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Synthesis and spectroscopic characterization of emerging synthetic cannabinoids and cathinones

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"Ett problem är bara möjligheter i arbetskläder"

- Mulle Meck

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Abstract

The application of different analytical techniques is fundamental in forensic drug analysis. In the wake of the occurrence of large numbers of new psychoactive substances possessing similar chemical structures as already known ones, focus has been placed on applied criteria for their univocal identification. These criteria vary, obviously, depending on the applied technique and analytical approach. However, when two or more substances are proven to have similar analytical properties, these criteria no longer apply, which imply that complementary techniques have to be used in their differentiation.

This work describes the synthesis of some structural analogues to synthetic cannabinoids and cathinones based on the evolving patterns in the illicit drug market. Six synthetic cannabinoids and six synthetic cathinones were synthesized, that, at the time for this study, were not as yet found in drug seizures. Further, a selection of their spectroscopic data is compared to those of already existing analogues; mainly isomers and homologues. The applied techniques were mass spectrometry (MS), Fourier transformed infrared (FTIR, gas phase) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. In total, 59 different compounds were analyzed with the selected techniques.

The results from comparison of spectroscopic data showed that isomeric substances may in some cases be difficult to unambiguously identify based only on their GC-MS EI spectra. On the other hand, GC-FTIR demonstrated more distinguishable spectra. The spectra for the homologous compounds showed however, that the GC-FTIR technique was less successful compared to GC-MS. Also a pronounced fragmentation pattern for some of the cathinones was found.

In conclusion, this thesis highlights the importance of using complementary techniques for the univocal identification of synthetic cannabinoids and cathinones. By increasing the number of analogues investigated, the more may be learnt about the capabilities of different techniques for structural differentiations, and thereby providing important identification criteria leading to trustworthy forensic evidence.

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List of Papers

This thesis is based on the following papers which are appended.

PAPER I

Andreas Carlsson, Sandra Lindberg, Xiongyu Wu, Simon Dunne, Martin Josefsson, Crister Åstot and Johan Dahlén

Prediction of designer drugs: synthesis and spectroscopic analysis of synthetic cannabinoid analogues of 1H-indol-3-yl(2,2,3,3- tetramethylcyclopropyl)methanone and 1Hindol- 3-yl(adamantan-1-yl)methanone

Drug Testing and Analysis, **2016**, *8*, 1015−1029

PAPER II

Andreas Carlsson, Veronica Sandgren, Stefan Svensson, Peter Konradsson, Simon Dunne, Martin Josefsson and Johan Dahlén

Prediction of designer drugs: synthesis and spectroscopic analysis of synthetic cathinone analogues that may appear on the Swedish drug market

In manuscript

Preface

The recent increase of many new substances on the illicit drug market has been challenging for forensic laboratories and placed focus on the importance of the capability of different analytical techniques to identify and differentiate between compounds. When I started working with structural elucidation of NPS at the Swedish National Forensic Centre (NFC) in 2007, one of my first encounters was with the synthetic cathinone mephedrone. Since then hundreds of compounds have appeared. During this time, NFC developed a fast GC-MS screening method for narcotics. GC-FTIR, which had formally been the primary method of analysis, became almost outdated. However, the huge amount of new substances with a high degree of spectral similarities changed this viewpoint over the years and showed that this complementary technique was indispensable. The incidence of analogues also pointed to the applied analytical criteria for identification of unknowns as well as access to reference compounds.

Present, when many new compounds annually are introduced on the drug market, it has become evident for many forensic laboratories, that there is a need for strategies for drug analysis and that the application of complementary analytical techniques often are needed. This is evident for laboratories with established general screening methods utilized for a large number of substances where changes, in order to adopt it for new compounds, are almost impossible due to tedious re-validation. There are in these cases normally two options. Use a complementary technique or develop selective methods using the same technique. Either way, it's a compromise.

The main focus of this thesis was to synthesize analogues to already existing compounds and investigate the abilities of different techniques to differentiate between them. Chapter 1 gives a brief introduction to narcotics and their legislation, whereas chapters 2-4 provide overviews of NPS; especially the synthetic cannabinoids and cathinones. The basic principles of the applied analytical techniques are described in chapter 5, while chapter 6 focuses on concepts and approaches facing the analysis of new substances, stressing some key concepts. An aim can be found in chapter 7. The synthesis of analogues is briefly outlined in chapter 8, whereas chapter 9 deals with the differentiation of analogues using spectroscopic data. A conclusion is found in chapter 10. Finally, chapter 11 highlights some future perspectives based on this work.

1 General Introduction

In the ancient Roman cities *forum* denoted a public place or square that was used for e.g. judicial matters. With these roots, modern *forensic science* aims at providing the legal system with investigations and answers to questions, based on well-established scientific methods. Since the first application of more structural forensic investigations in the late 19th century, many disciplines have been formed, as expressed by Anthony Longhetti: *"There is literally no end to the number of disciplines that become 'forensic' by definition. Nor is there an end in sight to the number of present or future specialties that may become forensic. The examples are many."* [1] In the following pages the use of spectroscopic methods, aimed at distinguishing analogues of narcotic substances, is presented within the field of organic chemistry with a forensic application.

1.1 The Crime Scene

A crime scene can be anything from the remains following the detonation of an explosive to a zip-lock bag with white powder. In most cases they have one thing in common; they contain traces which may play a decisive role as evidence in a forthcoming criminal investigation. The role of the forensic scientist is to investigate such evidence to generate results that can be elucidated based on a pre-defined hypothesis and provide the conclusions in a comprehensive way to the judicial system. Depending on the scientific field of expertise, the achievement of this is conducted in a number of ways.

Sweden has traditionally applied a strict drug policy, which implies that almost all direct or indirect involvement is considered illegal and thereby a criminal offence. This is in line with the Swedish Vision Zero objective for narcotics and is expressed in the Swedish government's strategy within the alcoholic-, narcotic-, doping- and tobacco politics (Prop. 2010/11:47). Therein, the overall objective is proposed to be *"a society free from narcotics and doping, with abating medicinal and socially related damages caused by alcohol and a decreasing use of tobacco."*. Even though the question relating to narcotics is central and important in Sweden, this issue is regarded differently in other countries.

1.2 Narcotics

In describing the theory and to initiate a discussion regarding narcotics, a good starting point may be to ask rhetorically: *"What do we mean by a drug?"* The term *drug* is widely used rather loosely even by professionals. The definition is dependent on the context where it is used. For example, drug, in a medicinal context, means any

substance with the potential to prevent or cure disease, whereas it refers to a substance (i.e. amphetamine) classified in Schedule I or II of the 1961 *Single convention on narcotic drugs* in an international drug control context. In the same way, Narcotics (Lat. Narkoticum) was originally defined as *"a chemical agent that induces stupor, coma or insensibility to pain."* [2]. This alludes mainly to opioids and is in medicine referred to as *Narcotic analgesics*. More generally, narcotics are defined as the substances included in the *Single convention on narcotic drugs* as substances that possess a euphoric effect, but aren't narcotics from a medicinal viewpoint. From a legal stand point narcotics are defined in such way so as to protect the citizens from a public health and socio-economical point of view.

