CHAPTER 1
Drugs of abuse

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1.1 Introduction

The aim of this chapter is to provide a brief overview of illegal drugs as they are controlled internationally, the specific nature of some of these compounds and how they can be analysed. The United Nations Office on Drugs and Crime (UNODC) provides an annual report (the World Drug Report) that collates international data to develop a picture of global drug markets in terms of production and use (UNODC, 2014a). The UNODC also provides an excellent international resource for information relating to analytical procedure and data interpretation that the reader is directed towards.

Generally speaking, controlled drug production and use tends to concentrate around four drugs or drug types: cannabis, opiates, cocaine and amphetamine-type substances. Each of these is discussed together with a brief overview of the emerging new psychoactive substances (NPS). These substances can also be categorized through their mechanisms of production as naturally occurring materials (cannabis), semi-synthetic materials derived from natural products (opiates and cocaine) and synthetic products (the amphetamine-type substances and the NPS).

Chemical substances controlled under International or National laws were used by between 190 and 324 million people worldwide in 2012 with 16–39 million users categorized as regular drug users (UNODC, 2014a). The prevalence of drug use varies regionally and the main problem drugs at the global level continue to be the opiates (notably heroin), followed by cocaine. For most of Europe and Asia, opiates continue to be the main problem drug; in South America, drug related treatment demand continued to be mainly linked to the abuse of cocaine; and in Africa the bulk of all treatment demand is linked to cannabis (UNODC, 2014a).

Forensic Chemistry: Fundamentals and Applications, First Edition. Edited by Jay A. Siegel.

1.2 Law and legislation

Over a century ago, the international effects of the "national" Chinese heroin problem were addressed with the creation of The Shanghai Opium Commission in 1909. Out of this was born the first mechanism for the international legislation of drugs, The Hague Opium Convention of 1912. Countries party to this convention were required to implement domestic legislation restricting drugs to medical use. The Shanghai Opium Commission and the Hague Opium Convention were the catalysts for international drug control as it is today.

Half a century later, the United Nations (UN) created the first of three major international drug control treaties that are still in effect: the Single Convention on Narcotic Drugs 1961. As with any international convention, signatories are required to implement domestic legislation in accordance with the recommendations set out therein, and they are obligated to evaluate the policies against the international standard. More than 180 countries are party to the UN treaties. The UN regularly names the countries that have not signed the treaties and continually urges them to do so.

The 1961 Single Convention on Narcotic Drugs was formed out of a need to combine many different nations' legislation on narcotic drugs into one primary instrument. It seeks to limit the manufacture, possession and use of drugs to science and medicine. Countries that are party to this convention are required to provide annual statistical returns and estimates for the use of, and need for, drugs covered by this convention. It also encourages international cooperation as a tool for fighting illicit international drug trafficking (UN, 1961; UNODC, nd).

The second major international treaty on drug control is the Convention on Psychotropic Substances 1971, which was written in response to the widening range of drugs of abuse. This convention establishes control over the use of psychotropic substances, including many synthetic drugs, based on their potential harm and therapeutic value (UNODC, nd; UN, 1971). A psychotropic substance is defined by the convention as "any substance, natural or synthetic, or any natural material in Schedule I, II, III or IV" of the convention (UN, 1971).

In contrast to the 1961 and 1971 treaties that seek to limit the trade in and use of controlled substances to legitimate scientific and medical purposes, the third major treaty was formed in an effort to deal with the growing problem of drug trafficking. The Convention Against the Illicit Traffic in Narcotic Drugs and Psychotropic Substances 1988 provides for the extradition of drug traffickers, and it offers provision against money laundering and the divergence of precursors (UNODC, 1988).

The effectiveness of this international drug control system is difficult to evaluate due to patchy data over the years; a concealed, illegal activity does not lend itself to simple quantitative monitoring. However, reports published annually by the UN now highlight the worldwide drug situation in yearly increments based in part on statistics provided by signatories to the major conventions.

On occasion, enough data are available to allow analysis of a drug trend over the last century. For example, The Shanghai Commission of 1909 was formed when the worldwide production of opium was estimated to be 30,000 metric tons, with 75% originating in China. Over one hundred years later, the global production has fallen by 80%, yet the world's population has tripled. Therefore, in terms of opium, it is reasonable to conclude that the global drug problem is contained and the major international conventions are effective.

Chemicals are essential to the manufacture of narcotic drugs. They are an integral component in the case of synthetic drugs and are required for the processing of coca and opium into heroin and cocaine. Only marijuana, of the major illicit drugs of abuse, is available as a natural, harvested product.

Chemicals used in drug manufacture are divided into two categories, precursor and necessary chemicals, although the term "precursors" is often used to identify both. Precursor chemicals are chemicals that are essential to the production of a controlled substance and for which no substitution can be made. Essential chemicals are used in the refining of the chemical processes, for example the refining of coca and opium into cocaine and heroin. Although some remain in the final product, the basic raw material is the coca or opium. Many essential chemicals required for illicit drug manufacture have extensive commercial applications, are widely traded, and are available from numerous source countries (King, 2003).

One of the major shortcomings of forensic science in general, is a lack of uniform consensus and international standards for the analysis of particular types of evidence. DNA typing has probably come the closest to achieving international consensus; however, in more recent times this has fallen short in the interpretation of mixed DNA profiles. The area of seized (abused) drugs, however, has come a long way in the development of standards and guidelines for analysis. This effort is personified by the work of organizations such as the UNODC, the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG; http://www.SWGDRUG.org) in the United States. Because of the major advances the latter group has made, its widespread adoption, especially in the United States and its international consensus, it is discussed in some detail.

During the last decade of the 20th century, the US Federal Government sponsored a group of discipline specific Scientific Working Groups (SWGs). With one exception, the SWGs were funded and managed by the Federal Bureau of Investication. The SWG that was created to set up standards and guidelines for the analysis of seized drugs (SWGDRUG) was set up and managed by the US Drug Enforcement Administration (DEA) in 1997 and still remains very active. It contains approximately twenty members, nearly half of which are international. During the intervening years, the SWGDRUG has been funded by the DEA, the US Office of National Drug Control Policy (ONDCP), and the National Institute of Standards and Technology (NIST).

The SWGDRUG was created to develop standards and guidelines for the analysis of seized drugs. Many of its recommendations have been submitted to the ASTM (American

Society for Testing and Materials, now ASTM International) for adoption as standards for the analysis of drugs.

The SWGDRUG's recommendations fall into four major categories:

- Code of Professional Practice
- Education and Training
- Methods of Analysis
- Quality Assurance.

The code of professional practice includes guidelines for professional conduct, characteristics of casework and standards for reporting the results of drug analysis.

The section on education and training specifies the minimum educational background and training for an analyst as well as provisions for continuing professional development. Initial training requirements are also spelled out.

The methods of analysis section is quite comprehensive. It has subsections on sampling considerations, methods of drug analysis, clandestine laboratory evidence and a new section on drug analogs and structural class determination. The section on methods of analysis has had a major impact on the field of seized drug analysis. It groups the most common analytical techniques into three categories based on the probative value and reviewable data of each one and then assigns analysis protocols to each category.

The last major section of the SWGDRUG's recommendations is on quality assurance. This is very comprehensive, covering general practices of quality assurance, validation of analytical methods and uncertainty.

As the SWGDRUG develops new guidelines, it provides ample opportunities for public comment before they are adopted.

1.3 Sampling

The taking of samples either at a crime scene or thereafter has obvious consequences for any subsequent investigation of that crime. The consequences of incorrect sampling may result in unusable data and potentially incorrect and improper conclusions where the adage of "rubbish in equals rubbish out" may easily apply. There is much published literature regarding sampling theory and the methods of sampling, much of which relates to specific disciplines or problems. Additionally, a specific aspect of sampling relating to the forensic sciences is that of sample integrity (being able to state that the sample gathered is the sample analyzed and the result obtained is from that sample),which must be maintained, as, if not, subsequent analytical results become questionable. Information regarding the sample is paramount, whether it has been gathered at the scene and transferred to the laboratory for analysis or has been generated *in situ* in the laboratory. Correct recording of the circumstances of the sample are essential: Who has handled it? For how long? Why?

Two things generally dictate sampling protocols:

- A legal obligation that may require that all items in a seizure are described and sampled.
- A policy left to the expert that will include a description of the items and the selection of a sample from the items.

The sampling strategy is fully dependent on the question being asked and, thus, the problem that has to be solved. There may be different needs for possession, production, or trafficking:

- Is a drug present? Minimal sampling (this may require just one positive result).
- Is a drug present in (more than) a specified proportion of the items?
- Is a drug present in all the items? (All samples to be examined.)

The criteria for selecting the type of sampling protocol undertaken should include a balance between the loss of completeness in terms of information versus time saving and that each new sample analyzed should produce new information.

The first stage in any procedure where sampling is to occur is an understanding of what it is that needs to beachieved. This will depend upon what type of sampling is being undertaken, whether sampling is occurring at the crime scene and the nature of that scene, or sampling at the laboratory. Whichever scenario is faced there are a few universal objectives:

- The sample should be representative of the material in question.
- The sample should be randomly chosen as appropriate
- Samples should be of adequate size for the analysis to be performed and to allow subsequent analysis to take place.

The basis of sampling is that the composition found in the samples taken reflects – in principle – the composition of the whole. As a consequence, only a fraction of the total packages in a seizure should need to be investigated.

Many laboratories have developed their own sampling protocol for drug samples. In all cases, samples should be taken from items that have the same morphology (also called sampling by attributes). This means that if a seizure is found to contain different types of materials, then each type should be sampled separately. This is particularly true for samples containing tablets with different logos or of different colors, or items that have been packaged separately from each other. Where blocks of resin are being sampled this should be away from the edges of the block, as such edges can potentially be used to make physical fits between the blocks.

In cases where samples of powder are seized, it is necessary to ensure that the powder is homogenous. This is generally accomplished using one of three methods:

- Cone and square method – the sample is ground up first and then poured onto a flat surface and flattened. The material is divided into quarters at right angles and one quarter shaped into a cone. The cone is quartered again at right angles and a quarter chosen and so on. This process is continued until a sample of sufficient size is prepared.

- Shaking in a plastic or nylon bag – the sample is placed in its entirety into the bag and shaken to mix it. This can result in larger particles falling to the bottom of the bag or particles of the sample preferentially adhering to the sides of the container because of charge differences depending on the size of compounds that may be present.
- Blending – the sample is placed into a blender and mixed mechanically. This method is very effective in homogenizing samples but it must be ensured that the blender is completely clean.

Sampling protocols will also be dictated by the nature of the analysis, identification/quantification, profiling or sampling at clandestine laboratories. An international system of sampling that has been adopted and promoted by the UNODC is:

Powders and tablets and packages Single package of material: The material should be removed from all packaging and weighed to a constant dry weight. The sample is then homogenized and a sample removed.

More than one package: The material in each package should be examined by eye for color differences. The contents of each package should be weighed (cleaning the balance in between each weighing) and each package should be tested using a color spot test or thin layer chromatography. If it is assumed that all packages contain the same material then:

- if there are less than 10 packages, all should be tested;
- if there are between 10 and 100 packages, 10 should be tested at random;
- if there are more than 100 packages, the square root of the total should be tested at random.

Liquids If all of one phase, then a sample of the liquid is removed for testing. If there is more than one phase present, then a sample of each phase should be removed for testing.

Control/comparison samples There are normally two types of control samples, positive controls (comparison samples) and negative controls (sometimes the same as blank samples).

