberately. Certain impurities identified in illicit drugs are route specific impurities (e.g. intermediates).

The type of information provided through the identification of impurities include, determination of the synthesis route [51], identification of precursor chemicals used [56], and determination of compounds which may represent public health risks [5].

Clandestine production of 3,4-methylenedioxymethamphetamine, MDMA was discussed in detail [51, 56] and many different synthetic routes such as the reductive amination route, the Leuckart method, the bromopropane route, the Ritter reaction, the nitropropene and the cinnamic acid routes were reviewed. Safrole, isosafrole, piperonal, and 3,4-methylenedioxy phenyl-2-propanone are the main precursors used. MDMA is almost always illicitly manufactured and because of the lack of quality control in clandestine laboratories, impurities arising from precursor chemicals, intermediates, and other impurities are also produced and will be present in the final product. In a review [51] mass spectral data of impurities in MDMA samples synthesised by the reductive amination route, the Leuckart reaction, the nitropropene route, and the bromopropane route were reported. Impurities in illicit MDMA samples were also isolated by TLC and detected by mass spectrometry and

<sup>1</sup>H-NMR [54]. Renton et al [52] provided data for precursor intermediates, and reaction by-products in illicit MDMA. They used these results for establishing the route of synthesis in actual casework. Verweij reported [51] structure elucidation for impurities in illicit MDMA prepared by the low-pressure reductive amination of 3,4-methylenedioxy phenyl propanone with methylamine. The presence of the intermediate imine (1,2-methylenedioxy-4-2-N-methyimino propyl benzene) provided information about the synthetic route.

In this chapter confiscated ecstasy tablets containing MDMA as the principal component were extracted using an optimised, solid phase extraction procedure. In this procedure extraction and concentration of impurities in ecstasy tablets were done to allow for their detection and identification by gas chromatography-mass spectrometry.

## **7.2. EXPERIMENTAL**

### **7.2.1. Chemicals and reagents**

Samples of ecstasy tablets (12 samples) used in the study were provided by the Forensic Science Services, Metropolitan Laboratory, London. All reagents used were of analytical or HPLC grade. Solid phase extraction columns (Bond Elut®) were obtained from Varian Associates, Harbor City, CA, USA.

### **7.2.2. Extraction procedure**

Solid Phase Extraction was the method of choice for extracting intermediates and reaction by-products from the finished products. This procedure was used to extract

and concentrate intermediates and reaction by-products for their analysis by gas chromatography-mass spectrometry.

About 50 mg of ground and homogenised ecstasy powder were added to 1 ml phosphate buffer of pH 9. The suspension was then mixed on a rolling extractor for 30 min., centrifuged for 5 min. and the supernatant was taken off. A period of 30 min. was required for complete equilibrium to be achieved. SPE procedures were carried out using Varian Bond Elut C18 (200 mg) columns. The SPE column was conditioned with 10 ml of deionised water (to remove water-soluble impurities due to manufacture of the cartridge), 10 ml methanol (to solvate the column and remove other impurities), and 10 ml deionised water (to remove excess solvation solvent). The sample was then applied to the column at 1-2 ml/min. The column was then washed with 10 ml deionised water (to remove sugars and other unwanted water-soluble compounds) and dried under maximum vacuum (15 psi) for 5 min. The cartridge was subsequently eluted with 0.7 ml isopropanol (to remove any remaining water and recover neutral analytes retained only by lipophilic interaction with the sorbent surface) followed by 1 ml ethyl acetate with 2 % ammonia (to elute basic analytes retained by ion-exchange interactions). These two fractions were combined and evaporated in a nitrogen stream to about 100 µl. Care was taken not to evaporate to dryness to avoid evaporating the amphetamine impurities present in ecstasy samples.

### **7.2.3. Instrumental**

#### ***7.2.3.1. Gas chromatography-mass spectrometry***

Gas chromatography was carried out using a Hewlett-Packard 5890 Series II instrument fitted with a mass spectrometry detector and equipped with a fused silica

capillary column (HP5, 30 m x 0.32 mm i.d., 0.25  $\mu$ m film thickness). Samples (volume 1  $\mu$ l) were injected with an injection port temperature of 270°C at a linear velocity of 30 cm/sec. The column oven temperature was programmed from an initial temperature of 80°C (1 min) at 35°C/min to 180°C (held for 18 min) then at 50°C/min to 300°C (held for 2 min). The mass spectrometer was a VG Analytical 70/250 S double focusing mass spectrometer operated at a resolution of 1000 and data was recorded by a Maspec 1 data system. The MS was used in the EI mode with electron energy of 70 eV, and was tuned daily using PFK according to the manufacturer's recommendations. Mass spectra were obtained by scanning from 40 to 600 amu at 0.3 s/scan.

### 7.3. RESULTS AND DISCUSSION

In this chapter the objectives were to perform comparative analysis on street ecstasy tablets seized between 1995 and 1996. Two different approaches were performed to establish a comparative study on the illicit ecstasy samples. The first approach was to identify the major impurities present in the impurity profiles obtained for the samples after subjecting them to solid phase extraction and instrumental analysis using gas chromatography mass spectrometry. Identification of the main impurities allowed for elaborating on the synthetic routes of these samples. The second approach was to use a comparison procedure based on visual comparison of impurity profiles to cluster the samples into smaller groups and relate those with similar impurity profiles to common batches.

Pure standards of MDMA, MDEA, safrole, piperonal, and 3,4-methylenedioxy phenyl-2-propanone (PMK) were analysed on GC-MS and their data including

retention indices and mass spectra were used for identification of impurities in street samples. Figures 7.1a to 7.1e illustrate the mass spectra for these standards.

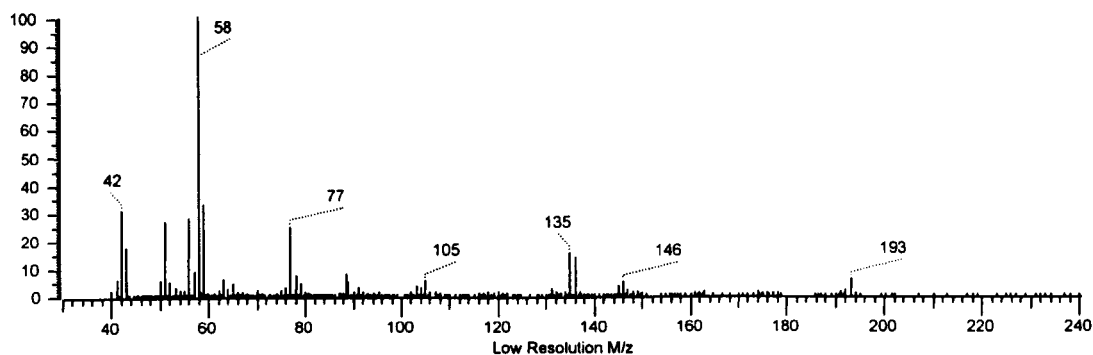


Figure 7.1a. Mass spectrum of MDMA.

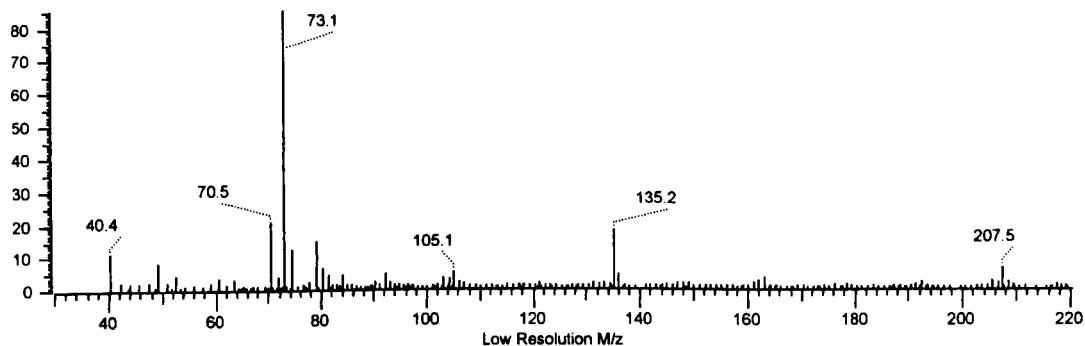


Figure 7.1b. Mass spectrum of MDEA.

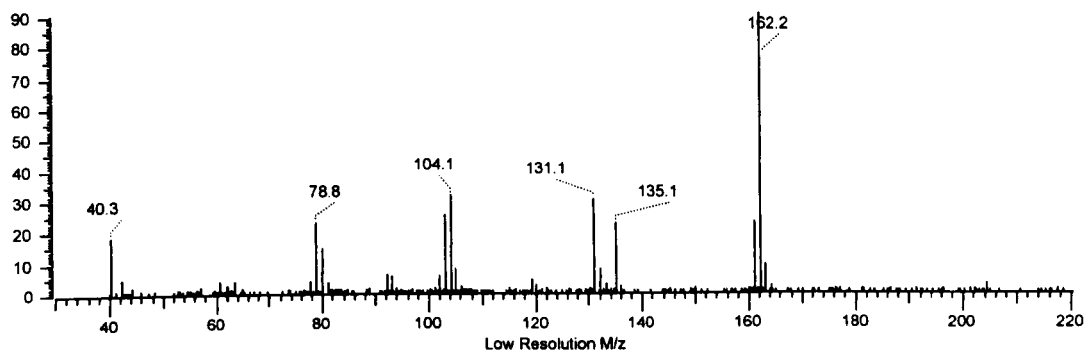


Figure 7.1c. Mass spectrum of safrole.



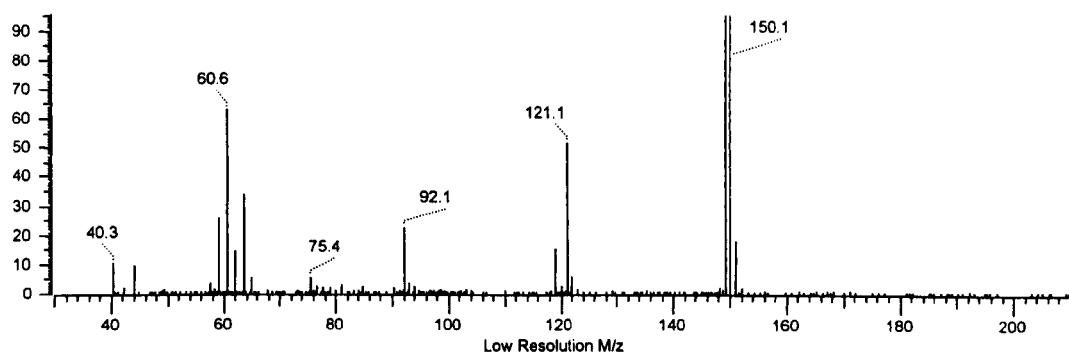


Figure 7.1d. Mass spectrum of piperonal.

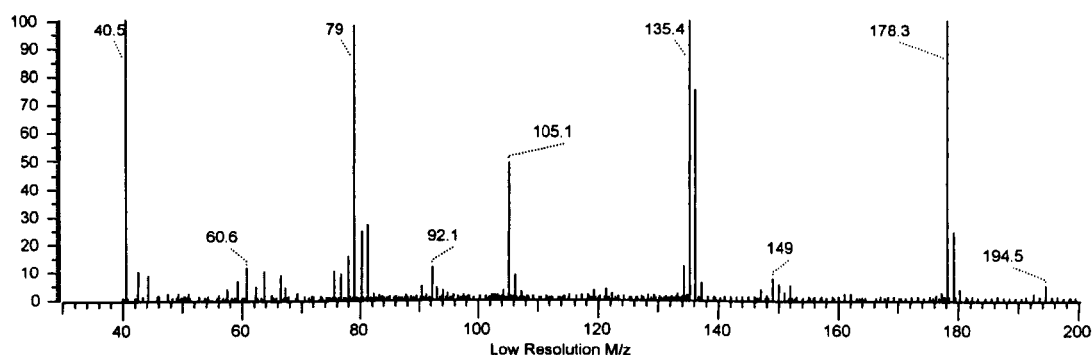


Figure 7.1e. Mass spectrum of PMK.

Impurity profiles are often observed in illicitly prepared drug samples, as they are not usually purified to any great degree after manufacture. In the case of illicitly prepared amphetamine, the presence of “route specific” impurities is used to establish the manufacturing processes [47]. Applying this analogy to MDMA, the identification of the intermediate N-formyl MDMA revealed that the Leuckart reaction (route 1) was used in the manufacture of samples 1, 2, 7, 8, 9, 10, 11, and 12. Samples 3, 4, 5, and 6 synthesis route’s were unidentified. The major impurities present in samples 1, 2, 7, 8, 9, 10, 11, and 12 were identified and their data are summarised in Tables 7.1.

	Compound 1	Compound 2	Compound 3	Compound 4
Retention time	3.49	5.04	5.28	8.28
Retention Index	1293	1517	1548	1983
Name	Piperonal	PMK	MDMA	N-formyl MDMA
Synthesis route: The Leuckart reaction				

Table 7.1. Data of compounds in sample 1

Impurity profiles of the analysed street ecstasy samples (Figures 7.2a to 7.2L) were used for the visual comparison of the analysed samples.