In Sweden, the definition of narcotics are stated by the *Penal Law of Narcotics (1968:64§8)* as drugs or goods dangerous to health that possess addictive properties or euphoric effects, or goods that easily can be converted to goods with such properties or effects. Further, the substance should either be listed in an international agreement (*Single convention on narcotic drugs*, 1961 or *Convention on psychotropic substances*, 1971) that Sweden has ratified or be declared as a narcotic by the government of Sweden (the current definition was agreed upon on the 1st of April 1999). Narcotic substances are not classified generically; instead each substance is noted in the statue LVFS 2011:10, issued by the *Medicinal product agency (Läkemedelsverket, MPA).* In this statue, an unambiguous chemical name is given for each substance, which then requires that the chemical structures of these compounds are known. This is a point where the legislation differs between countries where some practice a generic classification (e.g. Denmark and the United Kingdom).

1.3 The Drug Situation in Sweden

In 2015, more than 220 000 instrumental analyses of drugs of abuse were performed within approximately 35 000 cases arriving at the Swedish National Forensic Centre (NFC). The frequency of encountered drugs/drug classes is summarized in Figure 1.

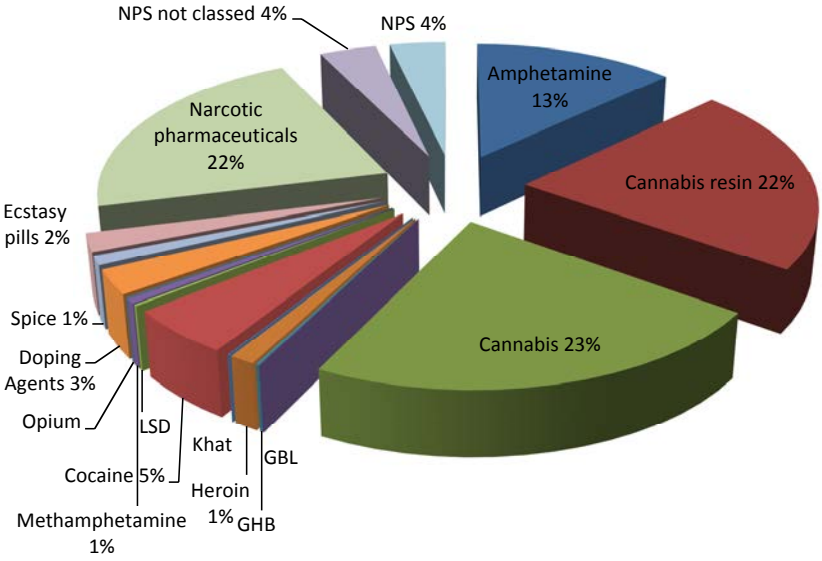


Figure 1. Frequency of narcotics seized in Sweden during 2015 (shares under 1 % not specified).

Even though the distribution of substances is more or less consistent over time, there are declines in some and increases within other areas. For example, new psychoactive substances (NPS) are a group of substances that are increasing. Most of the new substances that appear on the recreational market belong to the NPS, and therefore structural elucidation is required within this area. Since 2013, there are approximately 100 novel NPS encountered within Europe each year,[3] and there is typically little or no knowledge regarding their effect, harmfulness or toxicity. Therefore, statistics describing substances involved in drug related intoxications, the NPS constitute a much higher share than their corresponding appearance in seizures.

Illicit drugs have severe consequences on the community. In 2014, in the range of 700-900 people were suspected to have died from abuse of narcotics in Sweden.[4] In 2015, the National Board of Health and Welfare (Socialstyrelsen) changed their classification of drug related deaths, which makes this number and the comparison to earlier years somewhat uncertain. It is not possible to calculate the exact cost to society, but it has been estimated that the cost is about 24 billion SEK.[5] The cost for the healthcare makes up one-quarter of this sum, and the figures for the legal authorities are about the same. The remainder of the associated cost results from loss in productivity due to sick-leaves and early deaths caused by the drugs.

1.4 Classification Process

The Public Health Agency of Sweden (Folkhälsomyndigheten, FoHM) is responsible for the monitoring and investigation of needs for classification of narcotics, with the exception of narcotic pharmaceuticals that fall under the responsibility of the MPA (SFS 2013:1020). Further, the responsibility for the FoHM also includes the surveillance of *certain goods dangerous to health*, which is defined in *the Swedish Code of Statue (Svensk författningssamling, SFS)* 1999:42, as goods that, based on their inherent properties, cause risk for life and health and that are used, or presumably used, with the purpose of achieving intoxication or other effects. Such substances are recorded in SFS 1999:58 for which there are around 160. Narcotics, pharmaceuticals and doping agents, are excluded.

The process of classification starts with surveillance of drugs of abuse flourishing in the community. Sweden has a long tradition of consensus-based work, which is also reflected within this area, where several key authorities and institutes actively contribute with their expertise to a reference group called *the Network for the Actual Drug Situation in Scandinavia (NADiS)*. The NADiS has been established with the purpose of facilitating the FoHMs and the MPAs decision basis for classification (SOU 2011:66). In order to classify a substance as narcotics, several criteria have to be fulfilled. One is a confirmed chemical structure. This is a key issue and is provided by the Swedish National Forensic Centre (NFC) based on findings in seizures taken by *the* Swedish Police Authority (Polisen) and the Swedish Customs (Tullverket). Further, a documented abuse of the drug and/or a confirmed drug related death is needed and here the National Board of Forensic Medicine (RMV), the Swedish Poisons Information Centre and Karolinska Institutet (KI) contribute. The information gathered is also exchanged with the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) via its Early Warning System (EWS). When the FoHM suggest a classification to the Swedish Government, it is referred for consultation to the Commission of the EU regarding trade on the Single Market (EU 2015/1535). If no obstructions follow, the Swedish Government makes a decision, after which the MPA appends it to the narcotic statue. The whole process takes normally six months, but time spans up to twelve months occur. With the aim of speeding up the process, the Swedish Government decided to investigate if it is possible to rationalize the classification procedure (Dir. 2015:102). As described above, focus is set on several agencies ability to be updated regarding which substances that are flourishing in the community at any present time. This includes the structural elucidation process of unknown seizures that is denoted as a central element in the classification process (Figure 2).