1.3.1 Random sampling and representative sampling

A representative sampling procedure can be performed on a population of units with sufficient similar external characteristics (e.g., size, color). Many analytical methods require that random sampling of the item occur. A random sample is a selection of some members of a population in a manner such that each member of the sample is independently chosen and each member of the population has a non-zero probability of being chosen. Random does not mean haphazard but requires planning to ensure randomness. Random samples can be with or without replacement. Sampling without replacement (SWOR) means that once an item/element is removed from the population it is not put back and can, therefore, only ever be chosen once. Sampling with replacement (SWR) involves replacing items into the population after sampling.

The theoretical way to select a truly random, unbiased representative sample from a population is to individually number each item in the population and then use a random number generator to choose which item to select. This is not possible in practice, especially for large populations containing many thousands of units.

When sampling, two principles must be maintained:

- The properties of the sample are a true reflection of the properties of the population from which the samples were taken.
- Each unit in the population has an equal chance of being selected.

In reality, it is more difficult to adhere to these principles than it first seems because, when the population is high, it is impossible to number all the units and use a protocol based on a random selection of numbers. The practical solution is quite easy: after separating the samples by attributes all of the units in a group can be placed in a "black box" (plastic bag or similar) and samples selected. This kind of solution can be applied to practical cases, such as seizures of a thousand heroin street doses in similar external packages or a thousand tablets. This "black box" sampling method can reduce to a minimum any bias that may be introduced by the person selecting the samples and aims to prevent the sampler from consciously selecting a specific item from the population.

Any samples gathered must accurately reflect the material being tested (this is particularly true of control samples). The control sample and sample to be analyzed must, as far as possible, be adequate in size, so as to ensure that all presumptive and analytical tests can be carried out by forensic scientists acting for both the prosecution and defense. This can be difficult in the cases of trace amounts of illicit substances but there can, however, be no excuse for taking too little of a control sample. It is rare and in some cases impossible that an item can be re-visited in order to correct mistakes of inadequate sampling.

1.3.2 Arbitrary sampling

There are various arbitrary sampling methods that are used in practice across different drug analysis laboratories and work well in many situations. However, many have no statistical foundation and may lead to very large samples in cases where the original seizure is itself large. Not all existing sampling procedures are given here. Some laboratories use variations of these. In each case N = the total number of units and n represents the number of samples chosen (UNODC, 2009a):

1. All ($n = N$)
   Advantage(s): 100% certainty about the composition of the population.
   Disadvantage(s): Excessive sample sizes for larger populations.

2. $n = 0.05N$, $n = 0.1N$, etc.
   Advantage(s): Simple approach.
   Disadvantage(s): Excessive sample sizes for larger populations.

3. $n = \sqrt{N}$, $n = 0.5\sqrt{N}$, $n = \sqrt{\frac{N}{2}}$, etc.
   Advantage(s): Widely accepted approach.

Disadvantage(s): The samples may be too small when the population is small.

4. $n = 20 + 10\%(N - 20)$ *(*where $N > 20$*)*
   Advantage(s): Heterogeneous populations likely to be discovered before analysis is complete.
   Disadvantage(s): Excessive sample sizes for larger populations.
5. $$N < x \qquad n = N$$
   $$x \le N \le y \qquad n = z$$
   $$N > y \qquad n = \sqrt{N}$$

(where x, y and z are arbitrary numbers; $x < y$ and $x \le z < y$)

   Advantage(s): UN recommended method ($x = 10$, $y = 100$, $z = 10$).
   Disadvantage(s): Excessive sample sizes for larger populations.
6. $n = 1$
   Advantage(s): Minimum amount of work.
   Disadvantage(s): Least amount of information on the characteristics of the seizure.

1.3.3 Statistical sampling methods

There are two recognized approaches used in statistical sampling methods: a frequentist approach and a likelihood ratio approach (UNODC, 2009a).

1.3.3.1 Frequentist approach

The assumption behind a frequentist approach is that a fixed but unknown proportion of the seizure contains drugs and the proportion of drugs in a sample can estimate the proportion of drugs in the parent seizure. The sample proportion will, however, vary over different samples. One sample will give a higher proportion than another sample. Therefore, the frequentist methods provide a confidence, $(1-\alpha)100\%$ (for instance 95% if α is selected to be 0.05), that with a given sample proportion the seizure proportion is at least k × 100% (for instance 90% if *k* is selected to be 0.9). This means that if the sampled proportion is found to be as assumed then it can be stated with 95% confidence that the proportion of drugs in the whole seizure is at least 90%. The hypergeometric distribution can be used to calculate these probabilities (UNODC, 2009a).

1.3.3.2 Likelihood ratio approach

The assumption behind a likelihood ratio approach is that the proportion of drugs within the sample is known and fixed. This proportion is used to calculate probabilities on certain values of the unknown proportion of drug in the seizure, which at that point is still assumed variable. With this approach it is possible to incorporate some knowledge about the seizure that you may possibly have. The seizure proportion is not known but often some ideas about this proportion exist. For instance, if all plants in a cannabis

nursery appear similar they probably are all cannabis plants. It is also possible that there is no information about the amount and type of drugs in a seizure. These various forms of prior information will result in different mathematical models to estimate a desired sample size in the Bayesian approach (UNODC; 2009a).

1.4 Specific drug types

1.4.1 Cannabis

1.4.1.1 Introduction

Cannabis is the most consumed illicit drug in the world and, therefore, the subject of the most illegal trafficking in one form or another. The botanical name of cannabis is *Cannabis Sativa L.* and it is referred to as marijuana (herbal cannabis) and hashish (cannabis resin), terms associated with cannabis grown for its illicit use, or as hemp, a term usually associated with cannabis plants grown for their fiber content (UNODC, 2014a).

Cannabis is native to the mountainous areas of central and south Asia and man has used the plant for over 6,000 years. Cannabis grows over a wide variety of geographic terrains, altitudes and latitudes. It is grown in many countries and on all continents. Although it prefers the higher temperatures and longer growing seasons of the equatorial areas of the world, it has been cultivated as far north as 60° latitude. With the more recent prevalence for hydroponic growth, cannabis is known to be grown in most countries and remains one of the most used controlled substances globally (UNODC, 2014a).

A number of forms of the drug may be encountered, including plant material, resin and "hash oil." The active ingredient in all of these is Δ^9-tetrahydrocannabinol (Δ^9-THC). Also found is Δ^9-tetrahydrocannabinolic acid (THCOOH), which is converted to Δ^9-THC through smoking. Also present are the compounds Δ^8-tetrahydrocannabinol, cannabidiol (CBD), and cannabinol (CBN), as well as upwards of 50 minor cannabinols.

Plant material

Cannabis plant material can occur as live plants or as dried plant material. It is important when examining plant material that it should be in the dried state, as water content will affect any weight measurements, a crucial factor in determining the seriousness of any charges. Male and female cannabis plants are separate, with the male plant flowering before the female to ensure cross-pollination. The plant grows between 30 cm and 6 m in height; it grows from seed to maturity in about three months, although harvesting can occur after two months. The leaves are palmate with serrated edges and are opposite and alternate around a hollow four-cornered stem. The leaves are coated with upward pointing unicellular hairs called trichomes. Non-glandular trichomes are found on the stems and leaves together with a few glandular trichomes, which contain the Δ^9-THC. The greatest concentration of glandular trichomes is in the flowering tops of the plants and those of the female plant have a greater concentration of Δ^9-THC. Material prepared

from the flowering tops or leaves is commonly called marijuana and usually contains 0.5–5% Δ^9-THC. Cannabis plants grown under controlled hydroponic conditions, which are predominantly female plants (skunk), generally have a higher quantity of Δ^9-THC. (9–25%) and can reach full maturity in about 13 weeks.

Resin

This is also produced directly from the plant material. The glandular trichomes produce a resin that is scraped from the surface of the plant and pressed into blocks. Resin contains about 2–10 wt-% of the active constituent, Δ^9-THC. Usually between 100 and 400 mg of resin is used in a joint (cigarette), though this varies from user to user (UNODC, 2014a). The high increase in hydroponic growth, particularly in Europe, has seen a significant increase in the usage of highly potent plant material and a reduction in cannabis resin usage.

When examining cannabis resin several facts are recorded, including whether or not the blocks seized fit together, any striation marks (cutting marks) that may be present and whether these can they be linked to a cutting instrument. The color and different layers within the resin block should also be examined and recorded (Figure 1.1).

Hash oil

This is a manufactured product of cannabis produced by extracting the whole plant material using an organic solvent (usually alcohol, ether or benzene). On extraction the resulting oil can contain between 10 and 30 wt-% Δ^9-THC. The oil is used in various ways, including smoking in special pipes with tobacco.

1.4.1.2 The cannabinoids

There is a large number of known cannabinoids that can be divided into major and minor cannabinoids. The main cannabinoids (Figure 1.2) are cannabinol (CBN), cannabidiol (CBD), two isomers of tetrahydrocannabinol (Δ^8 and Δ^9-THC) and tetrahyrdocannininolic acid (THCOOH). Δ^9-THC is the cannabinoid that has the greatest pharmacological activity and it is this cannabinoid that needs to be detected in order to demonstrate that a sample is legally cannabis. CBD is the precursor of THC and CBN is the decomposition product of THC. THC is converted to THCOOH on smoking.

Other minor cannabinoids will exhibit similar oxidations; for example, THV (tetrahydrocannabivarin) will oxidize to CBV (cannabivarin). Both CBN and CBV are often absent from fresh plant materials, while the presence of CBD and its ratio to Δ^9-THC can often be a geographical indicator. The presence of THCOOH, THVA (tetrahydrocannabivarinic acid) and CBCh (cannabichromene) as well as others are also used in profiling, and it should also be noted that in gas chromatography (GC) analysis the THCOOH is converted thermally to Δ^9-THC (Lewis *et al.*, 2005; Le Vu *et al.*, 2006; Cadola *et al.*, 2013).

The major cannabinoids will decompose over time and, as such, the potential to link, for example, resin samples together using chemical composition is severely hampered.

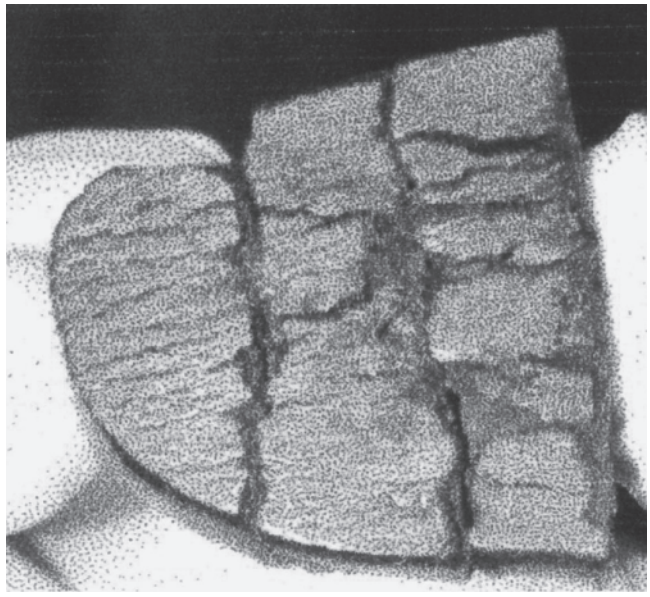


Figure 1.1 Three blocks of cannabis resin that physically fit together

Furthermore, studies have also exposed the issues associated with long-term storage of resin and plant extracts and demonstrated a steady decay in the concentration of Δ^9-THC and a rise in CBN levels (Trofin *et al.,* 2012; Lindholst, 2010).