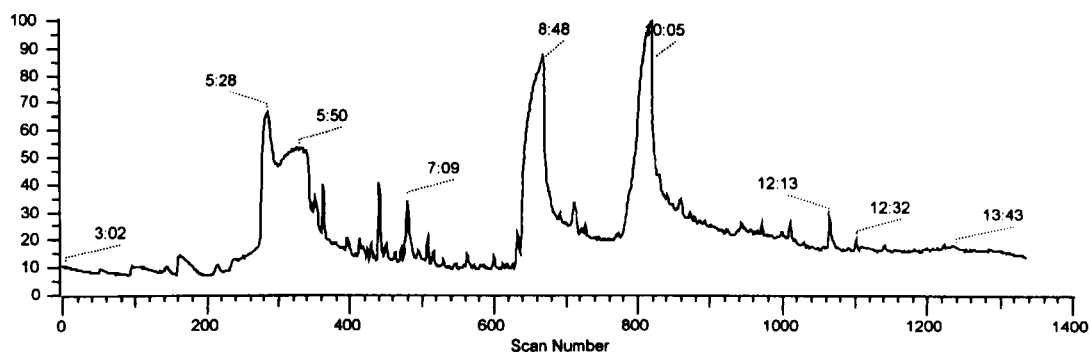


Figure 7.2a. Impurity profile of sample 1

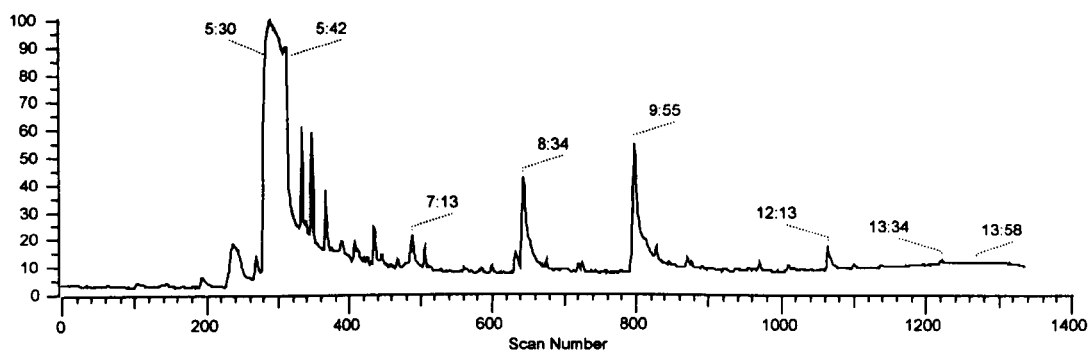


Figure 7.2b. Impurity profile of sample 2

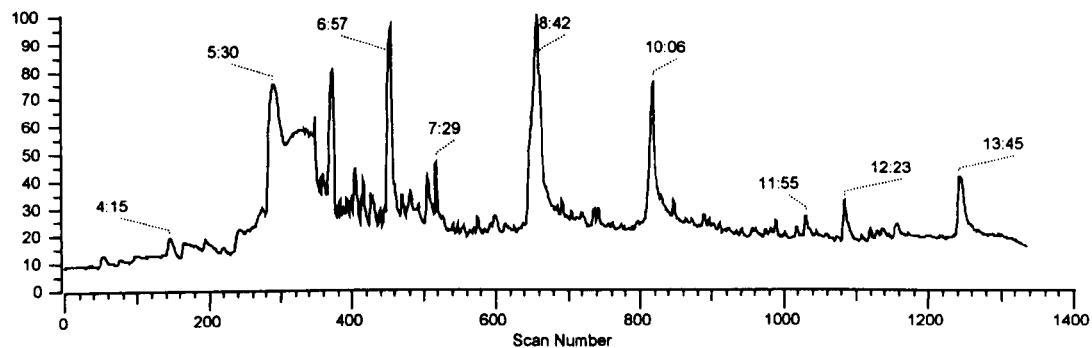


Figure 7.2c. Impurity profile of sample 3

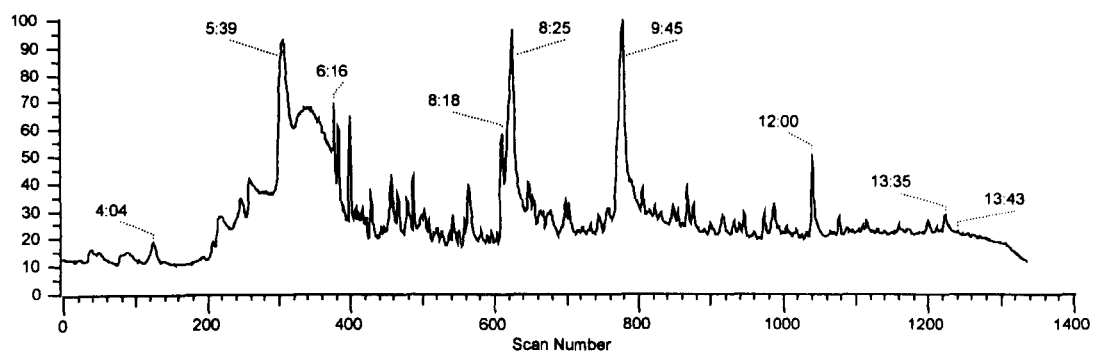


Figure 7.2d. Impurity profile of sample 4

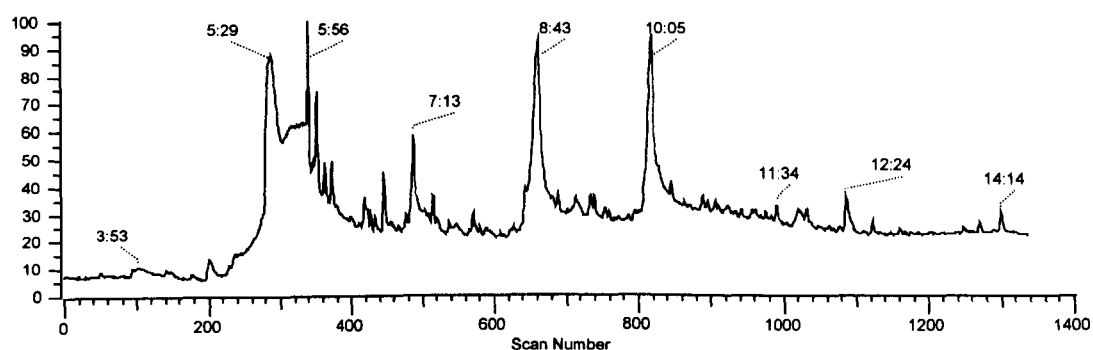


Figure 7.2e. Impurity profile of sample 5

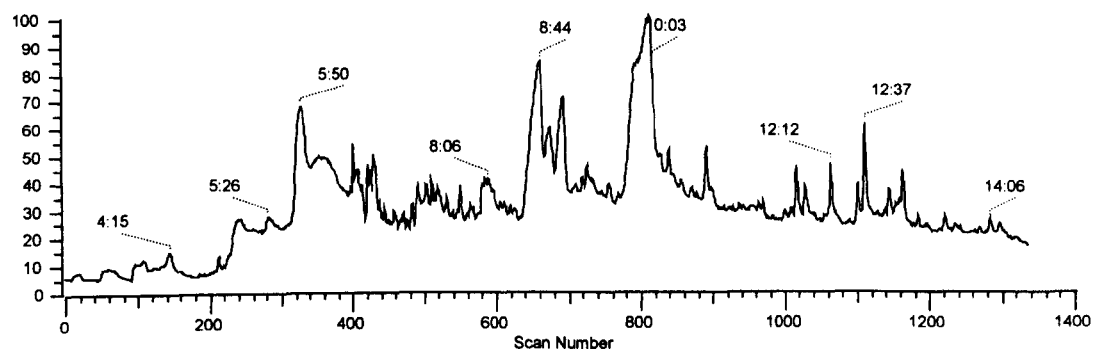


Figure 7.2f. Impurity profile of sample 6

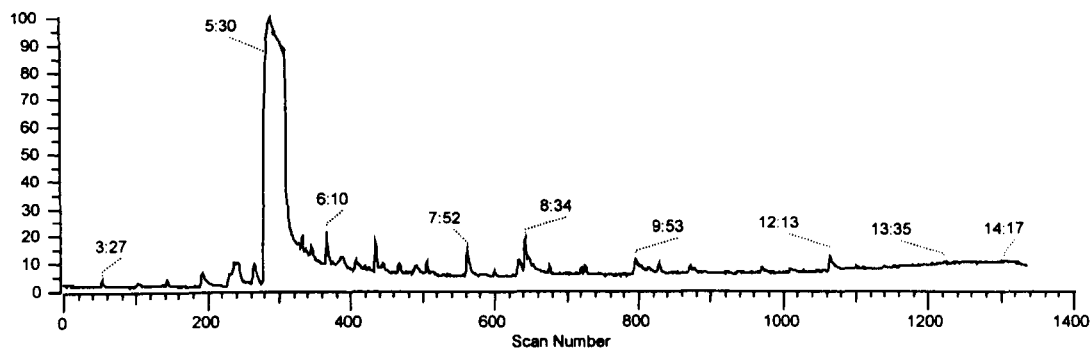


Figure 7.2g. Impurity profile of sample 7

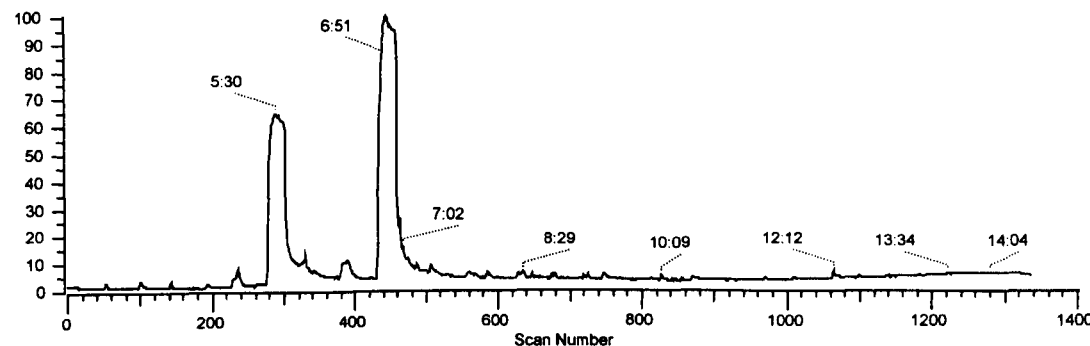


Figure 7.2h. Impurity profile of sample 8

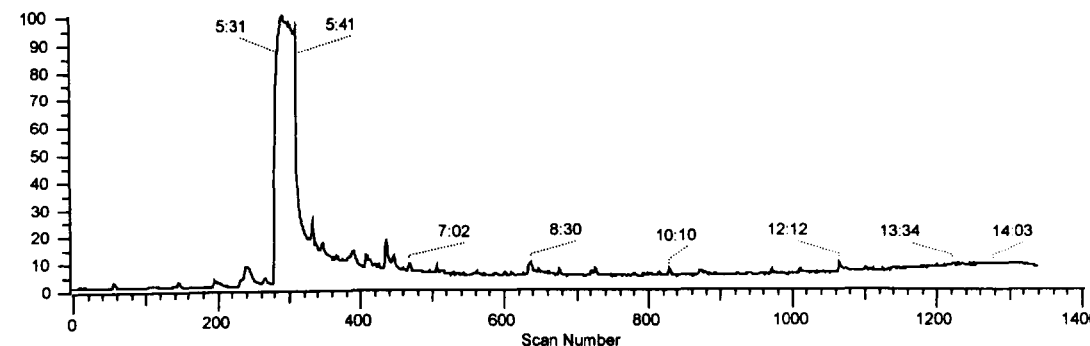


Figure 7.2i. Impurity profile of sample 9

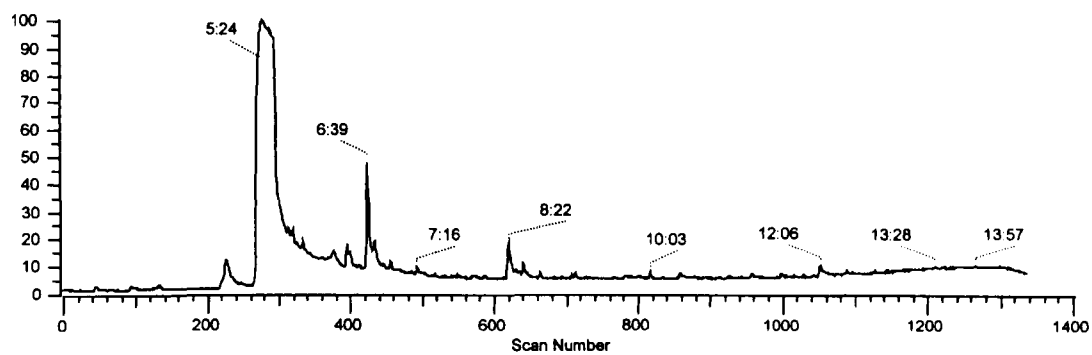


Figure 7.2j. Impurity profile of sample 10

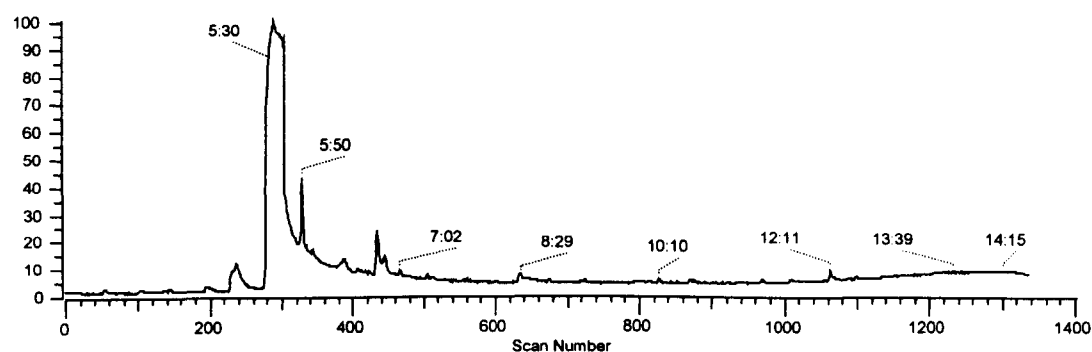


Figure 7.2k. Impurity profile of sample 11

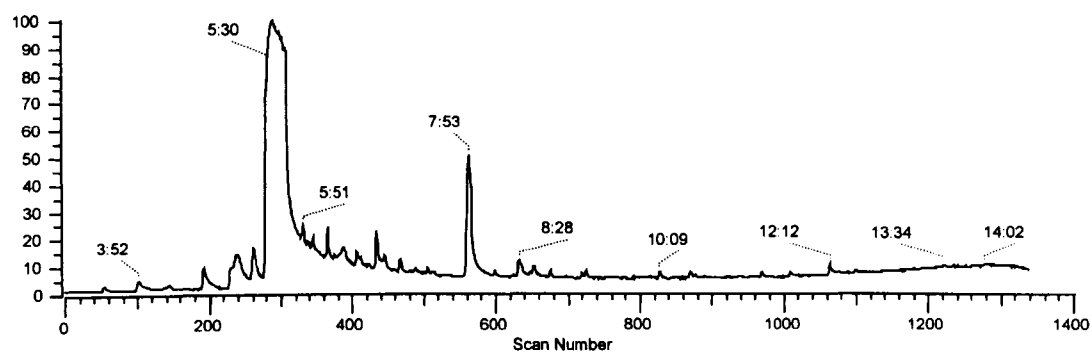


Figure 7.2L. Impurity profile of sample 12

The analysed samples were compared visually. Out of the 12 samples analysed, 7 samples were clustered into 3 different groups while the other 5 samples showed no similarity with other samples. Figures 7.3, 7.4, and 7.5 illustrate overlays of similar impurity profiles. This form of comparison of impurity profiles helps group samples together and with the aid of other analytical procedures such as physical analysis and other organic methods can provide useful information for drugs intelligence.

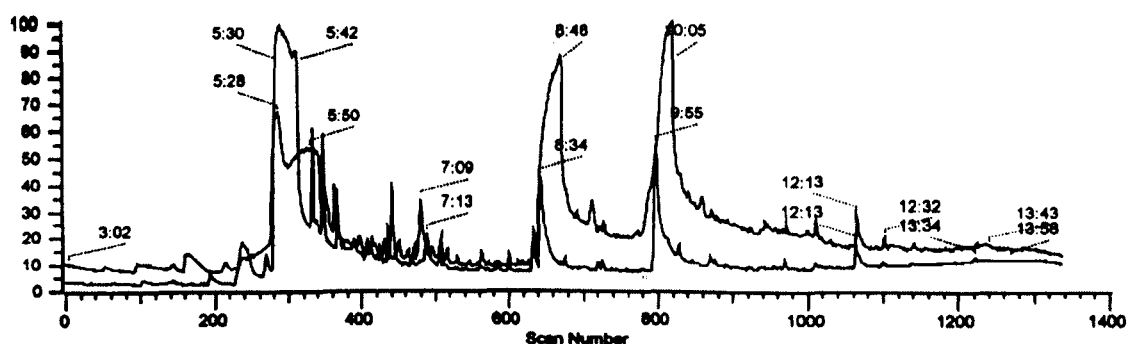


Figure 7.3. An overlay of impurity profiles of samples 1 and 2

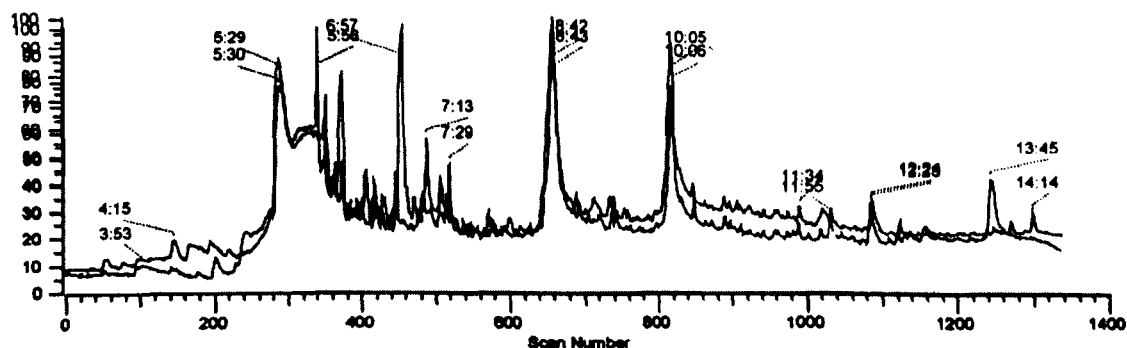


Figure 7.4. An overlay of impurity profiles of samples 3 and 5

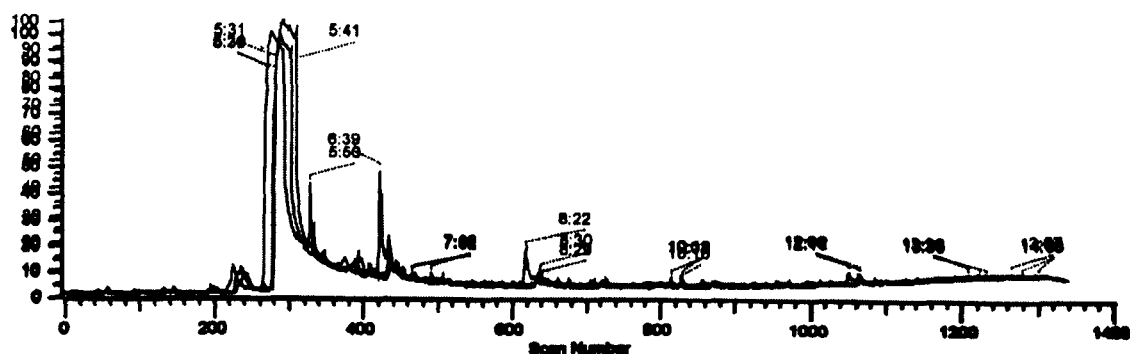


Figure 7.5. An overlay of impurity profiles of samples 9, 10 and 11

## 7.4. CONCLUSION

In this chapter 12 different ecstasy samples seized during the years 1995 and 1996 within the UK were subjected to comparative analysis through the use of solid phase extraction and gas chromatography-mass spectrometry. The chemical analysis included detection and identification of key impurities (route specific) which allowed for determination of the synthesis route for seven samples. Comparison of the impurity profiles of the analysed samples was also performed for the purpose of relating these samples to common source or batch. Visual comparison of the impurity profiles gave an indication of possible similarity of samples 1 & 2, 3 & 5, and 9, 10, & 11. Further analysis of the samples using other analytical tools may reveal more information that can help in drawing conclusions regarding the source of these samples.



## 8. CONCLUSIONS AND FUTURE WORK

### 8.1 WHAT HAS BEEN ACHIEVED?

This project has been on the subject of drug profiling with focus on the ring-substituted amphetamine; MDMA or ecstasy as it is widely called. Among the main objectives of this study was the development and optimisation of a new extraction procedure such as solid phase extraction, for impurities in seized ecstasy tablets. The instrumental analysis of impurities found in ecstasy tablets usually requires a preliminary extraction process to extract, isolate, and concentrate these impurities from the total tablet content. In the process, interfering materials are removed, and the required substances are concentrated into a solvent that is suitable for introduction into the instrument. Although lengthy and time-consuming, these procedures are of paramount importance to remove the principal component(s), diluents and adulterants, and concentrate impurities.

An evaluation of a comparison procedure of LLE and SPE for extracting impurities in ecstasy tablets for profiling purposes was done. Solid phase extraction of impurities in ecstasy tablets proved to be more efficient than the traditional liquid-liquid extraction. SPE provided impurity peaks with higher intensities than did LLE and a shorter extraction time.

Other area of research was the use of infrared technology for profiling ecstasy tablets since only chromatographic methods have been studied. Infrared spectroscopy and in particular fourier transform infrared, FTIR, can serve as another tool for comparing drug seizures. Each exhibit has its own characteristic spectrum, and comparison between the different spectra gives a gross estimate of

the similarity or dissimilarity between the different exhibits. FTIR has some discriminating capability to classify powders into a large number of groups, and consequently can be used for gross comparison or as screening tool. Drug intelligence involves two main streams with regard to comparative analysis or drug profiling. The first includes court cases such as sample/sample comparison and relating user to a dealer to prove conspiracy. The second task for drug intelligence involves establishing relationships between seizures and providing information on the networking of samples, which may be of use in investigating major trafficking operations. In such operation the laboratory may have to analyse many samples. FTIR analysis can be utilised for such operation as an elimination tool for its sheer simplicity and short analysis time. The developed method allowed for a short analysis at under 1 minute for each sample.