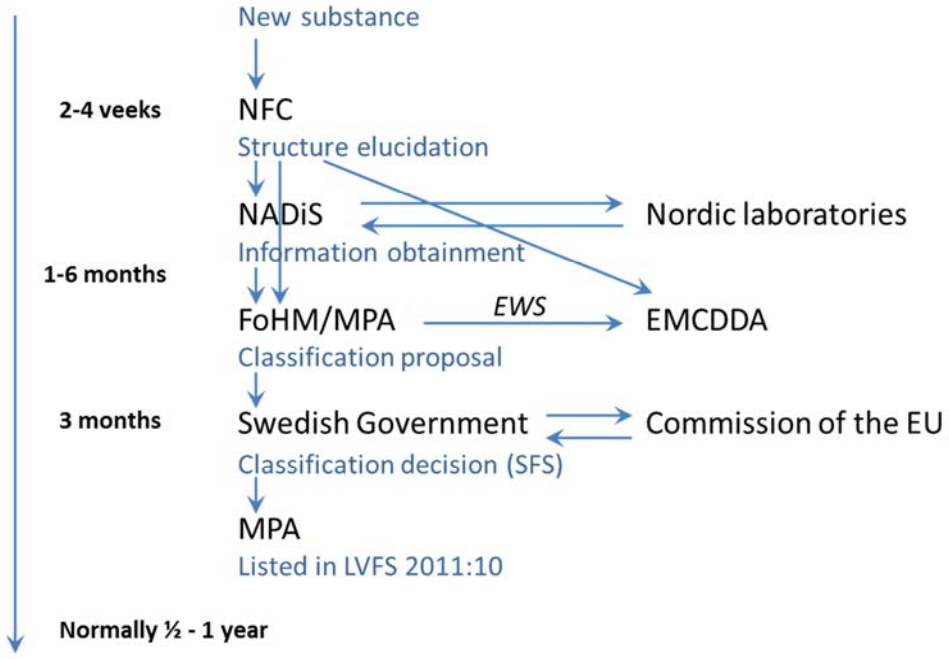


Figure 2. Schematic of the process applied for classification of narcotics in Sweden.

2 New Psychoactive Substances

2.1 Definitions

Since several years, there has been a huge increase in the number of new substances that resemble already established drugs of abuse. This change of the recreational drug market does involve a group of substances that are nowadays called *New Psychoactive Substances* (NPS). This group of substances is also referred to as *Designer drugs*, *Internet drugs* and *legal highs.*[6] NPS are defined as *"substances of abuse, either in a pure form or as a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat"*. The term "new" does not imply that the substances are new for the scientific community, but rather that they recently have emerged on the drug scene and have done so without legal control. Several of the compounds classed as NPS were synthesized several decades ago, so "new" is a relative concept also in this perspective.

2.2 Different Types and Classes of NPS

The NPS can be divided into subgroups based on their chemical structure. The most common are aminoindanes (Figure 3a), synthetic cannabinoids (Figure 3b), synthetic cathinones (Figure 3c), phenethylamines (Figure 3d), piperazines (Figure 3e) and tryptamines (Figure 3f). However, there are also other NPS subgroups including fentanyls (Figure 3g), benzodiazepines (Figure 3h) and arylcyclohexylamines (Figure 3i).

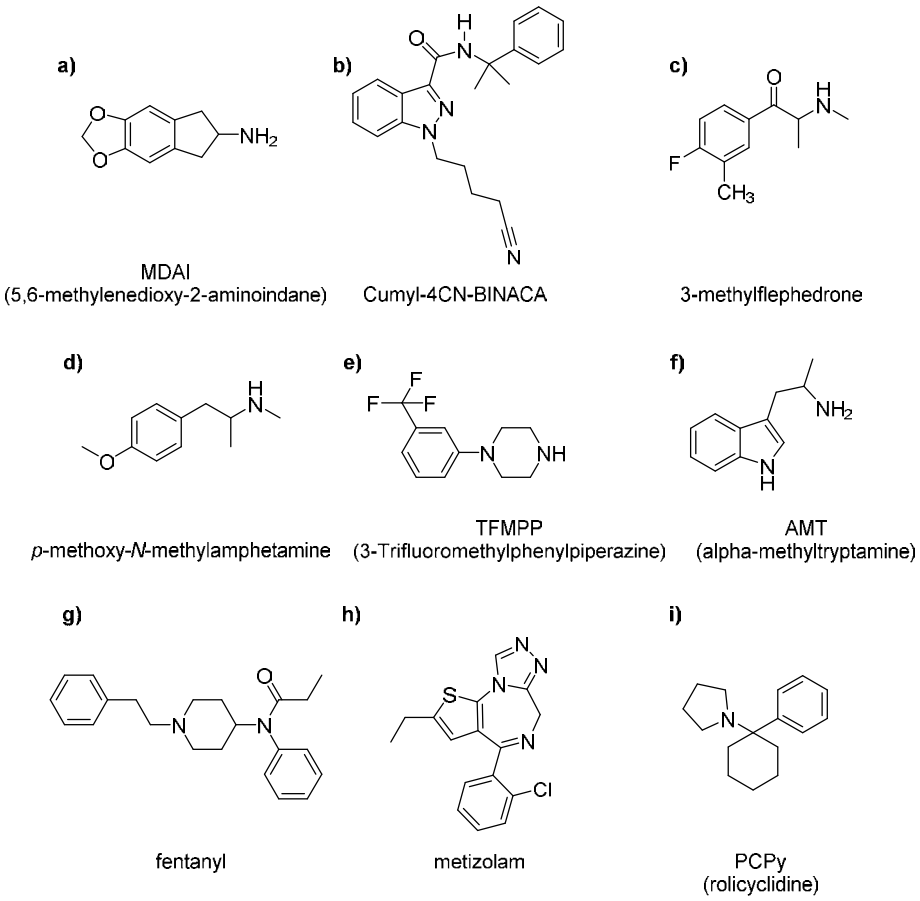


Figure 3. Examples of compounds belonging to different NPS subgroups. Lower case letters denoted chemical group a) aminoindanes, b) synthetic cannabinoid, c) cathinone, d) phenethylamine, e) piperazine, f) tryptamine, g) fentanyl, h) benzodiazepine and i) arylcyclohexylamine.

2.3 Legislation and Rate of Change

Although NPS rarely occurred in the 1980s and 1990s, those that were encountered were most commonly mimicking tryptamines or phenethylamines. These types of substances were often described by psychedelic drugs designers like Alexander Shulgin[7, 8]. In the last ten years, the emergence of NPS has provided a large number of new substances and an increase of forensic cases involving such compounds. As a consequence, the emergence of NPS has clearly affected the allotment of occurring drugs. More than 600 different NPS were reported to the United Nations Office on

Drugs and Crime (UNODC) Early Warning Advisory (EWA) on NPS until the end of 2015. The rapid increase of new substances on the recreational market is driven by the legislative procedures to bring a substance under control and the attempts of entrepreneurs and organized crime groups to continuously circumvent the legislation. Accordingly, the rate of change of the market is another factor to take into account.