1.4.1.3 Analysis of cannabis

A comprehensive text on the analysis of cannabis in all of its formulations is available from the UNODC Botanical analysis of Cannabis (UNODC, 2009b). Because cannabis is presented to the laboratories in plant form, the morphological characteristics of the plant are used to provide an identification of the material. Leaf shape and, in particular, the presence of the various trichomes of the plant are characteristic of *Cannabis Sativa L.* (UNODC, 2009b).

The main presumptive tests for cannabis are the Duquenois Levine test, which is carried out in test tubes and gives a violet layer in the presence of cannabinoids, and the Fast blue BB test, which gives a purple or red color change for cannabinoids. These tests are quite sensitive to the amount of Δ^9-THC present and can give negative results for "old" plant and resin samples, as the Δ^9-THC content may be reduced (UNODC, 2009b).

Thin layer chromatography (TLC) can be used to identify which drug is present within a drug class, as each different compound within the drug group can be separated on the basis of chemical structure, molecular weight, and so on. In many cases, the TLC plates need to be developed using chemical developers that give characteristic colors for the compounds under test.

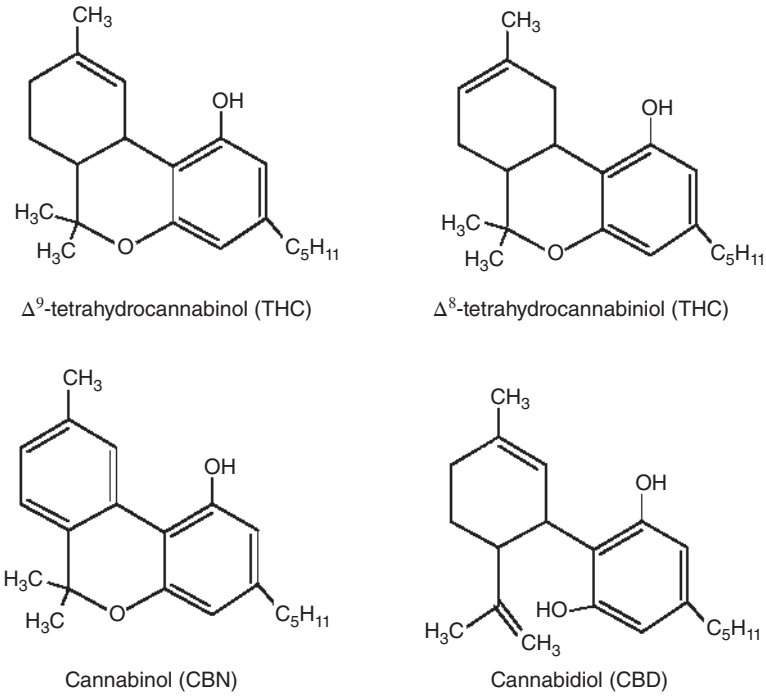


Figure 1.2 Chemical structures of the main cannabinoids

Both high performance liquid chromatography (HPLC) and gas chromatography or gas chromatography mass spectroscopy (GCMS) are also used as confirmatory analysis. However, in many cases microscopy, presumptive tests and TLC are deemed sufficient for court purposes.

Cannabinoids are weakly acidic compounds because of the phenolic group. This results in the compounds undergoing ionization in aqueous media forming compounds that will strongly interact with the stationary phase in HPLC analysis, causing tailing of the peaks when analyzed using liquid chromatographic methods. Because of this, ionization is suppressed by the presence of acetic acid in the mobile phase.

GC and GCMS analysis of cannabinoids tends to be favored over HPLC. However, the cannabinoids suffer severely from thermal decomposition and, as a result, do not chromatograph well (Lewis *et al.*, 2005). As a consequence, extracts from cannabis samples are often derivatized using, for example, N,O-bis(trimethylsilyl)acetamide (BSA). Figure 1.3 illustrates the main mass spectral fragmentation patterns for derivatized cannabinoids.

In more recent years, there has been a focus on the analysis of cannabis using DNA techniques. In particular, short tandem repeat (STR) DNA analysis has been used very successfully as a potential linkage tool between cannabis plants and does not suffer from the restrictions of chemical analysis (Gilmore *et al.*, 2003; El Alaoui *et al.*, 2013). The use of DNA analysis to explore the potential to link resin blocks together has also shown promise (Nic Daéid *et al.*, 2003).

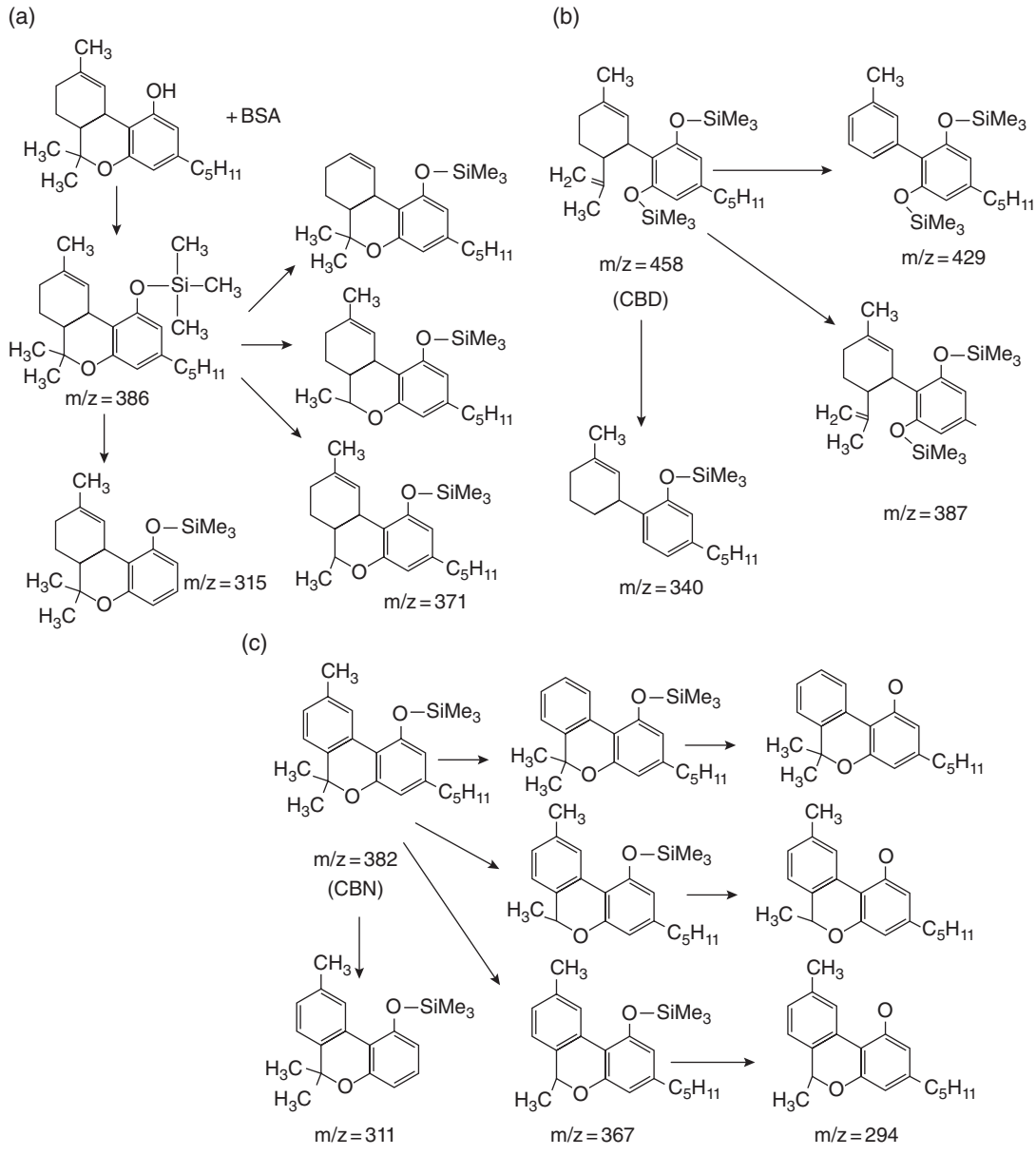


Figure 1.3 Fragmentation patterns for derivitized (a) THC (b) CBD (c) CBN

1.4.1.4 Recent issues in the control of cannabis

There have been a number of recent developments in the control of marihuana, principally in the United States, although some of these are occurring worldwide. The development of forms of so-called synthetic marihuana or synthetic cannabis has introduced serious issues of control and safety into the drug using community. Synthetic cannabis usually consists of substances that are designed to mimic the effects of cannabis

that are sprayed onto some type of plant material or herbal substances such as parsley. These formulations are sold as brand names such as Spice and K2. Synthetic cannabis first came on the scene in the United States in around 2000 and was initially thought to be comprised of all natural substances. A few years later it was discovered that the active ingredients were a large variety of synthetic substances. These include cannabicyclohexanol and other, more esoterically named, substances such as JWH-XXX or HU-XXX, where X = an integer. These substances were used to circumvent the laws that make cannabis illegal by substituting them for THC.

Another, more promising trend in the use of cannabis is the recognition that certain compounds, chiefly THC, may have significant medical benefits. The use of cannabis for medical purposes dates back thousands of years but little research had been done until recently to determine what diseases it may be used for, what doses and forms are best for medical treatment and what safety issues accrue with short or long-term use. In the United States, research has been hampered by the classification of cannabis in Federal Schedule I, which is reserved for drugs with a high potential for abuse and no accepted medical use, by the US Food and Drug Administration. Some medical agencies have urged the US Congress to move cannabis from Schedule I to Schedule II to facilitate research. A number of potential beneficial medical effects have been identified but most have not been proven. These include appetite stimulation, antiemetics (nausea reduction), control of involuntary spasms and even some pain management effects. It has also been experimented with in the control of some symptoms of AIDS. More than 30 States have adopted medical cannabis laws or are considering them. Bills have been introduced into the US Congress to remove Federal penalties against cannabis possession when it is used for approved medical purposes.

The most surprising development in the United States has been a move by three states (Alaska, Colorado, Oregon) and the District of Columbia to legalize outright the growing, possession and use of small amounts of cannabis. The initiative is most advanced in Colorado, where cannabis was legalized in 2013 and an entire growing and selling enterprise has been set up with the blessing of state government. The US Attorney General has directed the Drug Enforcement Administration (DEA) to suspend enforcement of Federal marihuana laws in states where it has been legalized by legislation. Although a large majority of citizens in DC voted in 2014 to legalize cannabis, elements of the US Congress seem bent on preventing implementation. Other states are now considering similar legislation.

1.4.2 Heroin

1.4.2.1 Introduction

Opiates belong to a family of compounds known as narcotic analgesics, narcotic meaning "tending to induce sleep" and analgesic meaning without pain. The natural opiate, morphine is extracted from the opium poppy; acetylation affords the semi-synthetic opiate diacetylmorphine (diamorphine). Heroin refers to a crude mixture of opium alkaloids

obtained from the extraction of morphine from opium and the subsequent acetylation reaction (Figure 1.4).

The psychological effects of opium have been known since 4000 BC and morphine was first isolated in 1805, followed by other opium alkaloids codeine and papaverine in 1832 and 1848. The pure alkaloids were prescribed for the relief of pain, coughs and diarrhea. In the 1860s, morphine was extensively used as a painkiller for wounded soldiers, often resulting in morphine addiction. Diamorphine was first synthesized in 1874 by acetylating morphine in an attempt to produce a new non-addictive painkiller; however, diamorphine was found to have narcotic and addictive properties far exceeding those of morphine. In 1914, the US Congress called for control of each phase of the preparation and distribution of opium, making it illegal to possess or supply these controlled substances. This, in turn, led to the start of illegal smuggling trades with the large scale smuggling of heroin into the United States in 1967 (Besacier and Chaudron-Thozet, 1999).