The second stage of the experimental part of the thesis dealt with synthesis of MDMA to acquire authentic intermediates and other impurities usually encountered in illicitly produced samples. These impurities were analysed and their retention indices and mass spectra were recorded. Synthesis of MDMA and MDA using different synthetic routes such as the reductive amination (the amalgamation and the cyanoborohydride schemes), the Leuckart reaction using two routes, the bromination route, and the nitroprpene route was also performed.

Theoretical data in the form of mass spectra or retention indices are useful. However, each analytical instrument has its own identity and therefore the results obtained can vary considerably. The synthesis of MDMA and MDA via the most widely utilised methods by the clandestine laboratories have provided reference data in the form of mass spectra and retention indices for most of the major compounds and impurities which are normally present in illicit preparations. This

data was used for the detection and identification of impurities in case samples. There are some impurities that can be considered as route specific and their identification can provide information as to the method of synthesis used by the clandestine laboratories for the preparation of illicit drugs. Once data on the authentic sample were established, ecstasy tablets confiscated within the UK containing MDMA were analysed and identification of marker impurities was performed which allowed for the determination of the synthetic routes for some of these exhibits.

### **8.1.1. Does it work?**

It is generally accepted that drug profiling is a complex science [70] and that it requires a great deal of expertise and knowledge on the subject. However, it can be a successful procedure if all the required parameters are met. The basis of comparative analysis for strategic and tactical intelligence purposes is the comprehensive examination of the chemical and physical characteristics of a drug exhibit towards the establishment of the degree of commonality of origin with another specific sample or group of samples. Therefore, it deals with the development of analytical procedures allowing the collection of valuable data on every drug seizure so as to obtain an exhaustive description of it. For this purpose the profiling procedure should not rely on a single analytical technique and a database covering the main features (criteria for comparison) of the analysed exhibits should be considered.

### **8.1.2. Is it useful?**

Characterisation and classification of illicit drug samples is an important objective for drug intelligence, It establishes relationships between seizures and may provide

evidence of links between dealers and users. Not only does this allow the matching of samples in local cases of dealing but also provides intelligence information on the origin and distribution of samples which may be of use in investigating major trafficking operations. Drug profiling methods also provide estimation of the clandestine manufacturing and illicit drug trafficking and distribution trends in addition to information on precursor chemicals used in illicit drug synthesis which will allow for their control. Drug profiling has proved that it is a useful science in real court cases [17] and in routine laboratory work [72, 74], as a tool for drug intelligence and for monitoring clandestine manufacturing of illicit drugs [5, 8, 56].

## **8.2. WHAT REMAINS TO BE DONE (FUTURE WORK)?**

Comparative analysis and drug profiling procedures as mentioned in chapter 3 involve the use of different methodologies and analytical analyses. Those concerned with impurity profiling include identification of key impurities or comparison of impurity profiles, usually involve set of procedures such as extraction of impurities by different extraction methods, instrumental analyses of the extracts followed by data acquisition, manipulation and interpretation. Each step can be performed by different approach. Extraction of impurities has been widely done using liquid-liquid extraction. In the study solid phase extraction has been used as an alternative method. Further work in the future can be done on examining the use of automated solid phase extraction procedure. The automated SPE procedure will provide an attended extraction saving precious analyst time and manpower, establishing more consistency and repeatability in the analysis, and allowing for a total automated procedure including extraction, chromatography and data and statistical analysis.

The use of other analytical instruments such as capillary electrophoresis (CE) gas chromatography-fourier transform infrared spectroscopy (GC-FTIR) may be explored as tools for drug profiling procedures. Other areas of future work also include developing new computer-assisted statistical analysis for comparison of impurity profiles that may allow for rigorous comparative analysis and higher power of discrimination.

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# **ANNEXES**

**PUBLICATIONS AND PRESENTATIONS  
IN SUPPORT OF  
THIS THESIS**

**Annexe 1.**

**SOLID PHASE EXTRACTION FOR PROFILING OF  
ECSTASY TABLETS**

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## Solid-Phase Extraction for Profiling of Ecstasy Tablets\*

**REFERENCE:** Rashed AM, Anderson RA, King LA. Solid-phase extraction for profiling of ecstasy tablets. *J Forensic Sci* 2000;45(2): 413–417.