The number of substances classified as narcotics or goods dangerous to health has lately increased in Sweden (Figure 4). As can be seen, there is a dramatic increase in the years 2015 and 2016, which follows the increased numbers of NPS during this period of time. Further, during the same period of time there were more than 280 NPS that were encountered in Sweden. Comparing that figure to the accumulated number of classifications, only about 150 were classified as narcotics. The corresponding value for classification as goods dangerous to health is also about 150 (Figure 5). Noteworthy however, are that during the described elapsed time some substances were re-classified from goods dangerous to health to narcotics.

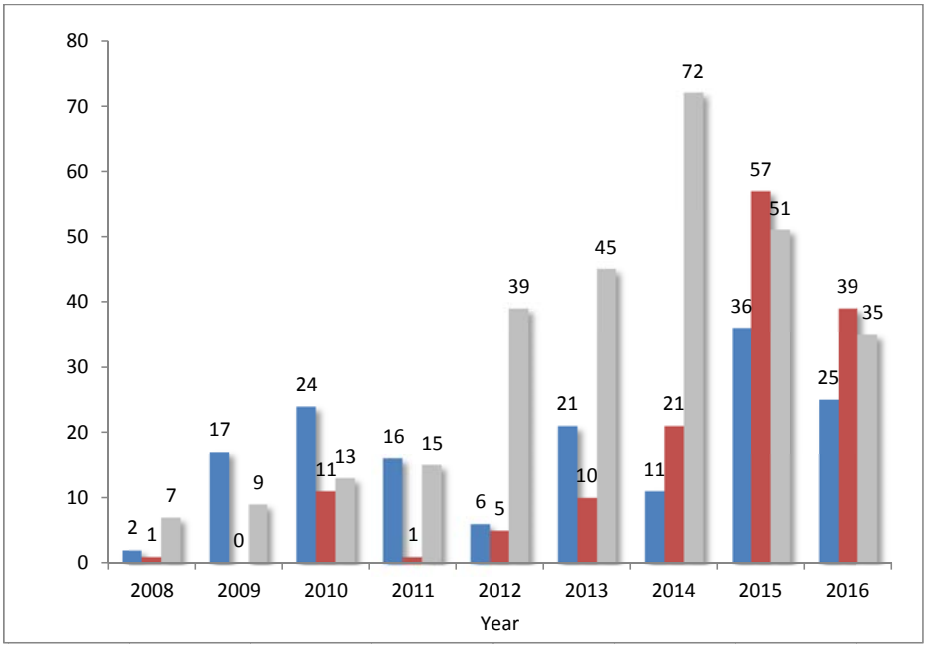


Figure 4. Numbers of substances classified as narcotics (blue), goods dangerous to health (red) and structure elucidated (grey) in Sweden on an yearly basis from 2008 to 2016 (September).

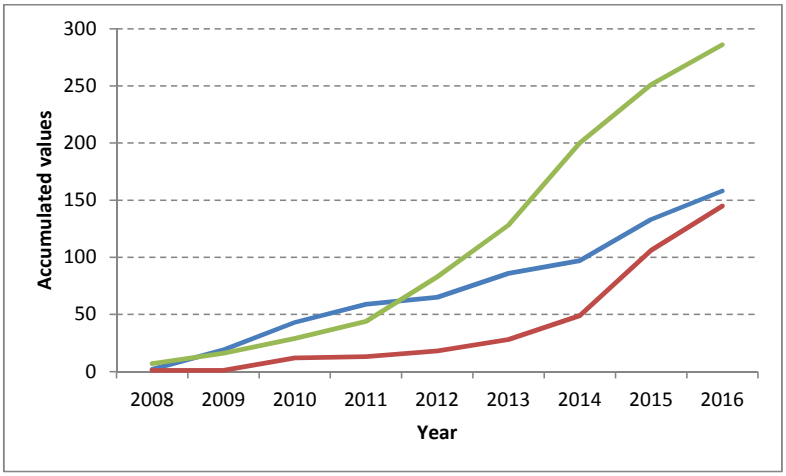


Figure 5. Accumulation of substances classified (blue=narcotics, red=goods dangerous to health) compared to substances that had their structures elucidated at NFC (green) during the period 2008-2016.

The emergence of unclassified NPS usually follows the classification of other, already known and abused NPS.[9] Often there is a fast decrease of incidence of these substances once they are classified, and at the same time a characteristic rise of other unclassified NPS.[10] Actually, the decline of some substances can be seen just prior to the announced classification date, which implies that the suppliers are fully aware of the legislation.

The occurrence of the various subgroups is subject to fast change. Since the emergence of Spice[11] the synthetic cannabinoids have been established as one of the major NPS subgroup. They comprised about 48 percent of the around 13 000 Swedish NPS seizures in 2014. At the same time, the synthetic cathinones had a share of about 13 percent. Just one year later (in 2015) the situation had changed greatly where the synthetic cathinones increased their part to 20 percent, while the synthetic cannabinoids had decreased to 20 percent. These trends become even more significant if data from the second half of 2015 is considered; during this period of time the numbers were 24 and 9 percent for the synthetic cathinones and cannabinoids, respectively (internal statistics at NFC).

The number of new substances per drug class (including the NPS subgroups) that were encountered in Swedish drug case samples in the years 2011-2015 is summarized in

Figure 6. In general, the Swedish trends is in agreement with those at the European[12] and global[13] levels (Figure 7).

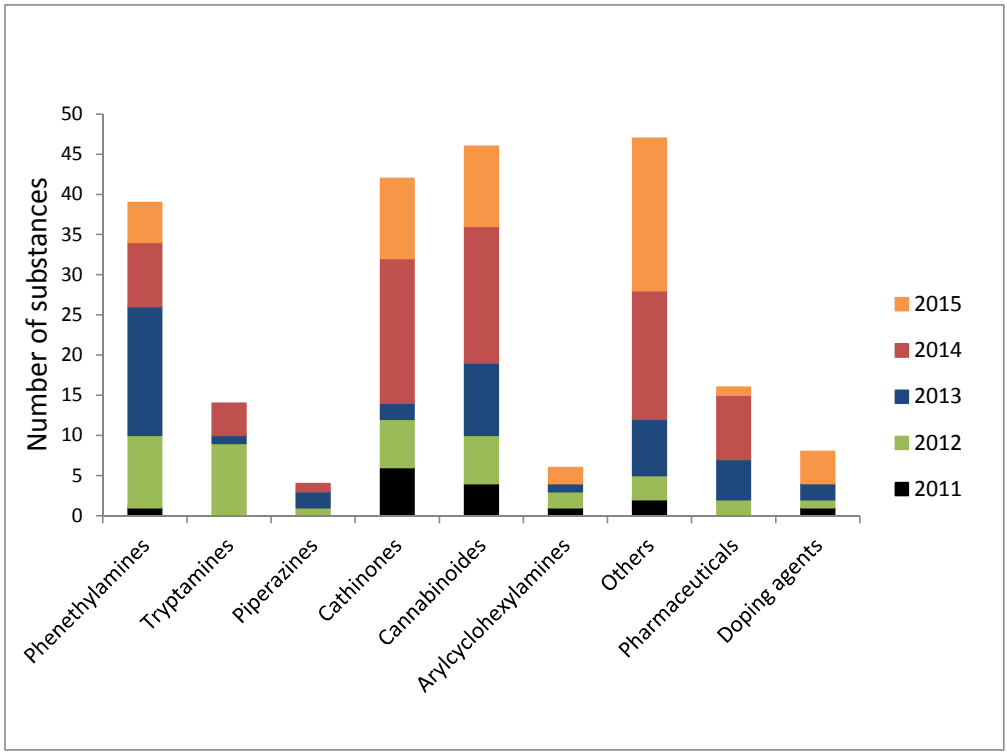


Figure 6. Number of NPS per drug group encountered in Swedish drug cases in the years 2011-2015.