Papaver somniferum (*var. album and Papaver somniferum var. glabrum*) are the two varieties of opium poppy plants cultivated for the illicit production of heroin because of their high morphine content (*Papaver setigerum* plants also contain morphine) within the opium latex produced from the poppy seedpods. The raw latex of 1414 poppy seed pods were analyzed by the United Nations International Drug Control Programme (UNIDCP) and in

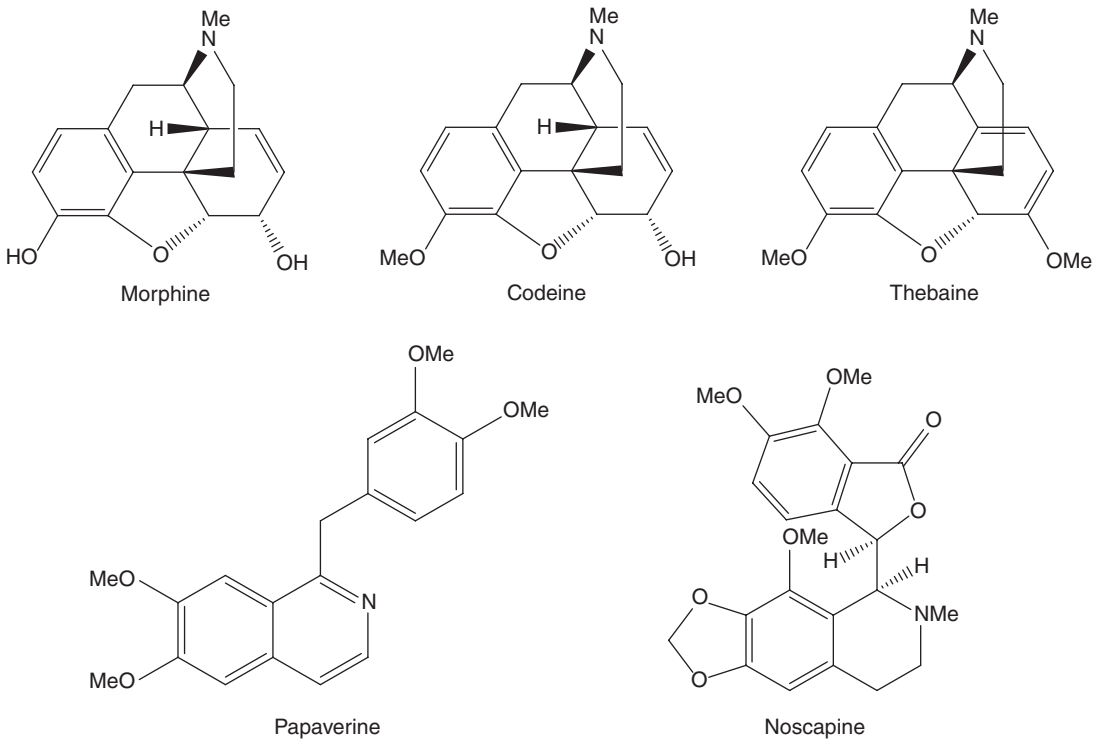
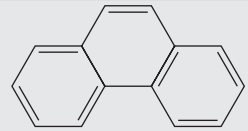
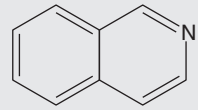


Figure 1.4 Chemical structures of major alkaloids

Table 1.1 Major alkaloid composition of opium

Alkaloid			
Class	**Structure**	**Name**	**Composition (%)**
Phenanthrene		Morphine Codeine Thebaine	3.1–19.2 0.7–6.6 0.2–10.6
Isoquinoline		Papaverine Noscapine	<0.1–9.0 1.4–15.8

total crude opium derived from *Papaver somniferum* was found to contain in the region of 25–30 alkaloids. The major alkaloid compositions are given in Table 1.1 (Drummer, 2001).

1.4.2.2 Heroin production

The raw material for the production of heroin is the opium poppy. The variety and age of the *Papaver somniferum* plant, together with the climate, altitude, soil fertility and moisture levels encountered during growth, affect the level and number of alkaloids found in the opium poppy.

The majority of clandestine laboratories extract morphine from opium using the "lime method." The raw latex from the opium poppy seeds is dissolved in boiling water to remove the insoluble plant material. Lime (calcium hydroxide) is added to the opium solution, converting water-insoluble morphine into water-soluble calcium morphenate; other insoluble alkaloids precipitate on cooling and are removed. The calcium morphenate solution is heated and the pH adjusted to 8–9 by addition of ammonium chloride. Upon cooling the precipitated morphine base is collected by filtration. Laboratories producing morphine as an end product perform further purification to remove traces of codeine, thebaine, papaverine and noscapine (Besacier and Chaudron-Thozet, 1999).

Clandestine manufactures typically acetylate the crude morphine base by addition of acetic anhydride, followed by heating to generate diamorphine via the intermediate 3-monoacetylmorphine (3-MAM). The cooled reaction mixture is typically treated with sodium carbonate to precipitate the heroin base. Dissolving the heroin base in acetone with the addition of hydrochloric acid generates the heroin hydrochloride salt.

Use of acetyl chloride is documented in New Zealand for the "home bake" preparation of heroin, which involves the initial production of morphine by demethylation of codeine with pyridine hydrochloride. Use of a mixture of trifluoroacetic anhydride and acetic acid or, alternatively, ethylene diacetate are reported; each of the different acetylation routes afford route specific markers (UNIDCP, 1998). The raw opium natural

product and subsequent synthetic steps involved in the illegal manufacture of heroin provide specific chemical profiles for the material.

1.4.2.3 Heroin impurities

Major opiate impurities include morphine, codeine, papaverine and noscapine. Thebaine is rarely observed in illicit heroin, as it decomposes during acetylation generating acetylthebaol (Figure 1.5). Minor opiate impurities from the opium include benzylisoquinolines (laudosine, narceine), tetrahydroisoquinolines, and cryptopine, as well as alkaloids of unknown structure. Non-opiate derived impurities from opium include meconin (Figure 1.5).

Impurities generated from the acetylation of codeine and thebaine are acetylcodeine (Figure 1.6) and acetylthebaol (Figure 1.5), respectively.

In addition, the incomplete acetylation of morphine using acetic anhydride affords 3-MAM while non-quantitative acetylation of morphine with acetyl chloride affords both 3-MAM and 6-MAM (Figure 1.6). The degree of skill of the illicit heroin manufacturer also determines the extent of diamorphine hydrolysis to generate predominantly 6-MAM. Huizer (1983) demonstrated that the reaction conditions used to convert diamorphine base to diamorphine hydrochloride also afford 3-MAM but further deacetylation to morphine occurs at a much faster rate than for 6-MAM. Occluded solvents trapped in the heroin matrix may include the acetone used in the conversion of diamorphine base to diamorphine hydrochloride or acetic acid generated during diamorphine deacetylation.

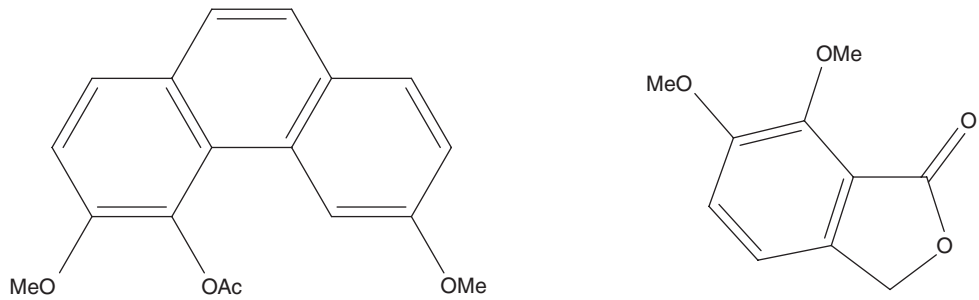


Figure 1.5 Chemical structures of acetylthebaol and meconin

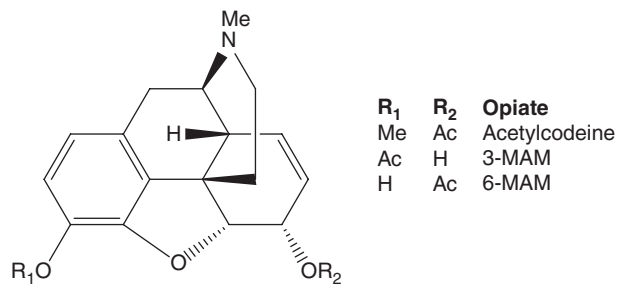


Figure 1.6 Chemical structures of acetylcodeine, 3-MAM and 6-MAM

1.4.2.4 Heroin cutting agents

Pharmacologically active adulterants are added to heroin. Caffeine and paracetamol are most common in Western European countries; paracetamol increases heroin base volatility, thereby increasing the effects from smoking heroin. Other adulterants include phenobarbitone, diphenyhydramine, procaine, and quinine. Inert diluents are also added to expand the heroin bulk and increase profit; the most frequently encountered diluents are sugars (mannitol, lactose and glucose) (Besacier and Chaudron-Thozet, 1999). Common cutting agents are listed in Table 1.2.

Kaa (1994) reported that heroin samples stored in the dark appear to be stable with no significant changes in their concentrations of acetylcodeine, papaverine and noscapine. However, storage for more than five years often resulted in diamorphine decomposition with increased 6-MAM and morphine content. Postprocessing diamorphine hydrolysis can also readily occur if the sample contains water that is not bound or excess acid (UNODC, 2005). Diamorphine decomposition studies varying pH and temperature have been conducted determining the ratio of 3-MAM and 6-MAM isomers obtained (Bernhauer *et al.*, 1983).

Although most opium poppy and opium production cultivation occurs in Afghanistan, there are three distinct production centers for opiates that supply three distinct markets. Trafficking flows tend to be from (UNODC, 2014a):

Table 1.2 Common cutting agents and their presence in heroin samples

			Heroin	
Cutting agent	**Description**	**Everyday use**	**Adulterant**	**Diluent**
Benzocaine	Local anesthetic	Throat lozenges and topical medicines	✓	
Caffeine	Stimulant	Ingredient in beverages and foodstuffs	✓	
Diazepam	Sedative	Medication for anxiety	✓	
Griseofulvin	Anti-fungal Agent	Medication for athletes foot	✓	
Mannitol	Sugar alcohol	Diabetic Sweetener		✓
Paracetamol	Analgesic	Pain relief preparations	✓	
Phenacetin	Analgesic	None	✓	
Phenolphthalein	Laxative and pH indicator	None as medicine	✓	
Procaine	Local anesthetic	Dentistry	✓	

- Afghanistan to neighboring countries and onwards to Europe through the Middle East ("Southern Route") or through the "Balkan route" across Eastern and Central Europe. There is increasing evidence to suggest other markets (Oceania and South East Asia) are also being targeted.
- Myanmar/Laos to neighboring countries of South-East Asia, (notably China) and to the Oceania region (mainly Australia).
- Latin America (Mexico, Colombia and Peru) to North America

There have been a large number of scientific investigations aiming to relate the relative and/or absolute alkaloid content of opium to the geographical source of the opium. Each major geographic source area produces a heroin that can usually be recognized as a chemically distinct type.

Southeast Asia heroin

Southeast Asia heroin is typically a white powder with high diamorphine purity (80%), as the hydrochloride salt, with few other alkaloids or adulterants. Papaverine and noscapine are seldom present, indicating effective purification of the intermediate morphine. The high similarity between chromatograms obtained from very pure samples results in poor resolution between subgroups (different areas of manufacture or processing techniques) in the Southeast Asia group.