**ABSTRACT:** A solid-phase extraction (SPE) procedure has been developed for impurity profiling of illicit tablets containing 3,4-methylenedioxy-N-methyl-amphetamine (MDMA, ecstasy). Following initial comparison of liquid-liquid extraction and solid-phase extraction, SPE was found to be preferable because it afforded higher extraction efficiencies and shorter extraction times. Procedure blank samples were also analyzed to identify constituents of the extracts which did not originate in the ecstasy tablets. The developed procedure was subsequently applied to 12 samples of seized ecstasy tablets and a comparison was made of these samples to determine similarities and obtain inferences with respect to commonality of origin.

**KEYWORDS:** forensic science, solid-phase extraction, ecstasy, profiling, impurities

Profiling of clandestinely manufactured drugs is concerned with the detailed chemical examination of impurities derived from their manufacture, if the drugs are synthetic, or co-extracted, if they are derived from natural products. These should be distinguished from diluents and adulterants which have been added deliberately. Many workers have reported on profiling of amphetamine (1–8), methamphetamine (9–12), heroin (13–18), cocaine (19–25), and 3,4-methylenedioxy-N-methyl-amphetamine (MDMA) (26–31). Profiling serves as a tool to relate different street drug seizures to a common source, to determine the origin of drugs manufactured from natural sources (32) or synthetic routes for synthetic drugs, and to identify impurities found in illicit drugs which may cause public health risks because of their inherent chemical or biological hazards (33).

"Ecstasy" is the common name for MDMA, a synthetic hallucinogenic amphetamine, which has become one of the most widely used illicit substances in Europe. It is almost always produced in clandestine laboratories and very often contains various impurities such as reaction by-products, synthetic intermediates and contaminants from reagents, which accumulate during the synthetic sequence because of the lack of quality control in clandestine laboratories. These impurities often represent a very small percentage of the total weight of the finished product and their analysis by in-

strumental methods usually requires a preliminary extraction process to isolate and concentrate the analytes from the total tablet content. In the process, interfering materials are removed and the target substances are concentrated into a solvent that is suitable for introduction into the analytical instrument selected. Although lengthy and time-consuming, these procedures are of paramount importance, resulting in the removal of the major constituents—the active drug substances, diluents and adulterants—and concentration of the minor impurities.

Liquid-liquid extraction (LLE) of impurities from street drugs has until now been the method of choice for sample preparation. Many problems are associated with LLE, including lengthy handling time and the need to concentrate the sample after extraction. Sample preparation techniques using solid-phase extraction (SPE) have not been widely used for the profiling of street drugs, and there are few reports in the literature (34). In this study, SPE and gas chromatography (GC) were used for the extraction and analysis of impurities in ecstasy tablets obtained from different street drug seizures, and comparisons were made of their chemical signatures (profiles).

### Experimental

#### Materials and Reagents

Sixteen different samples of ecstasy tablets used in the study were provided by the Forensic Science Services, Metropolitan Laboratory, London. All reagents used were of analytical or high-pressure liquid chromatography (HPLC) grade. Solid-phase extraction columns (Bond Elut<sup>®</sup>) were obtained from Varian Associates, Harbor City, CA.

#### Gas Chromatography

Gas chromatography was carried out using a Hewlett-Packard 5890 Series II instrument fitted with a flame ionization detector and equipped with a fused silica capillary column (HP5, 30 m × 0.32 mm inside diameter 0.25 µm film thickness). Samples (volume 1 µL) were injected with an injection port temperature of 270°C at a linear velocity of 30 cm/s. The column oven temperature was programmed from an initial temperature of 80°C (1 min) at 35°C/min to 180°C (held for 18 min) then at 50°C/min to 300°C (held for 2 min).

#### Solid-Phase Extraction Versus Liquid-Liquid Extraction

A comparison of SPE and LLE was carried out to evaluate the use of SPE as an alternative extraction procedure to LLE. Ten portions of a ground and homogenized ecstasy sample, 50 mg each, were separated into two groups of five portions. One group was subjected to LLE, the other to SPE. Both groups were analyzed by gas chromatography/flame ionization detection (GC/FID) using the conditions mentioned above.

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### Solid-Phase Extraction

The tablets were crushed and 50 mg of the homogeneous powder were added to 1 mL phosphate buffer at pH 9. The suspension was then mixed on a rolling extractor for 30 min, centrifuged for 5 min and the supernatant was taken off. A period of 30 min was required for complete equilibrium to be achieved. SPE procedures were carried out using Varian Bond Elut C18 (200 mg) columns. The SPE column was conditioned with 10 mL of deionized water (to remove water-soluble impurities due to manufacture of the cartridge), 10 mL methanol (to solvate the column and remove other impurities), and 10 mL deionized water (to remove excess solvation solvent). The sample was then applied to the column at 1 to 2 mL/min. The column was then washed with 10 mL deionized water (to remove sugars and other unwanted water-soluble compounds) and dried under maximum vacuum (15 psi) for 5 min. The cartridge was subsequently eluted with 0.7 mL isopropanol (to remove any remaining water and recover neutral analytes retained only by lipophilic interaction with the sorbent surface) followed by 1 mL ethyl acetate with 2% ammonia (to elute basic analytes retained by ion-exchange interactions). These two fractions were combined and evaporated in a nitrogen stream to about 100  $\mu$ L. Care was taken not to evaporate to dryness to avoid evaporating the amphetamine impurities present in ecstasy samples.

### Liquid-Liquid Extraction

Phosphate buffer (1 mL, pH 9) was added to 50 mg of the same homogeneous powder used for the SPE procedure. The solution was then mixed on a rolling extractor for 30 min. Ethyl acetate (1 mL) was added to the solution and the tube was again mixed on a rolling extractor for 30 min. A period of 30 min was required for complete equilibrium to be achieved. The sample was then centrifuged for 5 min at 3000 rpm and the organic layer was taken off and evaporated in a nitrogen stream to about 100  $\mu$ L, with care being taken not to evaporate to dryness. At this stage 100  $\mu$ L of the internal standard, triethylamine, was added. Only 1  $\mu$ L of the sample was injected in the GC-FID.

### Blank Extraction by SPE and LLE

Two blank aliquots of phosphate buffer were extracted by SPE and LLE using the same solvent and cartridges used in the rest of the work to ensure that analytical artifacts were not introduced.

### Application to Real Case Samples

Twelve different samples of seized ecstasy tablets were analyzed using solid-phase extraction and GC-FID. The extraction procedure and the GC-FID conditions were as described above.

### Comparison of Tablets from Same Seizure

Two tablets from the same seizure were chosen at random and were analyzed for the purpose of establishing common origin. Each tablet was ground, extracted, and analyzed separately to avoid cross contamination.

## Results and Discussion

### Solid Phase Extraction Versus Liquid-Liquid Extraction

Five portions of the same ecstasy sample were extracted by SPE and analyzed by GC-FID. The principal peaks of chromatograms

representing impurities of each portion were numbered. The ratios of their peak areas to that of the internal standard and the means of the relative peak areas were calculated. The same procedure was performed for the other five portions extracted by LLE.

To evaluate the data collected from the various chromatograms of ecstasy samples extracted by SPE and LLE, statistical analysis was performed to compare the means of the relative peak areas of seven major impurity peaks present in the chromatograms. The two-sided *t*-test was used to assess if means of the selected peaks were significantly different for each extraction method. In addition, a comparison was made of the means of relative peak areas of peaks number 1, 2, and 7 for each extraction method and the *p* value was calculated using a computer software package (Minitab). The *p* value of each *t*-test as shown in Table 1 indicated that there are significant differences between the evaluated means. Since peak areas for samples extracted by SPE were larger than those extracted by LLE, and the *p* values showed significant differences between the mean values, it is safe to conclude that SPE provided a better extraction yield of impurities in ecstasy samples.

Another illustration of the differences in the mean of relative peak area of peaks 1, 2, and 7 is shown in Fig. 1 where SPE yielded better peak recovery than LLE.

### Blank Extraction by LLE and SPE

Blank extracts showed no major peaks especially at the retention time as the ecstasy impurities peaks were present. Figure 2 demonstrates chromatograms of two blank samples extracted by LLE and SPE.

### Applications to Seized Samples

The magnitude of inter- and intra-batch variation of impurity profiles governs the establishment of a "common source." Intra-batch variation should be less than inter-batch variation since samples belonging to the same batch expected to show more similarities than samples belonging to different batches.

### Comparison of Different Seizures (Inter-Sample Variation)

Ecstasy samples from 14 different seizures (packages) were extracted by solid-phase extraction and analyzed by GC-FID for the purpose of establishing commonality. Figure 3 shows a comparison of impurity profiles of three ecstasy samples from three different seizures.

The above impurity profiles allowed for visual comparison of three different ecstasy samples. It is clear that they contain great variations and consequently do not belong to a common batch (source).

TABLE 1—Two sample *t*-test results of peaks 1, 2, and 7 for both SPE and LLE extraction methods.

Two Sample <i>t</i> -test for Peak 1
95% C.I. for LLE 1 – SPE 1: (–1.859, –0.12)
<i>t</i> -Test LLE 1 = SPE 1 (not=): <i>t</i> = –2.57
<i>p</i> = 0.030 DF = 9
Two Sample <i>t</i> -test for Peak 2
95% C.I. for LLE 2 – SPE 2: (–2.564, –2.053)
<i>t</i> -Test LLE2 = SPE 2 (not=): <i>t</i> = –20.82
<i>p</i> = 0.001 DF = 9
Two Sample <i>t</i> -test for Peak 7
95% C.I. for LLE 7 – SPE 7: (–0.176, –0.057)
<i>t</i> -Test LLE 7 = SPE 7 (not=): <i>t</i> = –4.63
<i>p</i> = 0.0025 DF = 9

*Comparison of Tablets from Same Seizure (Intra-Sample Variation)*

Impurity profiles of two randomly chosen ecstasy tablets from the same seizure (package) were obtained using solid-phase extraction and GC-FID. The generated impurity profiles showed similarities indicating that both samples belong to the same batch. Figure 4 demonstrates an overlay comparison of their impurity profiles showing little variation.

*Future Work*

Among the numerous advantages of using solid-phase extraction for profiling of illicit drugs are efficiency, no cross contamination of phases, and no emulsion problems, as with LLE, due to the presence of fatty acids in ecstasy tablets. Further profiling can be very difficult with a sample of high purity since the impurity profile is dependent on impurity abundance in the sample. An excellent extraction of impurities in high-purity drug exhibits by LLE requires repeated extractions and the use of a large volume of solvents, which can cause loss of analytes and consequently poor impurity profiles. SPE can provide excellent impurity profiles with superior selectivity, high recovery, and reduced organic solvents consumption. Finally, there is the possibility of automating the extraction procedure, which is more easily automated with SPE than with LLE. The automated SPE procedure will provide an attended extraction, saving precious analyst time and manpower, establishing more consistency and repeatability in the analysis, and allowing for a total automated procedure including extraction, chromatography, and data and statistical analysis.

*Conclusion*

Solid-phase extraction of impurities in ecstasy tablets proved to be more efficient than the traditional liquid-liquid extraction. SPE provided impurity peaks with higher intensities than did LLE and a shorter extraction time. Street samples of ecstasy were analyzed for profiling purposes using the SPE procedure. Samples from different seizures (packages) showed little variations and great similarities, indicating common batch origin, while those from different packages showed great variations, indicating different batch origin.

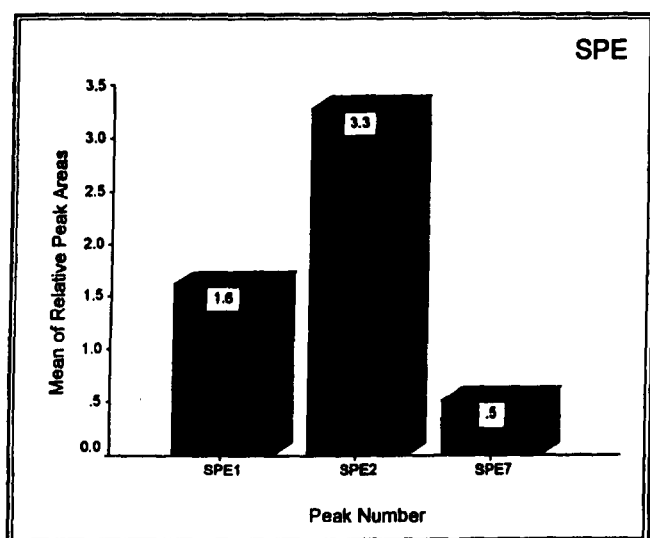
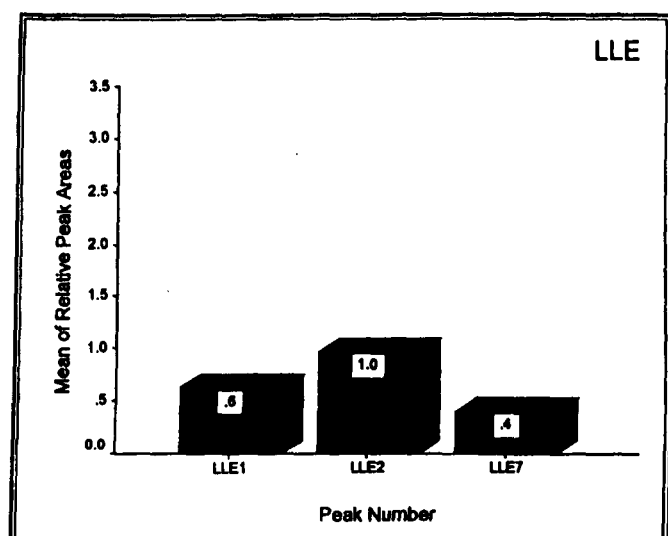


FIG. 1—Comparison of mean relative peak areas for three different peaks from chromatograms of an ecstasy sample extracted by LLE and SPE.