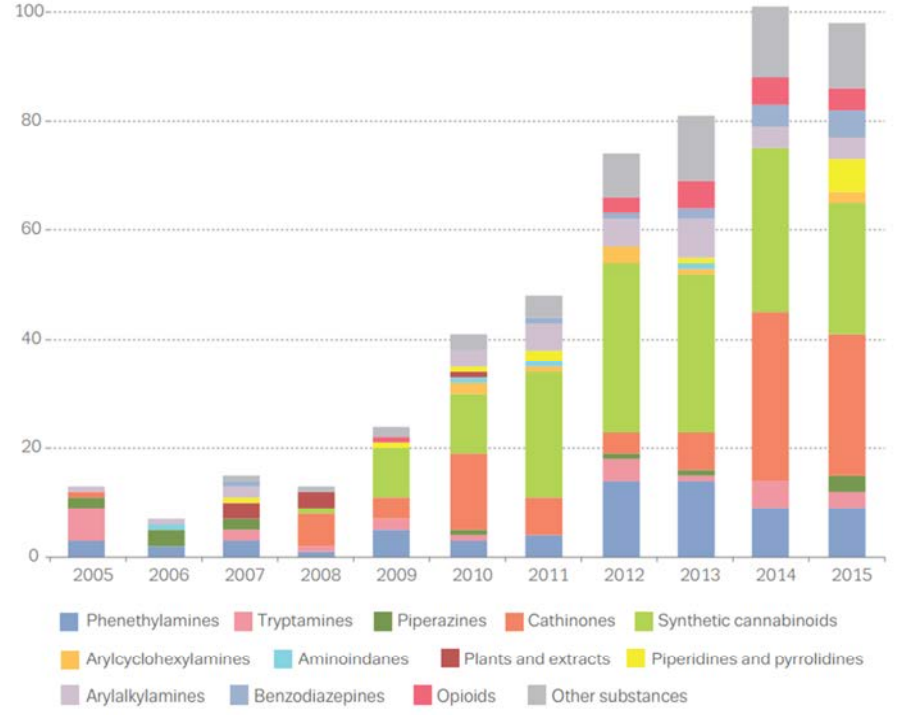


Figure 7. Reprint of the number of NPS notified for the first time to the EU EWS by category in the period 2005-2015[13].

The change over time can be exemplified by individual substances like methylenedioxypyrovalerone (MDPV). It took several years for MDPV to be classified as narcotics (Figure 8); its first appearance on the Swedish market was in 2006 and it was not banned until 2010. After its classification MDPV remained on the drug market.[14] This may imply that the drug had the time to get rooted among its users, so when the classification came into action, MDPV was already an established drug of abuse. Further, there could also be other reasons to the persistent of a drug even after its classification. Abusers may revert to a classified substance due to the non-familiar effects of other drugs, difficulties in kicking the habit or a high availability in the market. Based upon this reasoning, a faster classification process, which includes the structural elucidation of the substance as a fundamental part, may in some sense contribute to a faster disappearance of NPS.

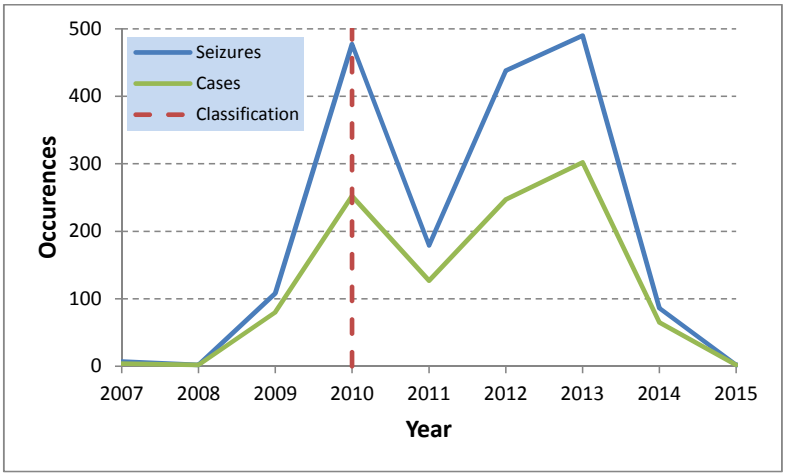


Figure 8. The number of Swedish cases and seizures containing MDPV during the period 2007-2015. The classification of MDPV as narcotics in February 2010 is indicated by the dashed line.

3 Synthetic Cannabinoids

3.1 *Cannabis Sativa* and the Cannabinoids

Cannabis Sativa L.[15] is an annual herb that seems to originate from Central Asia, supported by archeological findings showing that it was grown in China 10 000 years ago.[16] Historically, it has been used in folk medicine, as food, as a recreational drug and a source of textile fibers.[17, 18] In modern times, many research projects have focused on its pharmaceutical and therapeutic properties and its use in the biomass industry.[19]
In the secondary metabolism of plants a large number of substances are produced, of which the cannabinoids, terpenes, flavonoids, lignans, alkaloids and other phenolic compounds account for the majority.[20] The cannabinoids are defined as terpenophenolic C_{21} compounds, including their carboxylic acids, analogues and transformation products.[21] The cannabinoids are produced in the plant glandular trichomes, where they constitute part of the resin.[22, 23]
More than 110 different cannabinoids have been identified that can be divided into 10 different subgroups based on their chemical structure.[20, 24]

Cannabis is today the most frequently abused illicit drug in the western world.[13] It appears mainly in three forms, namely (i) herbal Cannabis (Marijuana), (ii) Cannabis resin (hashish), and (iii) Cannabis oil. The main cannabinoids in *Cannabis* are the acids derivatives from tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC) and cannabinodiol (CBND).[25] The other 90-100 cannabinoids usually occur in smaller amounts.[20] Normally the acids undergo transformation to their corresponding neutral substances, caused by time and environmental conditions such as heat. Among the above-mentioned cannabinoids, THC is the most psychoactive compound and is also the one that is present in highest concentration. Therefore, this compound is normally in focus in forensic analysis of Cannabis.
In humans, the cannabinoids affect the endocannabinoid system and interact with the first and second cannabinoid receptors (CB1 and CB2, respectively). CB1 is coupled to psychoactive effects, whereas the CB2 is involved in immune-regulation.[26, 27]

3.2 The Evolution of Synthetic Cannabinoids

The first generation synthetic cannabinoids originated from the pharmaceutical industry that attempted to develop new medicinal drug candidates targeting pain[28] and other diseases[29-34]. The main aim of these studies was to synthesize compounds with high specificity for the second cannabinoid receptor (CB2), transmitting anti-inflammatory effects and pain release[35-37]. However, these efforts resulted in a large

number of structurally different drug candidates that exhibited undesirable psychoactive effects[38-40] due to their binding affinity to the first cannabinoid receptor (CB1). These substances, which often were rejected as drug candidates, have instead appeared on the drug market as unregulated and illicit drugs.[41] Also, a large number of additional analogues have been derived from the published structures of these pharmaceutical candidates.