Southwest Asia heroin

In contrast, Southwest Asia heroin samples are far more variable than those from Southeast Asia. The most common form is medium brown and of lower diamorphine purity (40–60%) as the base with noscapine (20–30%), papaverine (2–6%), and acetylcodeine (5–9%), plus many trace level alkaloid-related impurities. The second most common Southwest Asia heroin is light brown with a higher diamorphine purity (60-85%) with proportional decreases in the remaining alkaloids. Huizer (1983) similarly reported that heroin from Southwest Asia characteristically has high levels of both noscapine and papaverine with further discrimination possible based on the levels of acetylthebaol present.

Mexican heroin

Mexican heroin is unique by its appearance as a sticky black tar (30–60% diamorphine purity) or, less commonly, as a dark brown powder. It is often called "Mexican mud" and is sometimes cut with cocoa powder.

South American heroin

South American heroin is characteristically of high purity (>90%) with low acetyl codeine (<3.5%) and a low total content of 3-MAM and 6-MAM isomers (<5%). Other reports indicate low thebaine content with high papaverine levels (Odell *et al.*, 2006).

Indian heroin

Heroin from India contains less acetylcodeine than heroin from other countries suggesting a lower proportion of codeine to morphine (Kaa, 1987).

The United Nations determined the alkaloid ratios for heroin samples of known provenance and applied the ratios to identify the geographical origin of unknown samples as southeast Asia, southwest Asia, Mexican or South American. The variance between the ratios was found to be quite significant and there was a large overlap for each data set across the different heroin producing regions.

1.4.2.5 Analysis of heroin

Various literature reviews have summarized the many different methods employed in the comparative analysis of heroin samples (Chiarotti and Fucci, 1999; Dams *et al*., 2001; Nic Daéid and Waddell, 2005).

Presumptive color tests provide an indication that heroin or other opiate alkaloids may be present but are not specific, as many other compounds also give similar colors with the test reagents, necessitating an additional confirmatory technique. The Marquis reagent (8–10 drops of 40% formaldehyde added to 10 mL concentrated sulfuric acid) (UNIDCP, 1998) is widely used as a heroin field test and gives a characteristic purple color with diamorphine, morphine, codeine, 6-MAM or acetylcodeine; papaverine gives no color and noscapine gives a bright yellow color (UNIDCP, 1998).

TLC is often used as a simple and rapid screening technique to identify the opiate alkaloids and other components that may be present in heroin samples prior to examination by other methods (Levy *et al*., 1996). Examples of solvent systems are cyclohexane/toluene/diethylamine (75:15:10) and toluene/acetone/ethanol/ammonia (45:45:7:3). Developed TLC plates can be visualized using UV light at 254 nm or various spray reagents (Iodoplatinate, Dragendorff and Marquis).

Infrared spectroscopy is a useful screening technique that is more appropriate for the analysis of pure samples, such as heroin, rather than multiple component mixtures. However, Fourier transform infrared spectroscopy (FTIR) has successfully been used for heroin profiling (Simonov *et al*., 2000; Drozdov *et al*., 2001; Cai and Wu, 2007).

Major IR peaks are listed below in order of magnitude of wave number absorbance (cm^{-1}) for the heroin base and heroin hydrochloride; The unique IR spectra enable their differentiation (UNIDCP, 1998):

Heroin base:	1243	1196	1727	1214	1444	1757	1054	1370
Heroin hydrochloride:	1245	1736	1177	1194	1448	1765	1157	1386

However, determination of the heroin form within a sample is often not practical if the sample contains mixtures of the heroin salt and base forms or if the heroin base is adulterated with different salts.

Gas chromatography coupled with mass spectrometry (GCMS) generates highly specific mass spectral data enabling the definitive identification of both known and unknown components within heroin samples. Brenneisen and Hasler (2002) used GCMS to study heroin pyrolysis; they obtained 72 pyrolysis products. Heating the heroin street samples from 250 to 400°C produced substantial diamorphine degradation, whereas morphine, codeine, acetylcodeine, papaverine and caffeine were heat stable. The fragmentation pattern for heroin is presented in Figure 1.7.

Liquid chromatography (LC) techniques employed to study and/or profile major and minor heroin components include high performance liquid chromatography (HPLC) (Collins *et al.*, 2006; Hibbert *et al.*, 2010), sonic spray ionization for liquid chromatography mass spectrometry (LCMS) (Dams *et al.*, 2002), and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Lurie and Toske, 2008). LC techniques eliminate the adsorption, heat instability and transesterification problems often associated with GC heroin analysis and sample derivatization is not required. LC limitations include the need for component solubility, poorer resolution, high solvent consumption and associated solvent waste disposal.

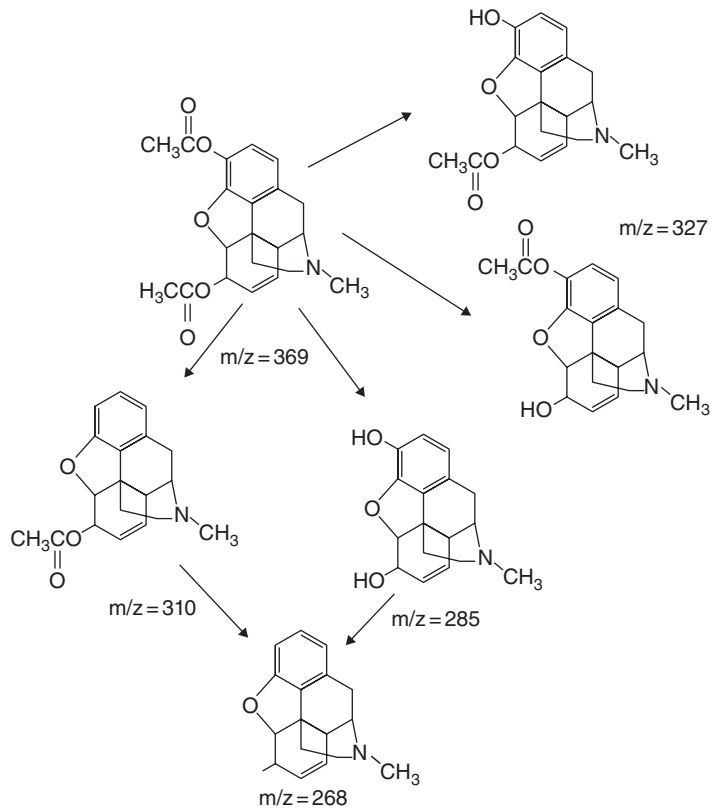


Figure 1.7 The fragmentation pattern for heroin (no derivitization)

Capillary electrophoresis (CE), capillary electrochromatography (CEC) with laser-induced fluorescence (LIF), and capillary electrophoresis-mass spectrometry (CE-MS) have been widely used to analyze heroin samples (Anastos *et al.*, 2005). The separation obtained by CE in narrow bore capillaries under the influence of an electric field is highly efficient, selective, rapid, and may be applied to both charged and neutral species. In addition, CE has been used to determine the concentration of carbohydrates (glucose, sucrose, lactose, mannitol and mannose) found in heroin samples (Lurie *et al.*, 2006).

A range of techniques have been studied to examine the trace inorganic impurities found in heroin samples originating from the elements present in the original opium poppy plus those introduced during the manufacturing process. Methods include elemental analysis (EA) to determine major metal (Ca, Mg, Al, Fe, Zn, Ba) and trace metal (Mn, Cu, Pb, Cd) concentrations (Bora *et al.*, 2003).

Isotopic analysis of heroin samples to determine isotope ratios of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$ as markers for their geographical origin has been extensively studied using gas chromatography-isotope ratio mass spectrometry (GC-IRMS) (Idoine *et al.*, 2005a, 2005b; Zhang *et al.*, 2005).

1.4.3 Cocaine

1.4.3.1 Introduction

Cocaine is a semi synthetic drug produced from the coca plant, mainly in South America. Extraction from the plant produces a large number of cocaine alkaloids, most of which do not appear in the final product. The main cocaine alkaloids found in street cocaine are shown in Figure 1.8.

No two illicit samples of cocaine will be exactly the same, though the variation from sample to sample is much less than for heroin, for example. Cocaine is commonly encountered in two forms: the salt (cocaine hydrochloride) and the base (cocaine). Certain forms of cocaine base are also known as "crack." Both forms are a white crystalline powder; however, the base form can appear as a more "waxy" solid.

The cocaine content in the plant is usually from 0.3 to 1.5% relative to dry leaf weight but 10–15% *cis*- and *trans*-cinnamoylcocaine is also present. The cocaine purity in coca paste varies from 30 to 80% depending on the extraction technique.

1.4.3.2 Cocaine production

There are over 250 species of the coca plant, two of which are known to produce cocaine: *Erythroxylum coca* and *Erthroxyylum novogranatense* (Nordegren, 2002). *Erthroxyylum novogranatense* is grown mostly in Columbia and other Central American countries, while *Erythroxylum coca* is grown in Boliva and Peru (Freye and Levy, 2009). Columbia, Bolivia and Peru are the three main areas for the production of cocaine (UNODC, 2014a). The coca plant grows in tropical rainforest climates between 100 and 1700 m above sea level.

The appearance of the coca leaves can vary depending on the variety of the coca plant, although there are some commonalities between them. For example, the upper side of

the leaf, which can be green or greyish in color, is always darker than the underside, which has two lines running parallel to the midrib (UN, 1986).

The growth cycle of the coca bush is anywhere from six to nine months and can be harvested between three and eight times during the year, but this is dependent on a number of factors, including seasonal variations such as rainfall and temperature. Other factors include disease or spraying of herbicides onto crops in counter narcotics operations, coca species, soil type and horticultural practices, for example, fertilization and irrigation (UNODC, 2014a). However, the main growth period is from December to April. Once picked the coca leaves must be dried so as to preserve the cocaine content (Nordegren, 2002).

The production of most illicit cocaine occurs in very crude and rudimentary settings, often by the coca farmers themselves, and, as a result, there can be a number of variations in the reagents and quantities used. The production process begins with the mixing of the leaves with water and a basic material, for example, lime, followed by the crushing of the leaves to form a pulp. Kerosene is then added and mixed with the pulp to extract the alkaloids from the coca leaves. The kerosene is then removed and separated from the leaves. Acidified water is added to the kerosene, extracting the alkaloids into the aqueous layer. The aqueous layer is removed and used to make the coca paste (UN, 1986). Calcium carbonate (lime) or ammonium is added to make the solution basic, precipitating out the basic alkaloids, which are then collected and allowed to dry, forming the coca paste (UN, 1986).

In order to produce cocaine, the coca paste is added to dilute sulfuric acid, dissolving it, and causing the hydrolysis of the alkaloids, which forms ecgnine. This is then reacted with 10% boron trichloride to form ecgnine methyl ester. Potassium permanganate is

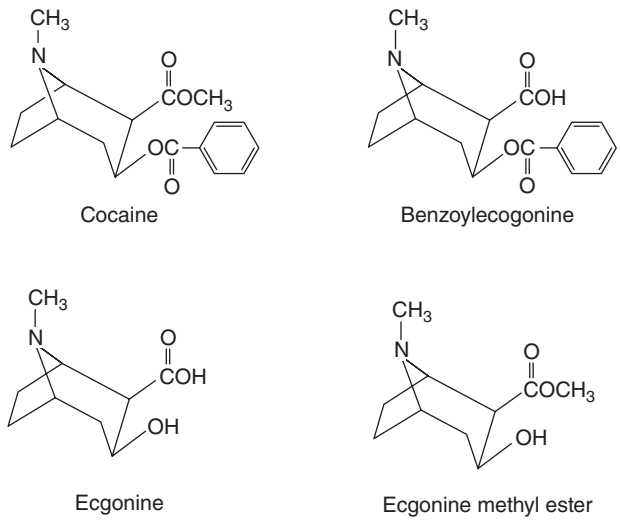


Figure 1.8 The main cocaine alkaloids

also added until the solution turns and remains pink, in order to eliminate any cinnamoylcocaine isomers present. The solution is then filtered and a base, such as ammonia, is added, causing the precipitation of the cocaine free base (UN, 1986).