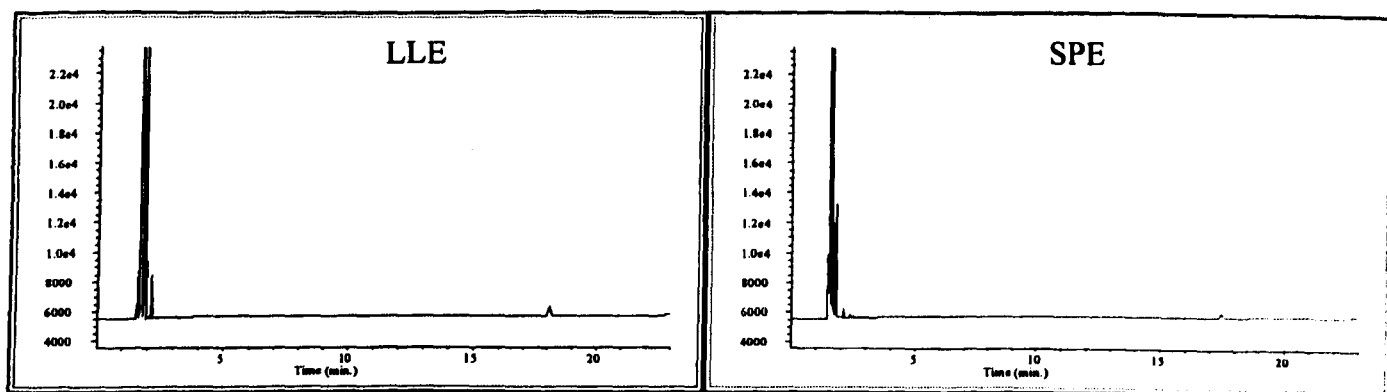


FIG. 2—Chromatograms of blank sample extracted by LLE and SPE.

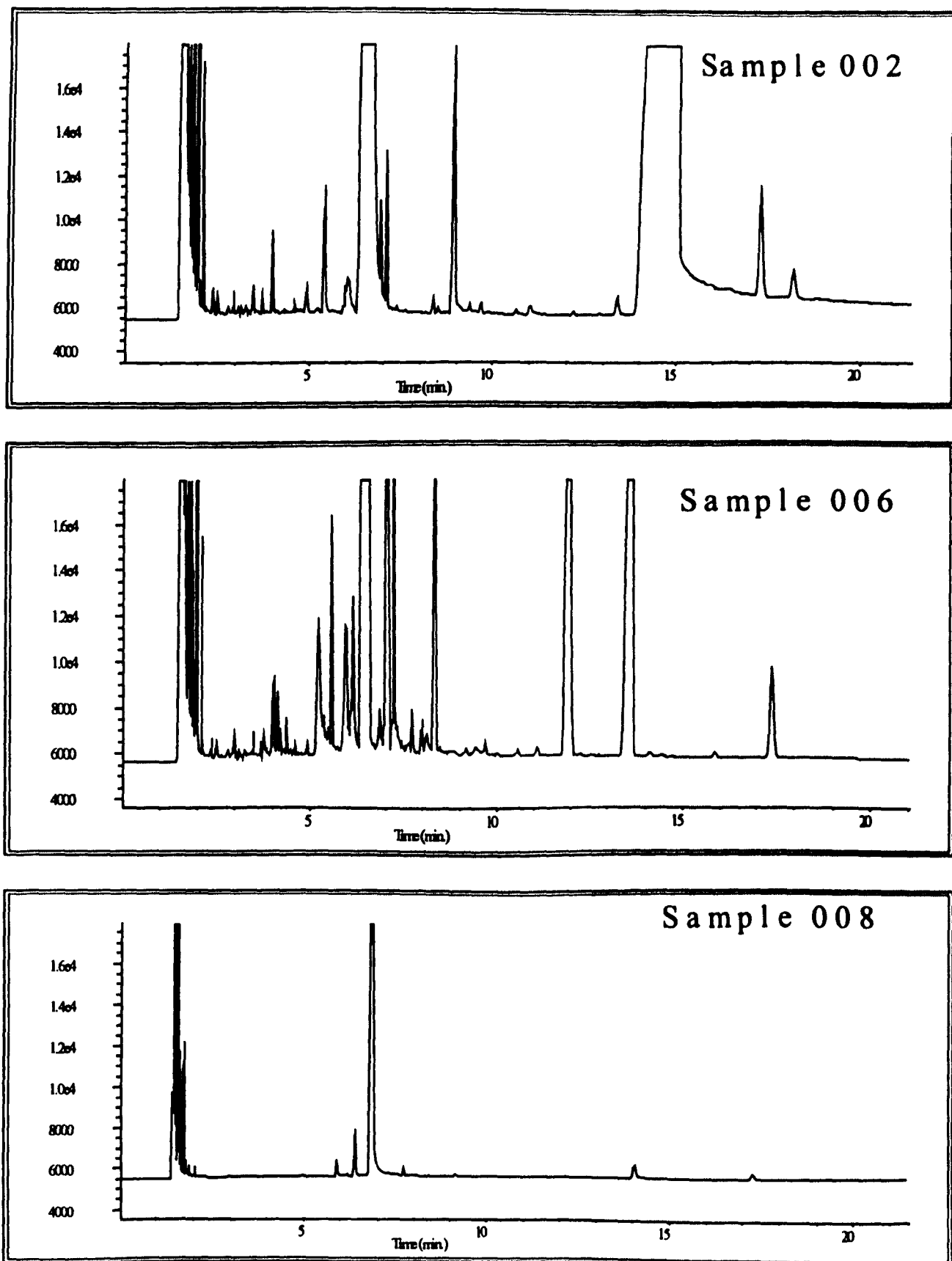


FIG. 3—Impurity profiles of three different samples 002, 006, and 008 from three different seizures.

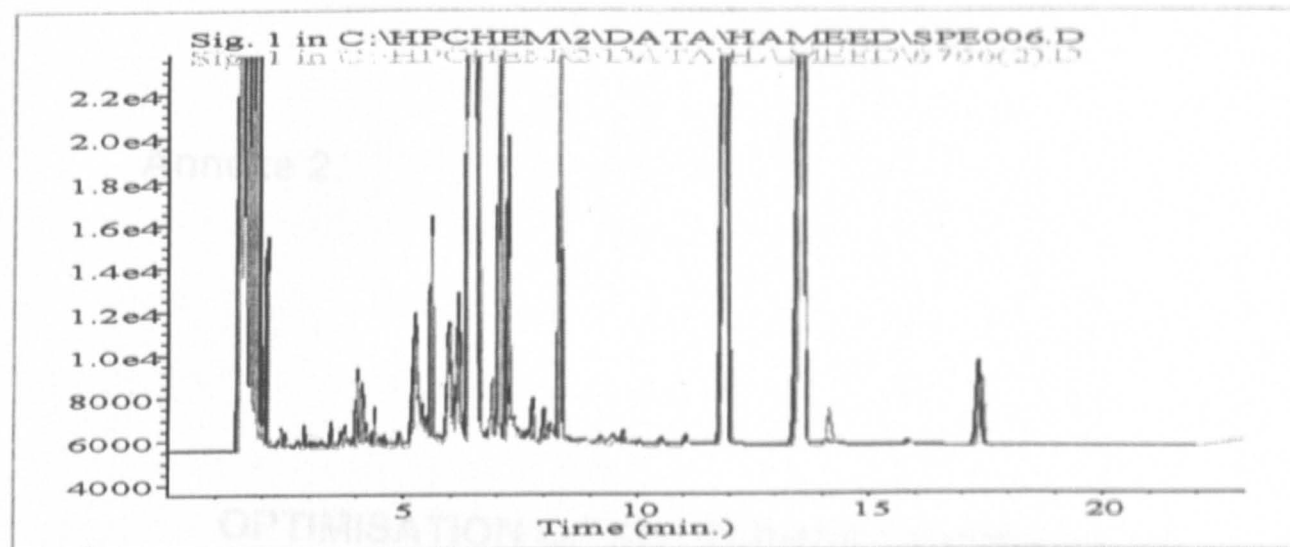


FIG. 4—Overlay of impurity profiles of two samples from the same seizures.

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## **Annexe 2.**

# **OPTIMISATION OF SOLID-PHASE EXTRACTION PROCEDURE FOR ECSTASY PROFILING**

Abdulhameed M. Rashed, Robert A. Anderson, Leslie A. King

In

Forensic Drugs Conference

Airth Castle, Scotland, 1999

# **Optimisation of a solid Phase Extraction Procedure for Profiling of Ecstasy Tablets**

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**Abstract:** Optimisation of a solid phase extraction procedure for the profiling of ecstasy tablets was done. Three main parameters of the extraction procedure were examined and evaluated. These parameters were pH, eluting solvents, and SPE columns. A range of pH values from 5 to 10 were examined for extracting impurities in ecstasy tablet and pH 9 was determined to be the optimum pH. Four different eluting solvents, ethyl acetate, dichloromethane, hexane, and isooctane, were evaluated. Ethyl acetate produced an impurity profile with higher yield than the other solvents examined. Out of eight different SPE columns from two different manufacturers, Varian Bond Elut C18 200mg produced the best impurity profile. The impurity profiles were stable over a period of four weeks.

**Keywords:** Optimisation, Profiling, Impurities, Ecstasy, SPE

## **1. Introduction**

Illicit ecstasy tablets produced in clandestine laboratories usually contain various impurities such as, reaction by-product, intermediates and contaminants within reagents, accumulate as a chemical reaction proceeds because of the lack of quality control in these laboratories. Many of these impurities have been isolated and identified [1-4]. These impurities provide chromatographic profiles that allows for comparison of different exhibits for the fact that samples originate from the same batch almost certainly have the same number and relative amounts of identical impurities [5].

The instrumental analysis of impurities found in ecstasy tablets usually requires a preliminary extraction process to extract, isolate, and concentrate these impurities from the total tablet content. In the process, interfering materials are removed, and the required substances are concentrated into a solvent that is suitable for introduction into the instrument. The impurities found in illicit tablets are extracted, after crushing the tablet and dissolving the fine homogeneous powder in deionised water or buffer solution which forms an aqueous matrix, into an organic solvent. Although lengthy and time-consuming, these procedures are of paramount importance to remove the principle component(s), diluents and adulterants, and concentrate impurities.

Solid-phase extraction (SPE) procedures have been used with various types of samples, from biological fluids to environmental pollutants, and the literature is full of articles on work done in this regard. But only one paper has been cited on the use of SPE for the extraction of impurities found in clandestine drugs [6]. In this project special emphasis will be given to the use of SPE for the extraction of impurities in illicit (ecstasy) tablets. This paper describes an optimisation method of a solid phase extraction procedure for profiling ecstasy tablets.

## **2. Experimental**

### ***2.1 Optimising SPE Procedure***

The optimisation of SPE procedure was done in three main steps

1. Examination of the best extraction pH
2. Evaluation of eluting solvents used for extracting impurities
3. Assessment of different SPE columns

### ***2.2 Examination of the best extraction pH***

The tablets were crushed and 50 mg of the homogeneous powder were added to 1 ml phosphate buffer at pH 9. The suspension was then mixed on a rolling extractor for 30 min., centrifuged for 5 min. and the supernatant was taken off. A period of 30 min. was required for complete equilibrium to be achieved. A SPE column (Bond Elut Certify 130 mg) was conditioned with 10 ml of deionised water (to remove water-soluble impurities due to manufacture of the cartridge), 10-ml methanol, and 10 ml deionised water. The sample (supernatant) was then applied to the column at 1-2 ml/min. The column was then washed with 10 ml deionised water (to remove sugars and other unwanted water-soluble compounds) and dried under maximum vacuum (15 psi) for 5 min. The sample was subsequently washed with 0.7 ml isopropanol (to remove any remaining water and reduce lipophilic bonding between the analytes and the sorbent surface) followed by 1 ml ethyl acetate with 2 % ammonia (to elute freed analytes by eliminating ion-exchange interactions). The collected sample was evaporated in a nitrogen stream to about 100  $\mu$ l, with care being taken not to evaporate to dryness. The pH values of the phosphate buffer ranged from 5 to 10.

## **2.3 Gas Chromatography**

The GC was an Hewlett-Packard 5890series II with flame ionization detector. One  $\mu\text{l}$  of sample was injected with an injection port temperature of  $270^{\circ}\text{C}$ , splitless mode. Gas chromatography was carried out using a fused silica capillary column (HP5, 30 m x 0.32 mm i.d., 0.25  $\mu\text{m}$  film thickness) programmed from an initial temperature of  $80^{\circ}\text{C}$  (1 min) at  $35^{\circ}\text{C}/\text{min}$  to  $180^{\circ}\text{C}$  (held for 18 min) then at  $50^{\circ}\text{C}/\text{min}$  to  $300^{\circ}\text{C}$  (held for 2 min).

## **2.4 Evaluation of eluting solvents for the one with most impurities extracted**

The same extraction procedure was repeated under the optimum pH determined above using four different eluting solvents, ethyl acetate, dichloromethane, hexane, and isooctane. The relative peak areas were calculated and bar charts were drawn for the main peaks to determine the best eluting solvent

## **2.5 Assessment of different SPE columns**

Once the optimum extraction pH and the best eluting solvent were determined an assessment of different SPE columns was done using the same extraction procedure as above. The columns used for the assessment study were, Bond Elut C18 200 mg, C18 500mg, Certify 300mg, and Certify 130mg (Varian) and Isolute C8 200mg, C8 500mg, C18 200mg, and C18 500mg<sup>9</sup> International Sorbent Technology). The relative peak areas were calculated and a bar chart was drawn for the main peaks to determine the performance of each type of SPE column. Throughout all the extraction procedures performed above only one type of ecstasy tablet was use to assure that any variations were due to different extraction procedure parameters not due to different samples.