More than 160 synthetic cannabinoid analogues[42, 43] have been identified since their first appearance in Spice products in 2008.[11, 44, 45] Briefly, Spice is defined as a plant material where these synthetic cannabinoids has been added by spraying or submerging (Figures 9 and 10). The structures varies, and have evolved into several different subclasses where indole and indazoles mostly comprise the base structure, even though some structures resembled traditional cannabinoids, while others contained other structural elements.[46] There are also several other synthetic cannabinoids that belongs to other compound groups. However, they all have (some) affinity for the CB1 cannabinoid receptor.

Figure 9. Examples of Spice seizures.

Figure 10. Items typically encountered in seizures made in conjunction with small-scale production of Spice.

Recent efforts by the EMCDDA, aimed at systematization of the chemical structures of the occurring synthetic cannabinoids, have resulted in a model describing of the diverse structural types. This model consists of four key structural elements, namely “the core and substituents”, “the link”, “the ring and substituents” and “the tail” (Figure 11) which denote altering positions. Even though most of the reported cannabinoids follows the general structure depicted in Figure 11, there are also other synthetic cannabinoids with affinity for the cannabinoid receptor that have other base structures.

Due to the extensive chemical modeling of the synthetic cannabinoid structures, leading to substances with structural and spectroscopic similarities, there are significant analytical challenges regarding their structure determination.

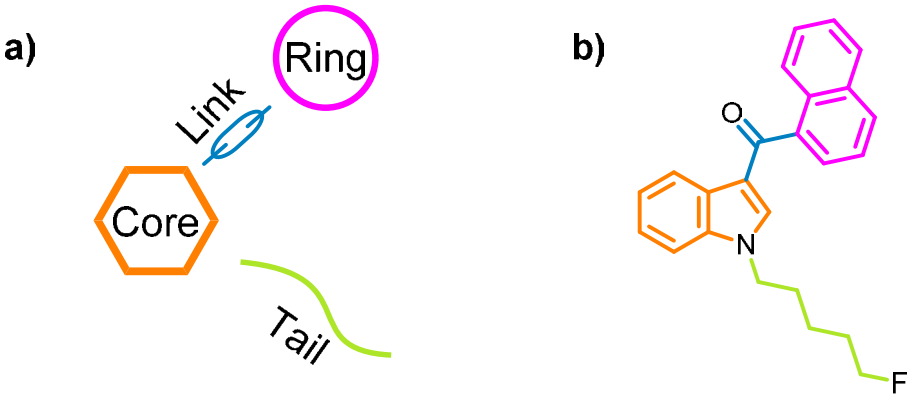


Figure 11. a) The EMCDDA model with four key structural elements of the synthetic cannabinoids and b) AM-2201 drawn according to this model.

4 Synthetic Cathinones

4.1 *Catha edulis* and Cathinone

The synthetic cathinones are, as the name implies, variants of the naturally occurring substance cathinone. It is found together with a cathine in the *Catha edulis* (Vahl) bush[47], where they account for the major central system stimulating effect (Figure 12). Kath, scientifically first described by Peter Forsskål during his journey in the Arabian countries in the beginning of 1760s[48], is a tree that measures quite variable height (1 to 10 m) depending on the geographical conditions. Normally the tree is pruned to bush of circa 5 m height by the Kath cultivators, which have their main production areas located in Ethiopia and Yemen[49], but they are also found in other places around the Horn of Africa and the Arabian Peninsula.[50] The use of *Catha edulis* consist in chewing the annually grown leaf shoots and the tender twigs. The Kath-chewing is widely spread among locals but is also occurring in remotely located countries, such as Sweden, where it's manly limited to certain ethnical groups. Due to dimerization of cathinone, the plant material is wrapped up, normally in leafs of *Ensete ventricosum* ("fake banana leafs"), maintaining moister and thereby slow down cathinone breakdown (Figure 12). The quantity of cathinone in fresh Kath is around 0.1 percent. During 2015, eighteen seizures of Kath were reported in Sweden.

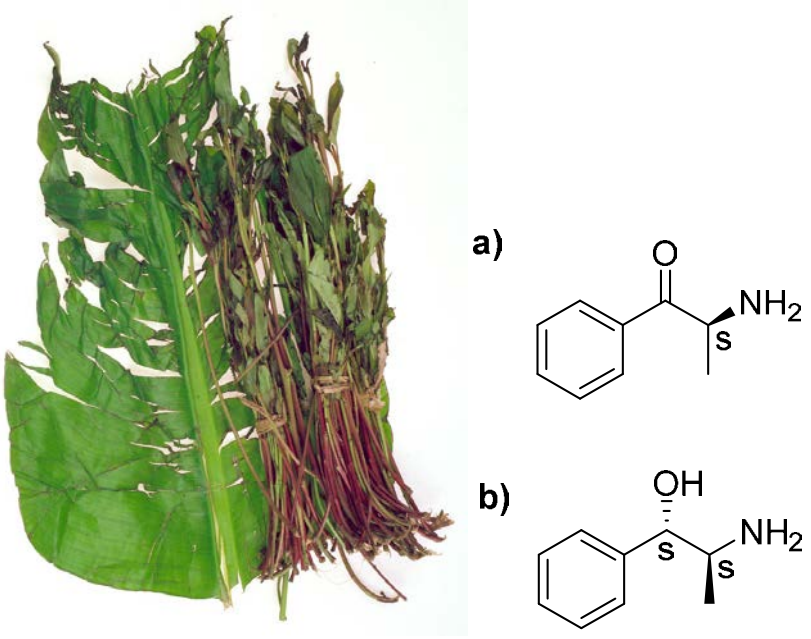


Figure 12. *Left:* Kath bundles and leafs of *Ensete ventricosum* used as wrapping, *Right:* Chemical structures of a) cathinone and b) cathine.