The cocaine free base is filtered off, washed with water and allowed to dry. It is then dissolved in ether and the mixture is filtered, after which hydrochloric acid and acetone are added to precipitate out the cocaine hydrochloride, which is then filtered off and allowed to dry.

The free base form is more potent than cocaine hydrochloride and, as such, it is often reverted back to its free base form using one of two methods. The less common and more dangerous method is called the ether wash method. The baking soda method is much more popular and involves mixing cocaine hydrochloride and sodium bicarbonate (baking soda) and heating. The result is a hard, crystal like material known as "rock cocaine" or "crack" (Freye and Levy, 2009).

1.4.3.3 Cocaine cutting agents

A range of impurities are present in cocaine, some of which are hydrolysis by-products, formed during the extraction and purification processes, while others are present in the solvents and processing chemicals used. Some may also due to the packaging materials used and the storage conditions under which the packages are kept (Casale and Waggoner, 1991).

Adulterants and diluents are also added to the cocaine before being sold. Common adulterants include lidocaine, procaine and benzocaine, and diluents include sugars such as mannitol, lactose or glucose. A table of common cutting agents is provided in Table 1.3.

1.4.3.4 Analysis of cocaine

Analysis of cocaine follows the normal methodologies of other drug samples where initial colorimetric tests provide a tentative identification, followed by TLC and then more specific chromatographic techniques. The main presumptive test for cocaine is the cobalt thiocynate test. A rapid development of a bright blue color indicates the presence of cocaine; however, a similar color may develop over time for barbiturates as well.

Gas chromatographic analysis of cocaine alkaloids is not straightforward and generally requires derivatization of the molecules in order to be successful. Casale and Waggoner (1991) developed a one-step derivatization method for the identification and quantification of a number of coca related impurities in unadulterated cocaine samples. They concluded that by using this method along with statistical analysis it was possible to identify samples of a common origin or batch, and that samples from the same batch gave "virtually identical chromatographic profiles," and while hundreds of batches were tested no two batches gave similar chromatographic profiles. Cocaine itself, however, does produce reasonably well defined peaks using GC or GCMS. Derivatization is generally accomplished with BSA or BSTFA (N,O-Bis(trimethylsilyl)trifluoro acetamide). The derivatization products and mass fragmentation are given in Figure 1.9.

Table 1.3 Adapted table of common cutting agents and their presence in cocaine samples

			Cocaine	
Cutting agent	**Description**	**Everyday use**	**Adulterant**	**Diluent**
Aspirin	Analgesic	Pain relief preparations	✓	
Benzocaine	Local anesthetic	Throat lozenges and topical medicines	✓	
Boric Acid	Antiseptic and insecticide	Acne medication and insect control preparations		✓
Caffeine	Stimulant	Ingredient in beverages and foodstuffs	✓	
Creatine	Dietary supplement	Body-building		✓
Diazepam	Sedative	Medication for anxiety	✓	
Glucose	Sugar	Ingredient in beverages and foodstuffs		✓
Lactose	Sugar	Filler in tablets and ingredient in foodstuffs		✓
Levamisole	Antibiotic	Medication for worm infections		✓
Lignocaine	Local anesthetic	Dentistry and topical medications	✓	
Mannitol	Sugar alcohol	Diabetic sweetener		✓
Paracetamol	Analgesic	Pain relief preparations	✓	
Phenacetin	Analgesic	None (withdrawn from use in the UK)	✓	
Procaine	Local anesthetic	Dentistry	✓	
Starch	Carbohydrate	Ingredient in foodstuff		✓
Sucrose	Sugar	Ingredient in beverages and foodstuffs		✓

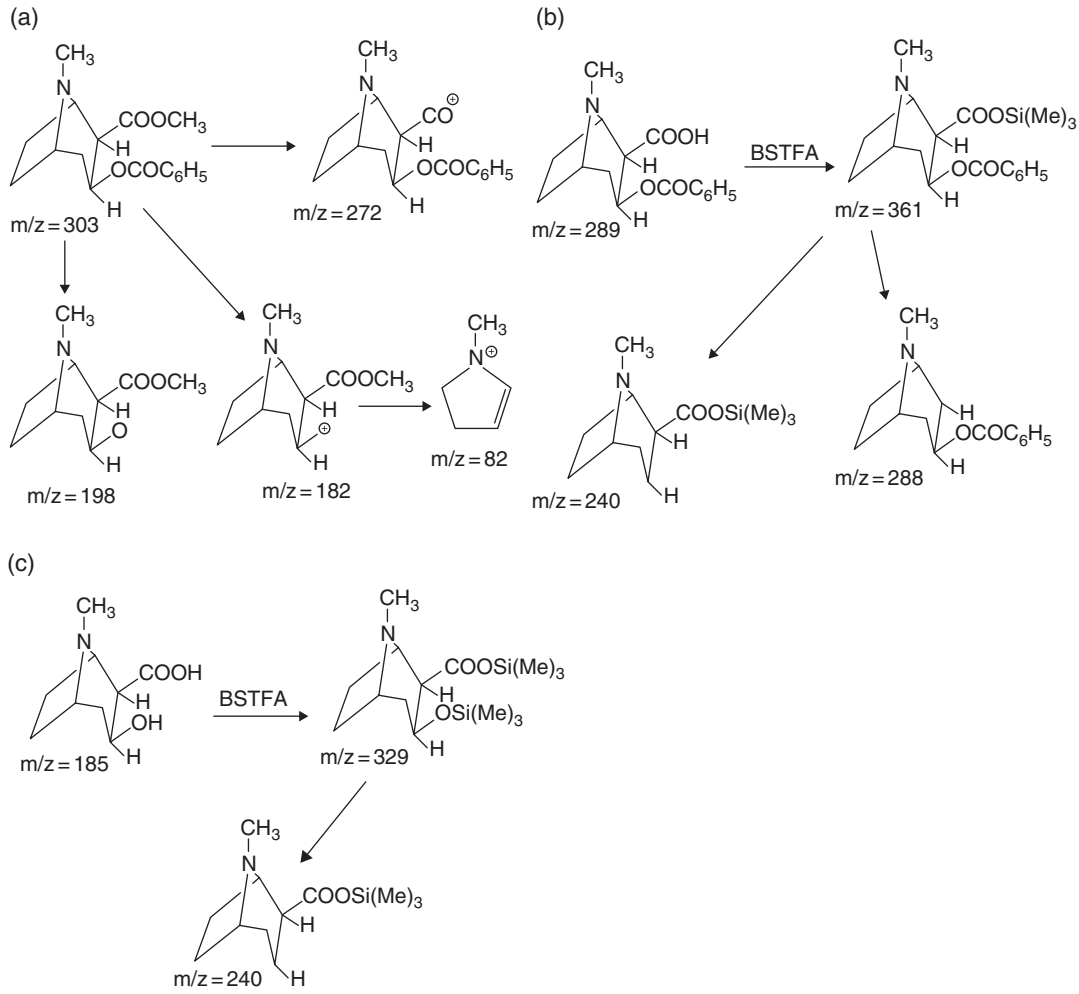


Figure 1.9 Fragmentation patterns for (a) underivitized cocaine (b) derivitized benzoyl ecgonine and (c) derivitized ecgonine

LeBelle *et al*. (1991) found that while the alkaloid composition may be similar between cocaine samples, the solvent residues found can be different, thus differentiating two samples as different solvents were used during manufacturing. They also concluded that from the profiles generated it was possible to differentiate between large production batches or manufacturers. Morello and Meyers (1995) successfully developed a method for the simultaneous identification and quantification of the residual solvents in cocaine a few years later.

A significant body of work has been undertaken in the use of isotope ratio mass spectrometry (IRMS) for cocaine analysis, particularly by Ehleringer and colleagues. In cocaine samples from the four major growing regions they found that the samples "differed by

0.6‰ and 7.1‰ in their $\delta^{13}C$ and for $\delta^{15}N$ values," and, as such, it was concluded that there is the potential for using isotope analysis to identify the geographical origin of cocaine (Ehleringer *et al.*, 1999). Ehleringer *et al.* (2000) also carried out a large study on 200 cocaine samples. They analyzed ^{13}C and ^{15}N and also the truxilline and trimethoxycocaine content. They found that there was a wide range in the $\delta^{13}C$ and $\delta^{15}N$ values obtained; the ^{13}C and ^{15}N values varied from –32.4 to –25.3 for ^{13}C and from 0.1 to 13.0 for ^{15}N. They also noted that "truxilline content and cocaine ^{15}N are positively correlated and that cocaine ^{13}C and trimethoxycocaine are negatively correlated." Combining these factors they correctly identified the country of origin of 192 of the samples. Casale *et al.* (2005) examined the isotopic fractionation of carbon and nitrogen and found that the processing of the cocaine only "caused minimal changes in the $\delta^{13}C$," although fractionation against ^{15}N does occur, during the conversion of the cocaine base to the hydrochloride salt form, but is less prominent the higher the yield of cocaine hydrochloride obtained.

1.4.4 Amphetamine-type stimulants

1.4.4.1 Introduction

Amphetamine-type stimulants (ATS), including amphetamines, methylamphetamine and ecstasy, remain the second most widely consumed group of illicit substances. Amphetamines are β-phenethylamine derivatives and totally synthetic drugs and, as such, are very different in terms of the potential for analysis, as in all cases there are many synthetic by-products carried through each stage of the synthetic process. These synthetic by-products can help to identify the specific synthetic mechanism used in their production. Amphetamine-type stimulants are generally amphetamine sulfate, methylamphetamine hydrochloride or free base and 3,4-methylenedioxymethylamphetamine hydrochloride (ecstasy).

ATS are commonly encountered in one of four forms: tablet, powder, free base or as crystaline material. Tablets normally contain 3,4-methylenedioxymethylamphetamine hydrochloride or methylamphetamine hydrochloride and caffeine. In the United States, methylamphatamine tablets are encountered in different colors and shapes that can be flavored, scented and stamped with various logos in a similar fashion to conventional ecstasy tablets.

The powder form is normally either amphetamine or methylamphetamine. It is bitter to taste and water soluble. The color of the powder ranges from off-white to reddish-brown depending on the chemicals used in the manufacturing process.

Crystalline methylamphetamine is usually obtained by recrystallizing the hydrochloride powder using isopropyl alcohol or water. This form of methylamphetamine has the highest purity compared to other forms. The structures of these compounds are provided in Figure 1.10.