## **2.6 Blank Extracts**

The same extraction procedure was used with blank extracts to ensure impurities other than those present in the tablets were not present.

## **2.7 Stability of Extracts during Storage**

Extracts were stored at  $4^{\circ}\text{C}$  in the dark, and were re-analysed over a period of 4 weeks to check their stability

## **2.8 Application to Real Samples**

Three ecstasy samples seized from different individuals at different locations were analysed on the optimised conditions.

# **3. Results and Discussion**

The optimisation procedure was done on a sequence to allow the use of the optimum determined parameter for the next step of the optimisation procedure.

## **3.1 Examination of the best extraction pH**

Six different pH values (5-10) were examined using the same eluting solvent (ethyl acetate) and the same SPE column (Bond Elut Certify 130mg). The SPE procedures were run for all pH range on portions of the same homogeneous ecstasy powder using the same steps all the time. Figure 1 illustrates the superiority of extracting with pH 9 over other pH values. The optimum pH at 9 yielded better impurity profile with more impurity peaks present allowing for a better chemical signature comparison.

### ***3.2 Evaluation of eluting solvents for the one with most impurities extracted***

In the evaluation of the eluting solvents procedure four solvents were used; ethyl acetate, hexane, dichloromethane and isooctane using the optimised pH 9 and Bond Elut certify 130 mg. Ethyl acetate yielded the best impurity profile providing impurities with higher relative peak areas as seen in Figure 2.

Although all four eluting solvents examined yielded acceptable profiles, ethyl acetate produced a profile for all the ten studied impurity peaks with reasonable relative peak area. Therefor ethyl acetate was the solvent of choice for the elution step.

### ***3.3 Assessment of different SPE columns***

Eight SPE columns from two manufacturers (Varian and International Sorbent Technology, IST) were evaluated. All SPE columns examined using the optimised pH 9 and the optimised eluting solvent, ethyl acetate. After evaluation of the determined impurity profiles, Varian C18 (200mg) provided the best impurity profile with the highest relative peaks areas as it is shown in the bar chart in Figure 3.

### ***3.4 Blank Extracts***

A blank sample consisting of only a buffer solution at pH 9 was extracted with the optimised eluting solvent, ethyl acetate and by the optimised SPE column, Varian Bond Elut C18 200m. This step was necessary to be certain that all the peak taken for the evaluation were not generated from any source other than the ecstasy homogenised powder. Figure 5 demonstrates a chromatogram of a blank sample extracted with the conditions mentioned above.

### ***3.5 Stability of Extracts during Storage***

A demonstration of the stability of extracts during storage was done for an ecstasy sample extracted with optimised procedure. The sample was analysed by GC-FID four times over a period of four weeks. The sample was stored in the dark at 4°C for the whole period and was only out briefly for the analysis. Figure 6 illustrates the stability study of the extracts profile over the four weeks period.

After four weeks of storage of extracts in the dark at 4°C, the impurity profiles showed no major changes and were still valuable for comparison purposes.

### ***3.6 Application to Case Samples***

Three different ecstasy tablets brought to the laboratory from different sources were crushed, homogenised and extracted with the optimised parameters. Figures 7a, 7b, and 7c show comparison of profiles of these different ecstasy samples.

Sample 001 and 002 showed similar profiles with minimum variation indicating that they have originated from the same source. Samples 001 and 002 showed no similarities with sample 003 indicating that they cannot be from the same source.

## **4. Conclusion**

The optimisation of the solid phase extraction (SPE) procedure for the purpose of profiling impurities found in ecstasy tablets manufactured in clandestine laboratories was done. The determined optimum parameters for the SPE procedure were as follows:

1. Buffers with pH 9 were found to be the optimum for extracting impurities of ecstasy tablets.

2. The eluting solvent with most impurities extracted was ethyl acetate.
3. Among all the eight SPE columns evaluated, Varian Bond Elut C18 200mg produced the best impurity profile allowing for easy comparison of profiles of real samples.

Blank samples were extracted using the optimised procedure to make sure that no impurities other than those present in the ecstasy sample were extracted and analysed. Extracts showed no major degradation after storing them in the dark at 4°C for four weeks and their profiles were still suitable for comparison purposes after this period.

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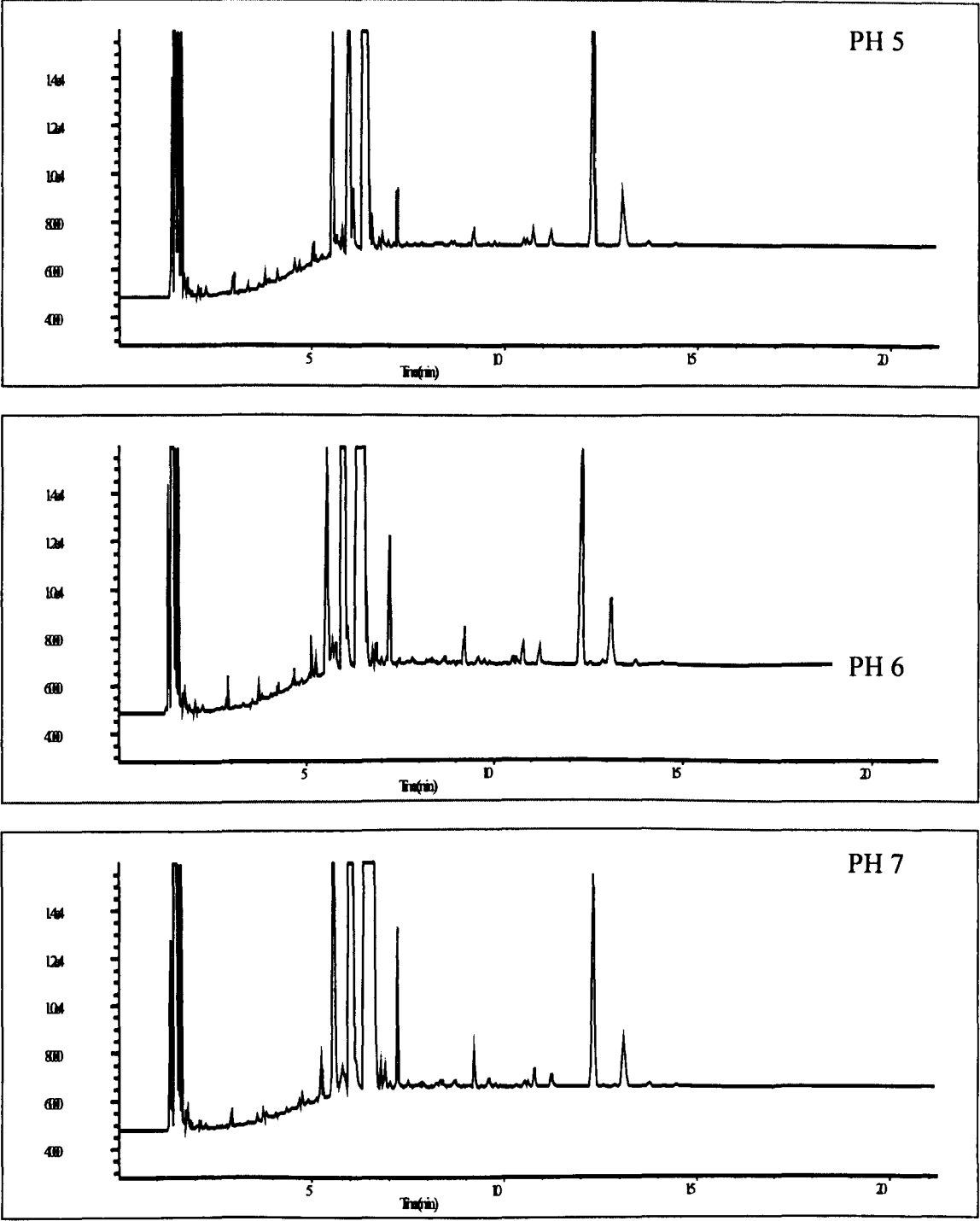


Figure 1b. Impurity profiles of an ecstasy sample extracted at different pH values

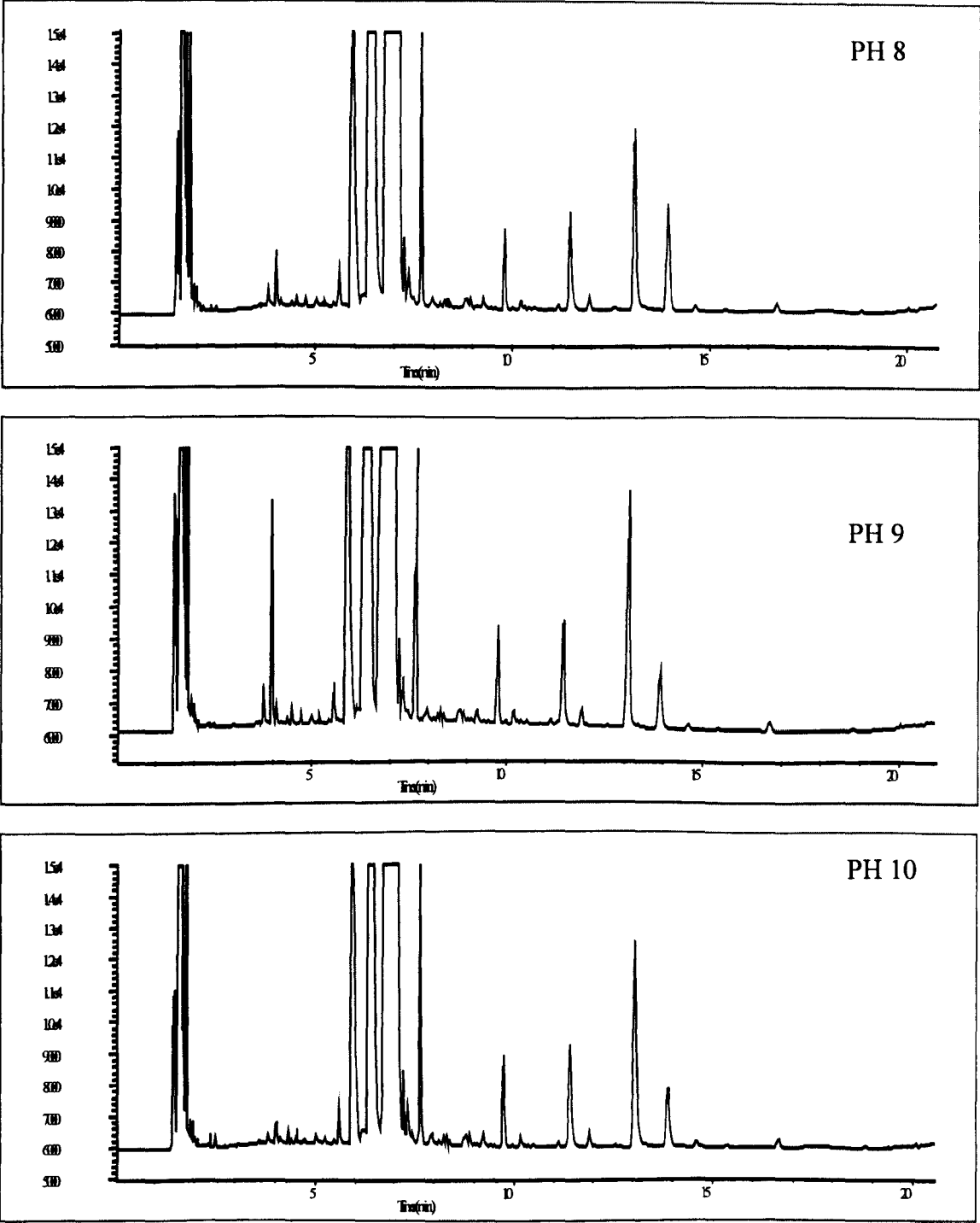


Figure 1b. Impurity profiles of an ecstasy sample extracted at different pH values