4.2 The Evolution of Cathinone Analogues

Methylone, first encountered in seizures in 2004, may be said to be the first of the modern synthetic cathinones. Followed by methylenedioxypyrovalerone (MDPV) in 2006 and mephedrone in 2007, these represent the beginning of the first generation synthetic cathinones. The first case with mephedrone in Sweden was sent to the Police on the west coast by concerned parents of a teenager. After the structural elucidation at NFC, the seizure was returned to the parents since it was not an illegal substance. This case pinpoints the availability, i.e. over the Internet[51], as well as the narcotic legislation circumstances at that time. Since then, more than 70 synthetic cathinones have been encountered in Sweden, appearing both as powders and tablets (Figure 13). The international trends and emergence patterns are in line with the Swedish findings, and the abuse is widely spread.[3, 52, 53]

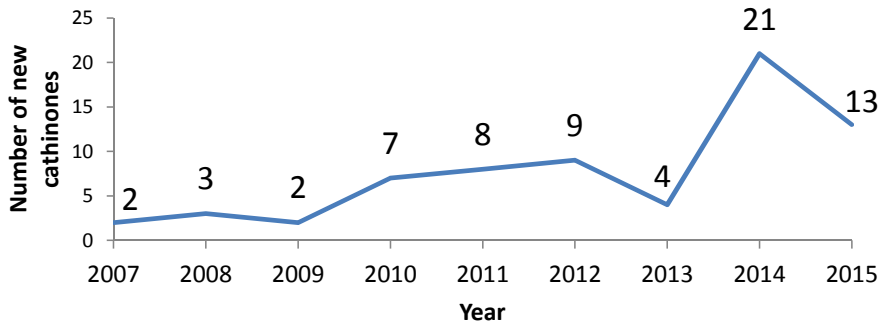


Figure 13. Number of first occurrences of synthetic cathinones found in Swedish drug seizures from 2007 to 2015.

The synthetic cathinones are associated with severe health effects. A range of different intoxications have been reported, ranging from cardiovascular and neurological to psychiatric effects, like tachycardia, arterial hypertension, hallucinations, aggressiveness, agitation, hyperthermia and locomotor behaviour alterations.[54-57] Further lethal intoxications have also been reported.[58-60]

Similarly, as with the synthetic cannabinoids, five structural positions are frequently exposed for alterations (Figure 14). Further, they have in common that the nitrogen atom is either alkylated or enclosed in a pyrrolidine ring. Obviously, there are almost an infinite number of possible structures, but to some extent limited due to their different psychoactive effects. Different aspectes of the analysis of the synthetic cathinones have been discussed in the literature.[61] Of particular note, are the limitations regarding the analysis of cathinone isomers.[62] A selection of synthetic cathinones are presented in Figure 15.

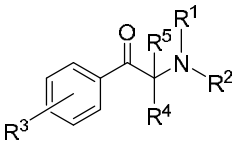


Figure 14. Positions R^1 to R^5 frequently altered in occurring synthetic cathinones.

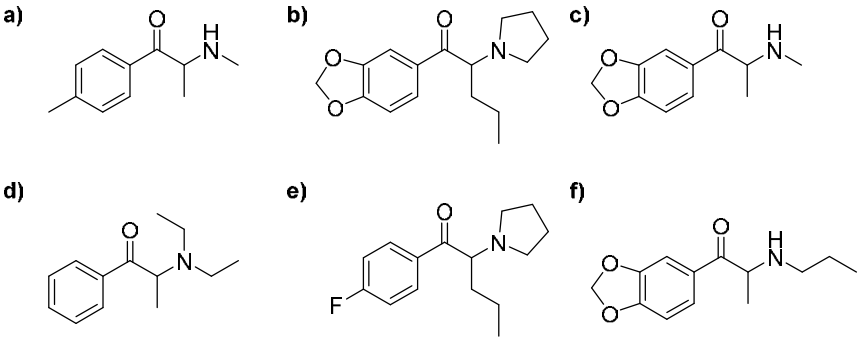


Figure 15. Selected structures of synthetic cathinones. a) 4-methylmethcathinone (mephedrone), b) metylenedioxypyrovalerone (MDPV), c) methylone, d) amfepramone, e) 4-fluoro-alpha-pyrrolidinovalerophenone (4F-α-PVP) and f) *N*-propylnormethylone.

5 Methodology

This chapter briefly describes each of the applied analytical techniques, gas chromatography – mass spectrometry (GC-MS), gas chromatography – fourier transform infrared (GC-FTIR) spectroscopy, liquid chromatography – high resolution mass spectrometry (LC-HRMS) and nuclear magnetic resonance (NMR) spectroscopy.

5.1 GC-MS

The hyphenated[63] technique GC-MS is widely used in forensic analysis and has become a "gold standard" in many fields of application.[64-72] Screening and confirmation of illicit drugs is often made by GC-MS, although there are alternatives[73].

Gas chromatography enables separation of organic compounds on a capillary column (typically 25 m long with an internal diameter of 0.25 mm) with an inner surface of stationary phase (e.g. polysiloxanes). The column is placed in an oven that controls the temperature at which the separation occurs. The separation is based upon the compound volatility and its affinity to the stationary phase. Compounds dissolved in an organic solvent are introduced, typically in volumes of 0.1 to 10 µL, onto the column via an injector. The by far most common is the hot split/splitless injector. The solvent and analytes are vaporized in the injector and thereafter transferred to an open tubular capillary column by a continuous flow of helium carrier gas. Due to differences in volatility and affinity to the stationary phase the compounds elute at different times, referred to as *retention time*. The effluent of the capillary column is connected to the mass spectrometric detector and enters first the ion source. In electron ionization (EI), which is the most common ionization technique for this application, the molecules are ionized and fragmented by formation of radicals via a 70eV beam of electrons. The ions formed are separated based on their mass-to-charge ratio (*m/z*) on a quadrupole to which constant and radio frequency oscillating voltages are applied. The ions are thereafter detected by an electron multiplier. In the resulting characteristic mass spectra the ion intensity is plotted versus *m/z*.[74]

The obtained mass spectra depends on the chemical structures of the analytes, which enables identification by comparison to a mass spectra library or by structural elucidation. The fairly high fragmentation energy used in EI creates a large number of fragment ions that are beneficial for identification. However, the intensity of the molecular ion is sometimes low, which in such cases makes determination of the molecular weight difficult. Chemical ionization (CI) is a softer ionization technique that generates less fragmentation and a more intense molecular ion. This technique is therefore suitable for determination of the molecular weight of unknowns.

GC-MS provide reproducible chromatographic retention times as well as reproducible mass spectra for individual analytes. These qualities have implied a combined use of accurate retention times and interchangeable spectral libraries for identification in high throughput GC-MS screening methods.