Most laboratories that have been dismantled by law enforcement have been manufacturing methylamphetamine and were concentrated in North America, East and Southeast Asia. Approximately half of the methylamphetamine seizures in the United States now

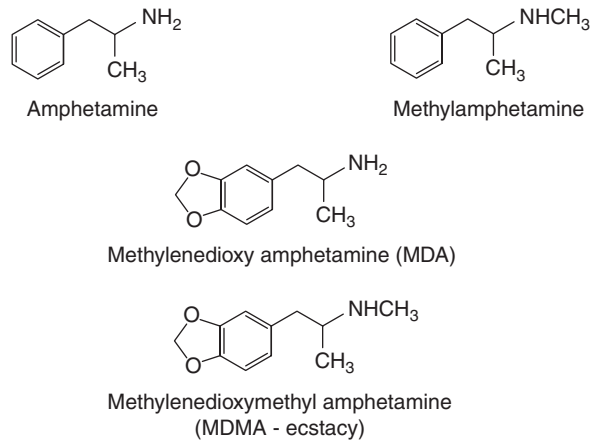


Figure 1.10 Amphetamine-type stimulants

occur at the US–Mexican border and large-scale production is now known to occur in Mexico (UNODC, 2014a). As Mexico respond has responded with strong counter methylamphetamine initiatives, manufacturing activities are tending to move south to Latin America, including Argentina, Guatemala, Honduras, and Peru. Similar shifts may also be occurring in South Asia, where India and Sri Lanka reported their first operational methylamphetamine laboratories in 2008, and reported seized manufacturing equipment and chemicals in 2007.

Large quantities of amphetamine seizures continue to be reported in the Middle East, in particular by Jordan, Saudi Arabia and the Syrian Arab Republic. Major quantities of "ecstasy" were seized in East and Southeast Asia, followed by Europe (Southeastern Europe and Western and Central Europe). All three regions account for nearly three quarters of global "ecstasy" seizures.

1.4.4.2 Synthesis of amphetamine-type stimulants

The most common methods used in the production of ATS usually involve more than one synthetic step and involve simple reflux apparatus or more complicated pressure controlled reaction vessels. Three categories of chemicals are used in the synthesis of amphetamine type stimulants: precursors, reagents and general purpose chemicals. The molecular structure of the precursor is generally quite similar to that of the final product. The purpose of reagents in the synthesis is to chemically modify or combine precursors in a chemical reaction. General purpose chemicals are used to facilitate the reaction and isolate the product. Examples include solvents, alkalis and acids. In many countries, the sale of these chemicals is placed under strict control and regulated by the individual legislation of the countries involved. Besides chemicals, another important tool required in the clandestine manufacture of ATS is appropriate glassware, which can be obtained either from chemical supply companies or made out of household items.

Amphetamine

The most common European method for the production of amphetamine is called the Leuckart synthesis which is a two-step formulation and hydrolysis of phenyl-2-propanone (P2P) (also known as benzylmethylketone [BMK]), phenylacetone and phenylpropanone. Reductive amination of P2P can also be used to produce amphetamine; this reaction is carried out under reduced pressure and homemade pressure reaction vessels are commonly used. The third common method is via a nitrostyrene intermediate to produce amphetamine. P2P is the starting product for each of these methods and as the major precursor chemical is itself under legislative control. Each synthetic route will produce a "family" of different reaction by-products and impurities. Some of these will be specific to the route used while others will be common across routes.

The Leuckart synthesis is one of the most common routes to synthesis of amphetamine as the sulfate salt. It involves a formylation reaction between P2P and formamide followed by a hydrolysis reaction to form the amphetamine.

Reductive amination of P2P is a popular method for the production of amphetamine in parts of Europe. P2P is reacted with ammonia to form an imine. The imine is then reduced under high temperature and pressure to the corresponding amine.

The nitrostyrene synthesis involves the use of lithium aluminum hydride as a reducing agent. The reaction is a two-step reaction involving the formation of nitrostyrene followed by a lithium reduction. The initial reaction involves mixing benzaldehyde with nitroethane and ammonium acetate in acetic acid. The mixture is refluxed to form nitrostyrene, which is purified by recrystallization in ethanol. This is followed by a dilution of the nitrostyrene in a solvent (this can be ether, toluene or THF) and the addition of this solution, drop wise, to a solution of lithium aluminum hydride, keeping the temperature of the reaction mixture cool. The reaction is refluxed, after which the excess lithium aluminum hydride is quenched and removed. Amphetamine free base is extracted into ether and precipitated as the sulfate salt with sulfuric acid.

Methylamphetamine

In general, methylamphetamine is synthesized from one of two different types of known precursors: *l*-ephedrine or *d-pseudo*ephedrine and phenyl-2-propanone (P2P). Ephedrine and *pseudo*ephedrine are used commonly in cold medication and sold as over-the-counter medication in various countries. To combat the isolation of ephedrine and *pseudo*ephedrine from medication, manufacturers increasingly add various inhibitors such as aminoalkyl methacrylate copolymer (Eudragit-E), ferrous gluconate, lactose, ethylcellulose, and hydroxypropyl cellulose, which interferes with the conversion of ephedrine or *pseudo*ephedrine to methylamphetamine.

Besides extracting ephedrine and *pseudo*ephedrine from cold medication, clandestine chemists also isolate the compounds from *Ephedra* plant based products. The stem and leaves of the Asiatic and Mediterranean species of *Ephedra* contain ephedrine and *pseudo*ephedrine as well as other alkaloids, such as norephedrine, nor*pseudo*ephedrine, methylephedrine, and methyl*pseudo*ephedrine (Massetti, 1996).

There are eight synthetic methods generally used in the illicit manufacture of methylamphetamine hydrochloride; these are presented in Figure 1.11. These methods are easily accessible through the Internet, the scientific literature, patents and books. The method used to synthesize methylamphetamine depends on various factors, such as availability of precursors, essential chemicals, the complexity of the process, equipment and the chemical hazards presented. With increasing Internet usage, many clandestine chemists can purchase the required supplies using Internet auction sites (Massetti, 1996).

MDMA

The clandestine synthesis of 3,4-methylenedioxy-methamphetamine (MDMA) usually begins with the synthesis of its major precursor, phenyl methyl ketone (PMK), which can be achieved in a number of ways (Dal Cason, 1990) A survey of "underground" web sites and recent literature on drug profiling (most of which used *PiHKAL* as a source) indicated that common routes to PMK originate from safrole or isosafrole and proceed via the isosafrole glycol to PMK (Figure 1.12).

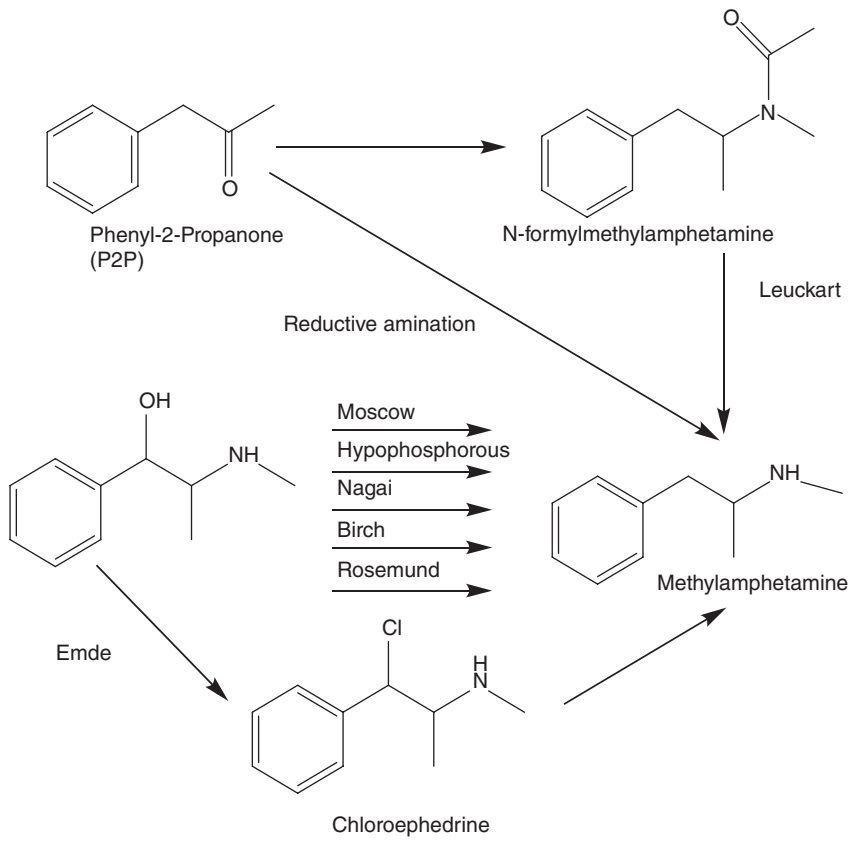


Figure 1.11 Outline of the synthetic routes used in the manufacture of methylamphetamine

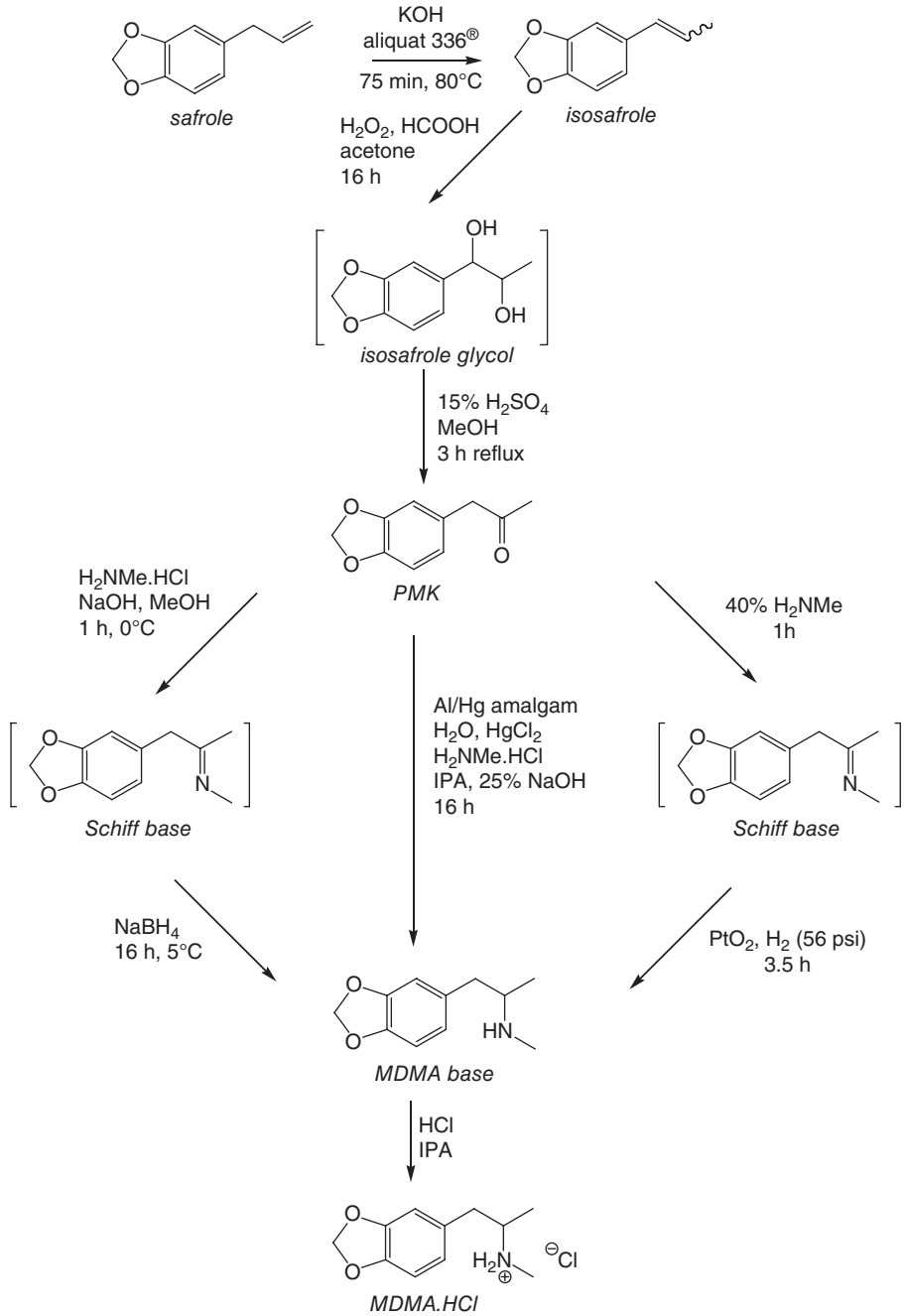


Figure 1.12 Summary of the synthesis pathways to MDMA

The root bark of the sassafras tree contains 5–9% aromatic oil, of which approximately 80% is safrole. Despite the suspected carcinogenic properties (both sassafras oil and safrole are banned as flavors and food additives by the United States Food and Drug Administration), sassafras oil can still be obtained as an "essential oil" in outlets such as health food shops.