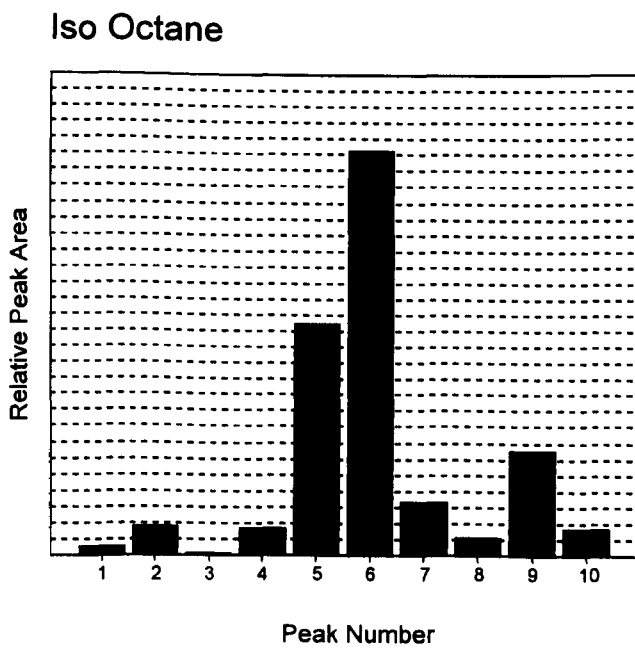
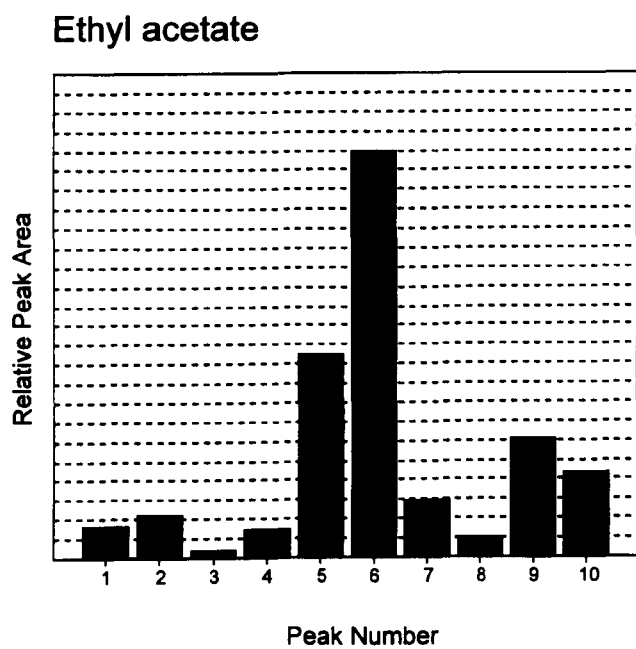
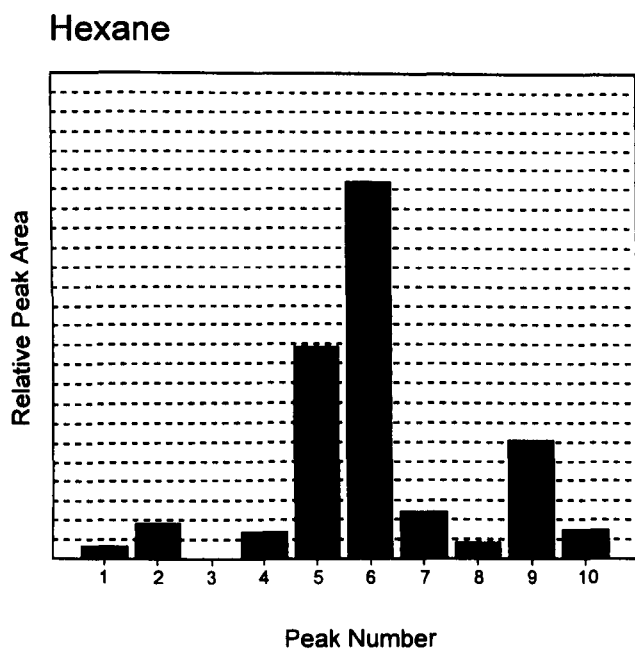
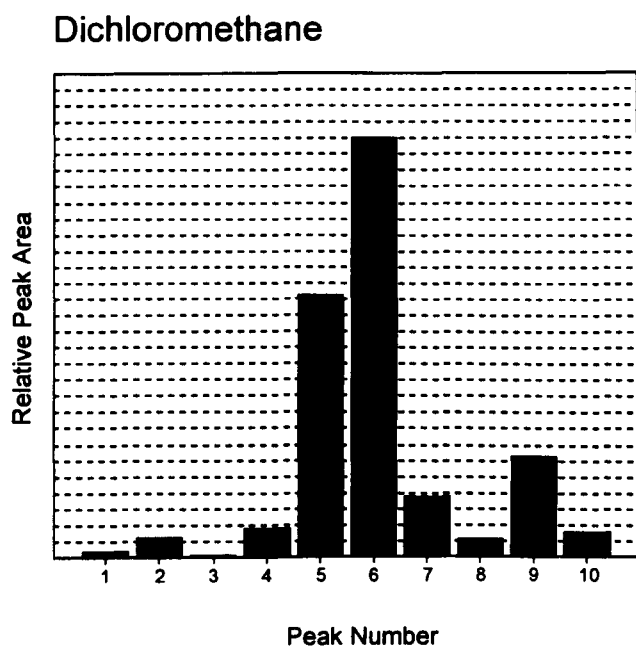


Figure 2. Bar charts demonstrate the extraction power of different elution solvents

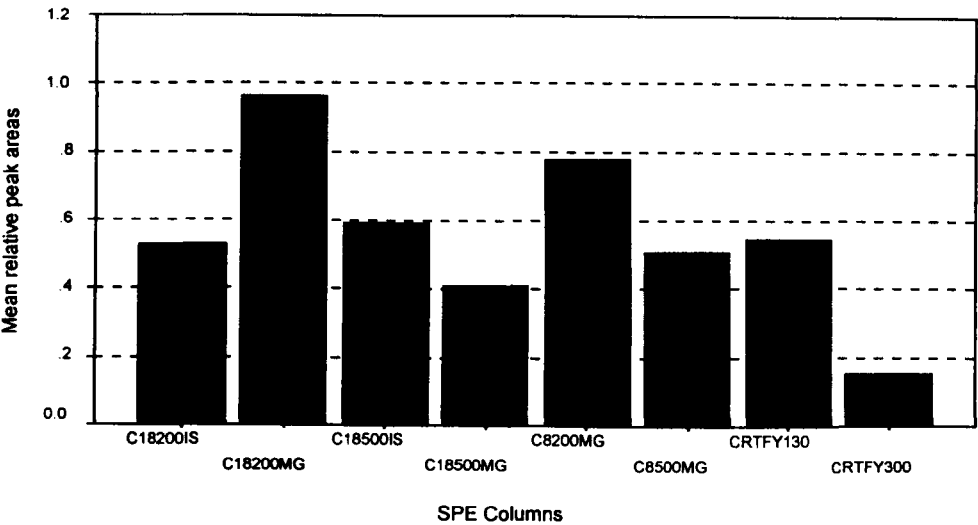


Figure 4. Means of relative peak areas Vs SPE columns

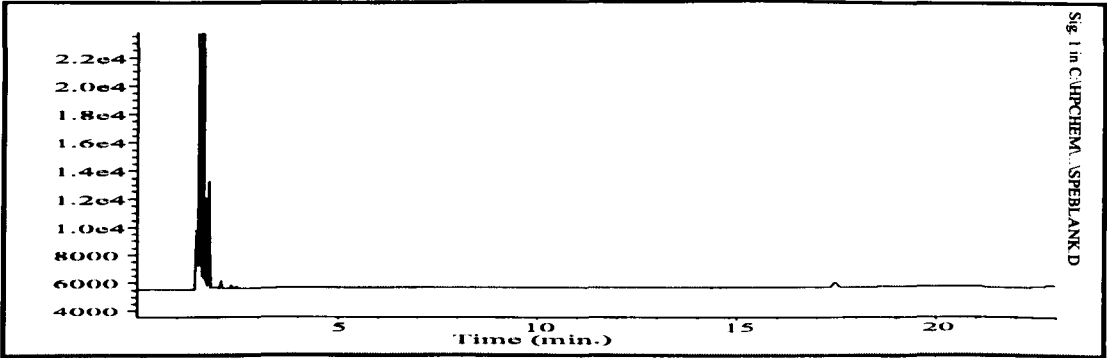


Figure 5. Chromatogram of a blank extracted with the optimised parameters

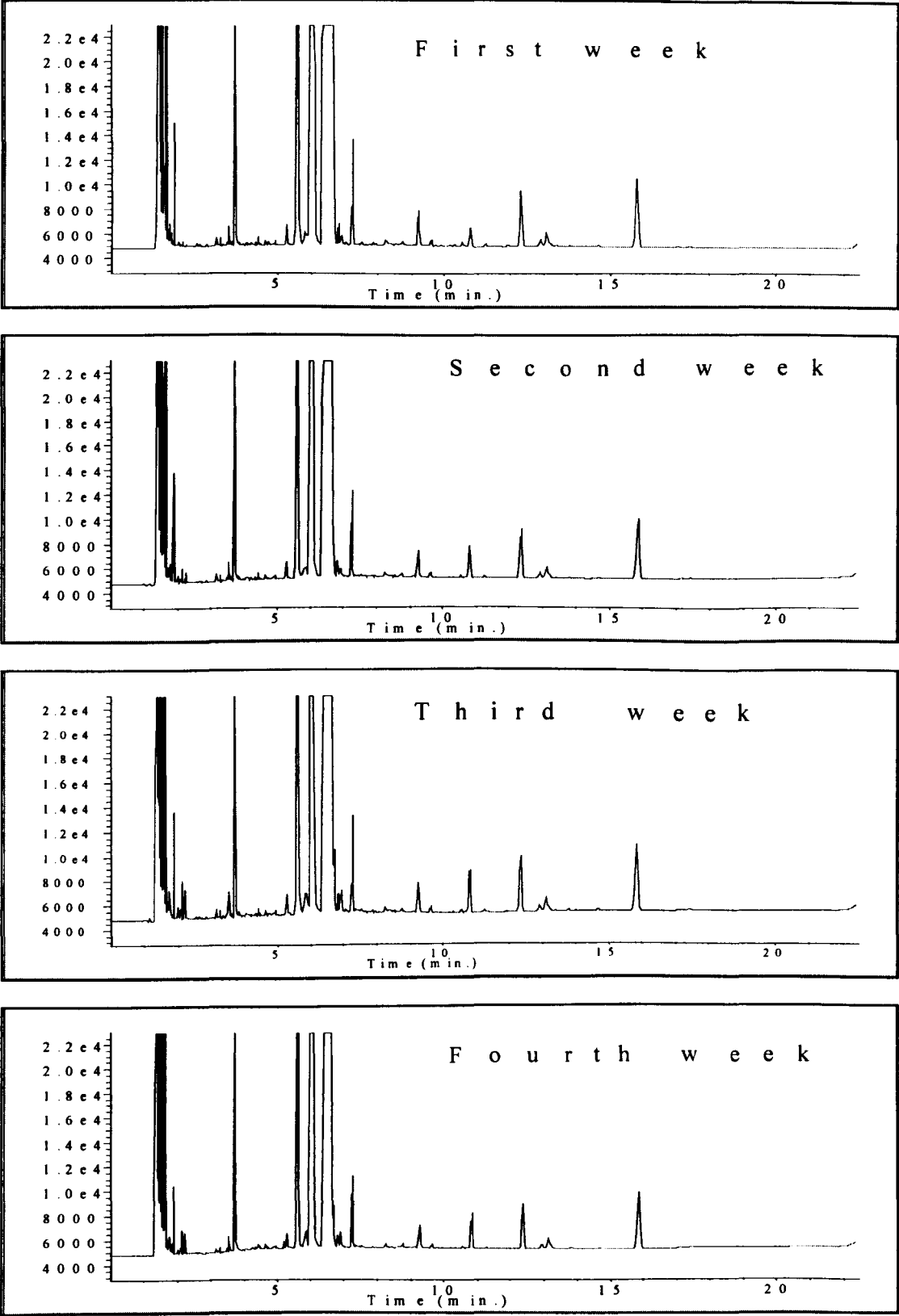


Figure 6. Impurity profiles of ecstasy sample demonstrating stability of extracts over a period of four weeks

## **Annexe 3.**

# **ECSTASY PROFILING USING FOURIER TRANSFORM INFRARED SPECTROSCOPY**

Abdulhameed M. Rashed, Robert A. Anderson, Leslie A. King

At the

15<sup>th</sup> Triennial meeting of the International Association of  
Forensic Sciences in Los Angeles 1999

# **Ecstasy Profiling using Fourier Transform Infrared Spectroscopy**

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## **ABSTRACT**

A method for profiling of ecstasy tablets through the use of fourier transform infrared spectroscopy (FTIR) was developed. Around 47 ecstasy tablets plus standards of MDMA, glucose, lactose, and caffeine were used for the study. Samples prepared as KBr disks were analysed directly without any extraction procedures or other sample preparations. Library search facilities were used to compare spectra using an algorithm of correlation coefficient. The library search provided best fits or closest matches from the library spectra to the unknown spectrum. A higher number means a closer match (1 being the highest and 0 being the lowest).

## **Introduction**

Chromatographic instrumentation has been used to perform drug profiling, in particular gas chromatography with flame ionisation detection [1-3], isotope-ratio mass spectrometry [4-5], and HPLC [6-7]. Chromatographic methods are powerful tools for comparing drug seizures, but they do have their inherent limitations. Infrared spectroscopy and in particular fourier transform infrared, FTIR, can serve as a viable elimination technique for comparing drug seizures. Each exhibit has its own characteristic spectrum, and comparison between the different spectra gives a gross estimate of the similarity or dissimilarity between the different exhibits [8-10]. FTIR has some discriminating capability to classify powders into a large number of groups, and consequently can be used for gross comparison of ecstasy exhibits. FTIR has some advantages over chromatographic methods such as minimum sample preparations and faster analysis time (under 1 min./sample).

Drug samples encountered in crime laboratories are normally exhibited in dosage units and presented as their own entities or as counterfeits of brand-name pharmaceutical products. Ecstasy is the common name for 3,4-methylenedioxy-methamphetamine (MDMA); a synthetic hallucinogenic amphetamine, which has become one of the most widely used controlled

substances in Europe. It is almost always illicitly produced in clandestine laboratories and often exhibited in tablet dosage.

This paper deals with the subject of ecstasy profiling (that is, attempt to carry out gross comparisons between ecstasy seizures) through the use of FTIR as a screening tool in drug profiling procedures.