PMK can be used to synthesize MDMA by three variations of the popular route of reductive amination. Three different reducing agents can be used: Al/Hg amalgam, $NaBH_4$ ("cold method"), and Pt/H_2 ("high pressure method"). Reportedly, reductive amination with the platinum catalyst has, in recent years, become in the most commonly encountered method of MDMA production in the Netherlands, with $NaBH_4$ sometimes encountered, and Al/Hg rarely encountered (Koper *et al.,* 2007).

1.4.4.3 ATS cutting agents

ATS also contain a range of substances being used as cutting agents. These vary considerably from country to country. Common materials include sugars (mainly lactose of glucose), caffeine, and other anesthetics, such as "Ketamine," and depressant drugs such as barbiturates.

1.4.4.4 Analysis of ATS

Characteristics such as logos, color, size and shape are all recorded as part of the initial examinations carried out. It may be possible to link the logo on tablets to provide intelligence information across drug seizures, although such data should be treated cautiously. The main presumptive tests used for amphetamine compounds are the Marquis and Mandelin tests providing a brown–orange or green response and green response to ATS, respectively. TLC can provide extra information, potentially identifying which of theATS may be present in a particular sample. In this case, is it common to use a developing reagent, such as ninhydrin, acidified iodoplatinate or Fast Black K, to visualize the separated samples.

Both HPLC and GCMS are used when quantification of amphetamines is required. The amphetamines generally chromatograph well on both systems. There can be some coelution of amphetamine, methylamphetamine, MDA and MDMA occurring and it is wise to always run standards separately to establish the specific retention time of each compound. Mixtures of amphetamines in powders or tablets are not usual but do sometimes occur. Many amphetamine samples also contain caffeine, which has a much greater molar absorptivity coefficient than amphetamine and, as such, will have a much greater peak area than amphetamine, even though the concentration of both within the sample may be similar. This can also cause problems in the resolution of peaks in HPLC analysis.

Amphetamines generally chromatograph well underivatized using GCMS instruments, though many laboratories still chose to derivatize the samples. Derivatization in this case occurs using, for example, trifluoroacetic acid (TFA) where chemical modification occurs at the amine functional group.

Other analytical methods, in particular IRMS and ICP-MS (inductively coupled plasma-mass spectrometry), have been used for the chemical profiling of ATS and, especially, methylamphetamine and MDMA; the reader is directed to the specific literature in this area, as it is beyond the scope of this chapter.

1.4.5 New psychoactive substances

1.4.5.1 Introduction

New psychoactive substances are synthetic substances that have recently become available on the market. As of yet, many are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic substances (UNODC, 2014b). They are often referred to as legal highs, designer drugs or bath salts or spice.

The use of NPS has grown rapidly over the last few years and there are an increasing number of reports on the availability and manufacture of these substances. By 2013, 348 NPS had been reported to UNODC, which is more than the 234 psychoactive substances currently under governance of the 1961 Single Convention on Narcotic Drugs and the 1971 Convention on Psychotropic Substances. New psychoactive substances have been reported in every region, a total of 94 countries worldwide (UNODC, 2014b). Synthetic cathinones make up 25% of the NPS reports to UNODC. Cathinones are analogues of amphetamines and the beta-keto derivatives of phenethylamines; each phenethylamine compound has a parallel cathinone analogue (Schifano *et al.*, 2011) (Figure 1.13).

Synthetic cannabinoids

Synthetic cannabinoids are compounds that have been prepared to have the same functionality as Δ9-tetrahydrocannabinol (THC) in terms of their interactivity with the cannabinoid receptors in the brain. In late 2008, several cannabinoids were detected in herbal smoking mixtures or so-called incense/room odorizers. These products are typically sold via the Internet and in "head shops." Many of the synthetic cannabinoids are structurally unrelated to THC; however, they do often contain a side chain containing between four and nine carbon atoms. The synthetic cannabinoids can be classified into six major structural groups:

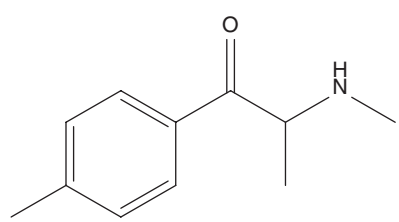


Figure 1.13 4-Methylmethcathinone (mephedrone)

- Naphthoylindoles (e.g., JWH-018, JWH-073 and JWH-398).
- Naphthylmethylindoles.
- Naphthoylpyrroles.
- Naphthylmethylindenes.
- Phenylacetylindoles (i.e., benzoylindoles, e.g., JWH-250).
- Cyclohexylphenols (e.g., CP 47,497 and homologues of CP 47,497).

Substituted cathinones

Substituted cathinones usually take the form of a white/off-white/yellow powder; they are found less frequently as tablets or capsules. The most common route of ingestion is insufflation but they can also be ingested orally, rectally and intravenously. When snorted the effects are felt immediately with a short high and a rapid comedown. Polydrug use is a common habit; it is where multiple drugs are taken in order to increase the high and prolong the effects, thus increasing the potential harm and toxicity effects.

The synthetic cathinone most commonly reported to the United Nations Office on Drugs and Crime in 4-methylmethcathinone, more commonly known as mephedrone. The first online reference to mephedrone was in 2003. However, it became increasingly popular and readily available for purchase in 2007. The rise in popularity has been attributed to a number of factors. Mephedrone was viewed as a legal alternative to illicit substances, meaning it was also viewed as being safer. The decreasing purity in ecstasy and cocaine also led to an increase in mephedrone consumption, as it was seen as a cheaper, more potent alternative. Mephedrone was also readily available to purchase over the Internet and in head shops and was largely driven by web-based marketing, exposing it to a whole new range of audiences (Schifano *et al*., 2011).

The rise of the Internet as a global marketplace for drug information, distribution and supply presents a unique challenge in disrupting the supply of NPS (EMCDDA, 2014). NPS that are not controlled under international drug laws are being produced outside of Europe and obtained through online retailers. The Internet is set to become the newest method of drug trafficking and law enforcement and policy makers must take this into account in the future.

The speed at which controlled substances are being replaced by new substances provides serious challenges for lawmakers. Once a substance is deemed to present a certain level of harm it is legislated against, but this gives rise to rebranding or development of another alternative with unknown toxicity. When a substance is removed from the drug market it is likely to be replaced almost immediately and the cycle begins again (Winstock and Ramsey, 2010). The time it takes to pass legislation controlling substances cannot keep up with the speed at which new psychoactive substances are appearing on the market, leading policy makers to demand new, faster and more effective ways of drug

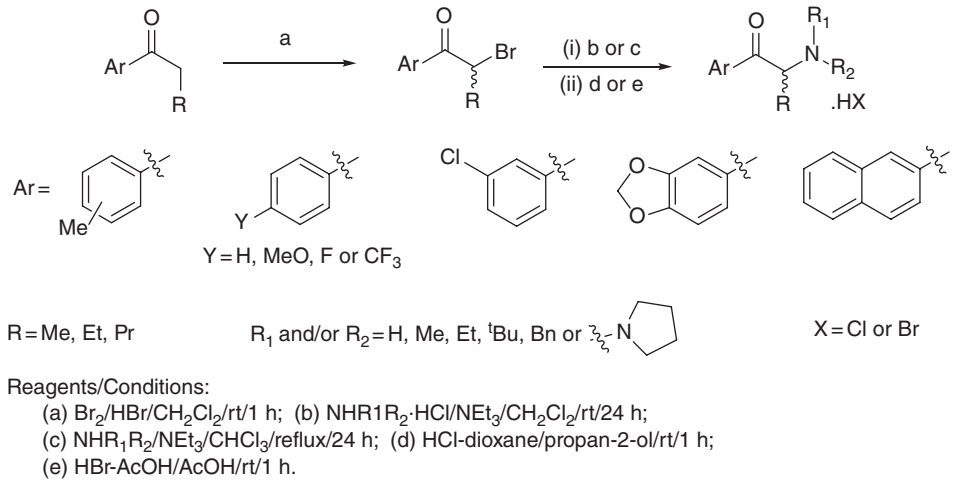


Figure 1.14 Reaction scheme for the preparation of cathinone derivatives

control. As fast as law makers can identify new substances and assess the risk, another one has already taken its place.

1.4.5.2 Synthesis of cathinones and synthetic cannabinoids

The synthesis of cathinones is chemically straightforward and usually follows a two-step synthesis process. The initial synthesis is of an α-bromoketone followed by synthesis of the relevant cathinone as a hydrochloride or hydrobromide salt (Figure 1.14).

The preparation of synthetic cannabinoids is much more chemically complex and beyond the scope of this chapter.

1.4.5.3 Analysis of cathinones and synthetic cannabinoids

The greatest challenge in the analysis of both cathinones and synthetic cannabinoids is the availability of validated or certified standards. Presumptve tests are listed for the analysis of cathinones. These include, for example, the Zimmerman test, which has been suggested as being consistently effective across a range of cathinone derivatives. There are no presumptive field tests as yet available for the synthetic cannabinoids. TLC analysis is also limited for both sets of compounds although some methods have been published (Nic Daéid *et al.*, 2014).

Both the cathinones and cannabinoids are readily resolved using gas chromatography, but their identification and quantitative analysis is limited by the availability of pure reference samples. In many cases, nuclear magnetic resonance (NMR) spectroscopy is now being used in an attempt to identify the structures of the target molecules. FTIR and Raman are also showing some promise in identifying the compounds once standards are available (Nic Daéid *et al.*, 2014).

1.5 Conclusions

The analysis of drugs of abuse is a constant component of a forensic science laboratory and is one of the most commonly encountered types of analysis preformed. Modern forensic chemistry laboratories are governed by accreditation standards, normally to ISO 17025 or similar. These ensure that the processes carried out are appropriately validated and fit for purpose.

The choice of instrumental technique used in any analysis depends upon the motive behind the analysis and on the nature of the sample. Normal procedure when dealing with bulk samples is to perform presumptive color tests to identify the drug class, possibly followed by TLC to identify the specific member of the drug class. When dealing with trace samples it is often the case that these non-confirmatory tests are circumvented and confirmatory tests only are employed where the choice of technique is based upon the experience of the analyst or laboratory protocol.

Until comparatively recently, our knowledge of the materials encountered has been straightforward and well grounded. However, the now global emergence of new psychoactive substances, where the nature of the compounds is constantly developing, provides new challenges, even in the simple identification of these materials. This requires information sharing across the forensic chemistry community in order to keep up to date on new trends. It also requires a movement towards using technologies such as NMR to confirm the identity of complex isomeric mixtures.

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