## **Experimental**

### *Materials*

Forty-seven ecstasy samples from twenty-four seizures were used in the experiment. The Forensic Science Service, Metropolitan laboratory, London, provided street samples of ecstasy tablets. Standards of MDMA, lactose, glucose and caffeine were maintained from Sigma.

### *Sample Preparation and Instrumental Analysis*

Samples were prepared using KBr disks. Around 1 mg of sample was ground by pestle and mortar into a fine powder. Then about 300 mg of oven dried KBr were added and ground again to a fine homogeneous powder. The finely ground sample was then removed and placed in a 13mm KBr die to sandwich the mixture between two polished surfaces and press the KBr and sample into a disk. High pressure (12000kg) was then applied by means of a lever to coalesce the sample into a transparent or semi-transparent disk. Vacuum was applied to the die to remove entrained air, and to a limited extent, entrained moisture. As entrained moisture can cause the finished disk to be opaque. The prepared disk was then mounted in the beam of the spectrometer for analysis. Infrared spectra were obtained using a Jasco FTIR-410 Spectrometer (Jasco UK Ltd., Essex, UK).

### *Spectra Comparison*

Once spectra for standards and all 47 ecstasy samples were obtained, comparisons of spectra using library search facility from a commercially available spectral program called Spectra Manager SSP-400 search program (Jasco UK Ltd., Essex, UK), were performed for the purpose of establishing similarity or dissimilarity between the different exhibits. An in-house library was created which contained spectra for standards and 47 ecstasy samples. The library search was done using an algorithm for correlation coefficient for comparison purposes. This method conducts a search by comparing the spectrum waveform of an unknown sample with the spectral waveforms of library spectra. The library search provides best fits or closest matches from the library spectra to the unknown spectrum. A higher number means a closer match (1 being the highest and 0 being the lowest). The comparison procedure was based on two main elements, 1) the library search results (best fit) and the visual comparison of spectra using an overlay diagram.

### *Repeatability of Analysis*

To test the repeatability of spectra, a single sample was analysed as above ten times using the same procedure and parameters including sample preparation as a disk and analysis on FTIR. Statistical analysis was then performed to calculate percentage relative standard deviation (RSD).

### *Comparison of tablets from different seizures*

Tablets from different seizures chosen randomly were analysed for the purpose of establishing common source. Each tablet was ground, extracted, and analysed separately to avoid cross contamination. The result spectra were compared by the use of library search facilities.



**Results and Discussion**

The aim of comparative analysis is the comparison of confiscated drugs for investigative purposes and court testimony. Drug intelligence involves two main streams with regard to comparative analysis or drug profiling. The first includes court cases such as sample/sample comparison and relating user to a dealer to prove conspiracy. The second task for drug intelligence involves establishing relationships between seizures and providing information on the networking of samples, which may be of use in investigating major trafficking operations. In such operation the laboratory may have to analyse many samples. FTIR analysis can be utilised for such operation as an elimination tool for its sheer simplicity and short analysis time.

*Repeatability of Analysis*

After analysis of sample number (M01) for ten times, reproducible spectra were obtained and the calculation of percentage relative standard deviation (RSD) showed excellent repeatability of results. The procedure included running a library search on ten different spectra of sample (M01) and using the fit factors (library scores) from the search results to calculate percentage RSD. Table 1 illustrates the statistical results for the repeatability study.

Column Statistics	Results
Mean of library scores	0.98
Standard Deviation	0.0098
Library score : minimum	0.974
Library score : maximum	1
n	10
RSD (%)	1.00

Table 1. Statistical results for the repeatability study.

The above study provided evidence of the excellent repeatability of our procedure. This shows that when using FTIR technique as a screening tool for drug comparison no false interpretation based on poor repeatability is made.

#### *Comparison of tablets from different seizures*

Comparison of spectra of samples from different seizures yielding different physical features such as colour and logos showed correlation of their spectra as seen in the example given in Figure 1 which shows an overlay comparison of two samples from different seizures. This technique can be used as a visual mean of comparing spectra of similar tablets from different seizures or comparing two different exhibits to establish similarity or dissimilarity.

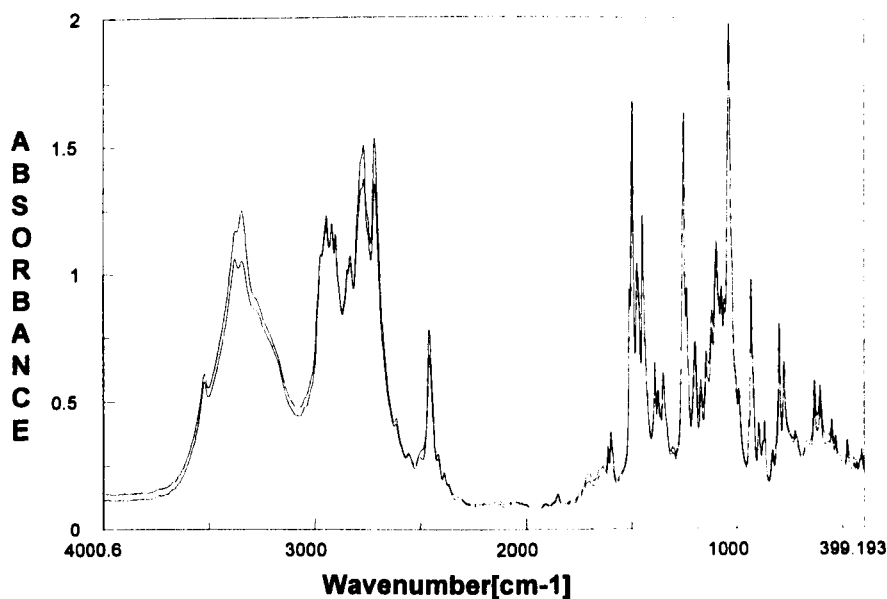


Figure 1. An overlay of spectra of sample (M01) and sample (M26)

The above method is useful when only small amounts of exhibits are compared. In the case of large amounts of seizures or in the case of building a database with many spectra included and samples to be related or grouped into smaller clusters, then use of computer

software is faster and more useful. Visual comparison is still handy as a confirmation to the library search results. In Table 2 an example is given of a library search results where a spectra of an unknown (confiscated ecstasy tablet) was compared with a database of spectra of street ecstasy tablets.

Order of Fit	Library Score	Sample Name
1	0.977	M13
2	0.971	M26
3	0.900	M32
4	0.789	M10
5	0.769	M14
6	0.761	M02

Table 2. Search results of comparing an unknown against a library database for the closest match

Figure 2 illustrates the library results in terms of spectra. Samples (M13), (M26), and the unknown showed similarities indicating their relation to a common batch.

Theses findings can be used positively to save time and laboratory work. The results of the FTIR analysis may be used to eliminate many samples before proceeding to more sensitive procedures such as chromatography. FTIR results can also provide a gross estimation of the different groups or profiles of drugs under investigation.

Although FTIR technique is not sensitive enough to detect minute amounts of compounds such as manufacturing impurities, which are essential to the profiling process, it can still be a useful tool when its results are combined with other analytical procedures including physical analysis and microscopy and information from police investigations.

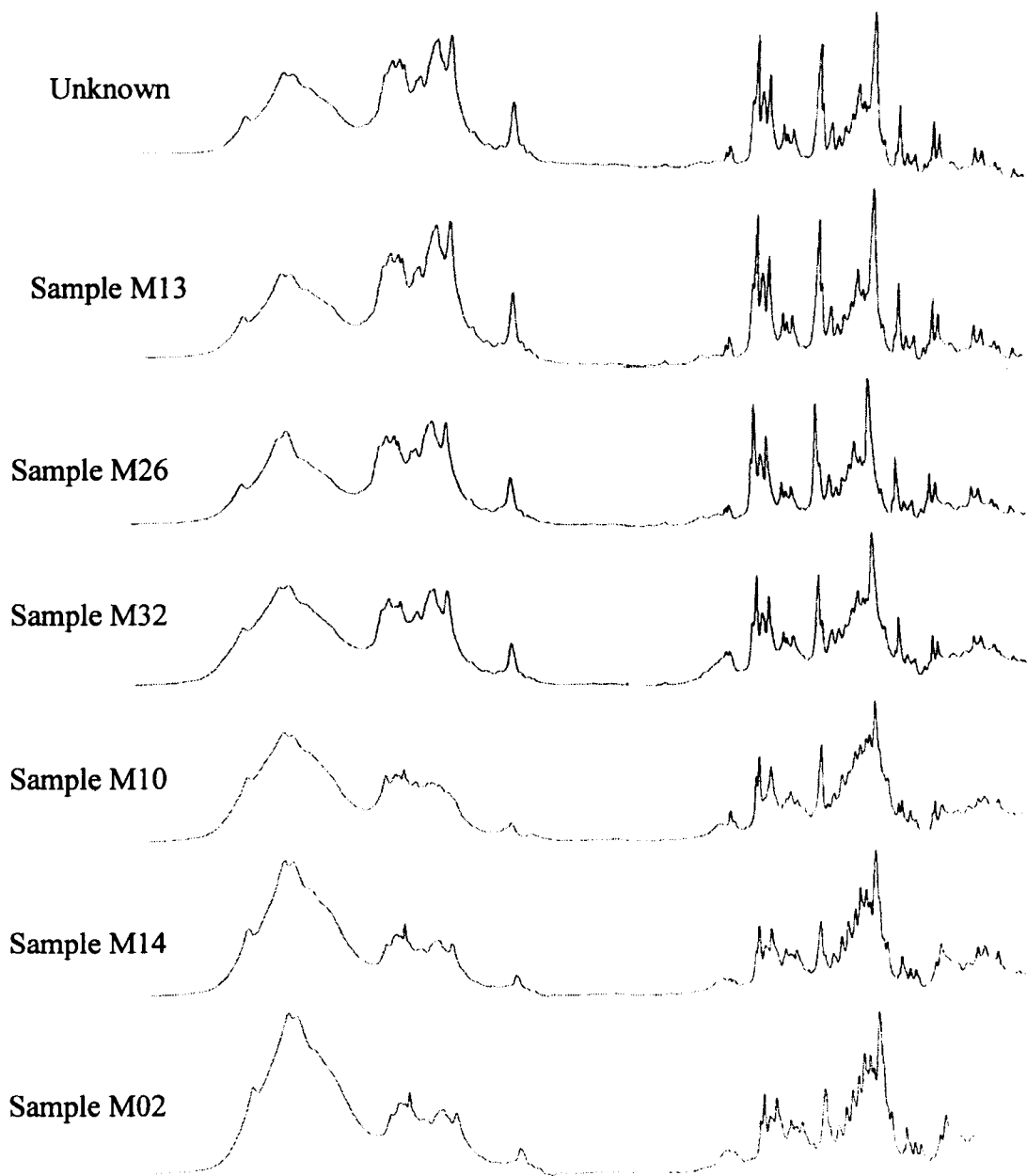


Figure 2. Library search results of matching an ecstasy exhibit (unknown) with a database of spectra of other ecstasy samples.

## Conclusion

FTIR can be a very useful tool for comparative analysis of confiscated drugs for its sheer simplicity with regard to sample preparation and instrumental procedure, short analysis time and

excellent repeatability of results. The library search facility provided a way to compare two samples and relate a sample to a group of samples from different seizures analysed previously. Comparative studies on confiscated ecstasy tablets through the use of FTIR when combined with a study of the physical features of tablets such as colour, size, logo, and microscopic analysis are possible and help provide information for the drug investigation process.

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## **Annexe 4.**

### **DRUG PROFILING: A MILESTONE**

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## **DRUG PROFILING: A MILESTONE**

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The objectives of this paper are to infer the area of drug profiling or comparative analysis of drugs of abuse and provide an overview of the developments on this science including the different approaches and procedures performed. In addition to identifying the presence of a specific controlled drug in an exhibit and measuring its concentration, forensic drug laboratories are requested in certain cases or as a routine to provide additional information that may be helpful to the investigation process. On the basis of their chemical and physical characteristics, seized drugs may be profiled and linked to common sources or routes of distribution. Not only does this allow the matching of samples in local cases of dealing but also provides intelligence information on the origin and distribution of samples. Scientists, specialising in illicit drug analysis in criminal or other analytical laboratories have been using many different approaches in the area of illicit drug characterisation / profiling since the early 1970s. These approaches varied according to the drug nature and the analytical philosophy or needs. Samples coming into laboratories in tablets or capsule form are usually subjected to physical analysis or so called "ballistics". Other exhibits such as powders are subjected to chemical analysis, which can be organic or inorganic.



Inorganic analysis of illicit drugs is concerned with the determination and comparison of the elemental content in exhibits. Organic analyses on the other hand are more varied with different types of analysis and approaches available to the forensic chemist including the following:

1. Determination and quantification of impurities or minor components present in the seized drug, which originate from precursors, natural raw materials, and reaction by-products.
2. Measurement of naturally occurring isotopic ratios.
3. Determination of diastereoisomeric or enantiomeric composition of drug exhibits
4. Determination of residual solvents for comparative analysis
5. Analysis of the controlled drug(s) and cutting agents
6. Drug profiling using infrared spectrometry

Impurity profiling procedures have by far dominated the illicit drug comparison methods used in forensic laboratories around the world. Two main approaches have been reported, (i) identification of trace impurities for establishing synthetic routes and identification of precursor chemicals used in synthesis and (ii) comparing impurity profiles (chemical signatures) to establish drug origin, grouping, and sample-to-sample comparison.