5.2 GC-FTIR

As in GC-MS, the chromatographic separation in the GC-FTIR is followed by detection with high specificity. Infrared radiation is passed through a Michelson interferometer and further to a light-cell that is connected to the continuous flow from the column. The eluting compounds absorb at different wave lengths depending on their vibrational and rotational modes. There are several types of vibrations[75] that correspond to various structural elements in the molecule. The wavelengths that are absorbed depend on the type of bond, which for instance allows for identification of various functional groups. Normally, absorption is recorded in a wave number range of 4000 to 500 cm^{-1}, where the region between 1500 and 500 cm^{-1} is referred to as the *fingerprint region* since this is where most of the unique absorptions occur. A simultaneous measurement of absorption energies enables a continuous recording of IR spectra that is required in GC-FTIR analysis. The use of a chromatographic separation before the recording of IR spectra enables simultaneous detection of several components also for complex samples[76]. This would for instance be difficult when applying FTIR analysis directly on mixtures of solids. The fact that the infrared measurements are performed in gas phase offers another advantage; there are less inter-molecular interactions that result in spectra with lower degree of interferences.

Like GC-MS, GC-FTIR enables the use of interchangeable spectral libraries that, in combination with accurate retention times, can be applied for identification of compounds. The technique is not as wide-spread as GC-MS, although GC-FTIR is superior for the some analytical challenges. In comparison to GC-MS, the sensitivity is lower for most substances, but there are exceptions (i.e. heroin).

5.3 LC-HRMS

Ultra-high performance liquid chromatography quadrupole time of flight tandem mass spectrometry (UHPLC-QTOF-MS) is novel analytical technique for accurate mass determination. The method enables high resolution mass spectrometry (HRMS) measurements, at a resolving power exceeding 10 000. The mass accuracy is typically 1 ppm or better.

The combination of a chromatographic separation in an aqueous phase with a mild ionization technique at atmospheric pressure enables determination of the masses of intact molecules. The method can be applied to determine the accurate mass of

different molecules ranging from smaller drug molecules to larger biomolecules like peptides and proteins.
Commonly, electrospray ionization (ESI) in positive mode is used, which generates protonated molecular ions (MH^+). Fragmentation can be generated by collision-induced dissociation (CID) by addition of energy (eV) in the presence of a collision gas (Ar, N_2). The QTOF detector is a pulsed type ion source that accelerates the formed ions over an electric field into a field free region known as the *fight tube*. All accelerated ions are subjected to the same amount of accelerating energy. This implies that ions of the same charge ideally possess the same amount of kinetic energy, and thereby gain different velocities depending on both their mass and inherent charge. Small and multiply charged ions will have high velocities, reaching the detector prior to heavier or less charged ones. The measured flight time is then transferred to a *m/z* value and further calibrated via an internal mass standard to acquire high mass accuracy.[77, 78]

In recent years, extensive efforts have been undertaken to improve the mass resolution and sensitivity. One measure involves improvements to the reflector and ion optics to correct for differences in velocity among ions of the same mass, which improves the resolving power. In comparison to low resolution methods, the good mass accuracy provided by HRMS instruments limits the number of possible sum formulas and thereby the number of possible compounds. Due to those abilities, UHPLC-QTOF-MS and other HRMS techniques are powerful alternatives for identification of unknowns.

5.4 NMR

Nuclear magnetic resonance (NMR) spectroscopy is used in a variety of applications such as structure determination as well as studying molecular dynamics and protein folding.[79] The exploration of the atomic nuclei provides information regarding chemical surrounding, the relative number and distance between atoms. The technique utilizes the phenomenon that isotopes with spin placed in a static magnetic field are able to absorb and subsequently emit specific amounts of energy (resonance frequency) that are isotope dependent and affected by its chemical environment. The static magnetic field is produced by a liquid helium cooled super conducting magnet consisting of complex twined coils. The magnetic field is highly homogenous and of high field strength, typically 7-20T. When applying short pulsed electromagnetic radiation (radio frequency in this case), the nuclei absorb energy and the measurement is made during the subsequent relaxation. The changes in the nuclei polarization state are achieved using different pulse-sequences including frequency and time modulations in advanced

order. This enables a variety of different NMR experiments, including the measurement of the relaxation of the nucleus. Normally the spin-active nuclei hydrogen (^{1}H), carbon (^{13}C) and (^{15}N) are examined in one- and two-dimensional experiments, where the latter also includes transfer of the magnetization between nuclei.

Proton NMR is the most common one-dimensional experiment. It generates information about the different proton magnetic environments, their relative abundance and which protons are adjacent to each other. This fairly short time experiment is used widely and the spectrum is rich in information. One-dimensional carbon-13 NMR experiments are also widely used of which there are several types of editable experiments probing the hydrogen substitution of the different carbons, like the attached proton test (APT) or the Distortionless Enhancement of Polarization Transfer (DEPT). In structural elucidations, a set of two-dimensional experiments is often used, notably correlation spectroscopy (COSY), *J*-resolved spectroscopy and nuclear overhauser effect (NOE) spectroscopy. The COSY is a through-bond correlation technique where coupling between 2-5 bounds are shown by cross peaks in the spectra that provides information on linages to neighboring protons. It is especially powerful when there are overlapping signals and complex coupling patterns in the one dimension proton spectra. Other through-bond correlation experiments are total correlation spectroscopy (TOCSY), heteronuclear single quantum correlation spectroscopy (HSQC) and heteronuclear multiple bond correlation spectroscopy (HMBC). Also commonly applied is the two-dimensional NOE spectroscopy (NOESY), which allows for the determination of the proximity of nearby spin systems and is a through-space experiment.[80]

The different NMR experiments utilized for structural elucidation provide a rapid and a wide range of knowledge about the chemical structure. These techniques are therefore extremely powerful for such purposes. Hence, NMR complements other techniques like GC-MS and GC-FTIR.

6 Narcotic Investigations and Structural Elucidations

6.1 Screening Concepts

Screening allows the selection of those compounds having certain properties within a large sample set. In analytical chemistry, a screening method generally refers to an application that provides information regarding the presence or absence of a specific compound or set of compounds. Within forensic toxicology, screening is usually a part of the systematic toxicological analysis (STA) strategy that is applied to samples with unknown content. This screening aims at revealing and identifying as many compounds as possible within each sample.

Another common screening approach is to first apply a simple and fast method to discard negative samples, and thereafter apply more sophisticated and resource demanding methods only on the potentially positive samples identified in the first step. This approach is normally a time and cost-effective alternative when there are many possible negative samples.

In forensic drugs analysis, screening is normally used in two ways: targeted screening (TS); and, untargeted general unknown screening (GUS).[81, 82] In TS the presence of a preselected set of compounds is screened for.
Tandem mass spectrometry like LC MS/MS assays is suitable for this approach as it measures the presence of predefined characteristic precursor and fragment ions. However, the emergence of HRMS has provided a tool that enables determination of the accurate mass and thereby the molecular weight and sum formula of unknowns. Consequently, HRMS is nowadays often used in the GUS approach, although availability is sometimes limited as the instruments are more sophisticated and expensive. HRMS is a complementary technique for both TS and GUS. The HRMS technique also allows for the reprocessing of historical analytical data that, for instance, could be triggered by the reporting of the presence of new substances on the recreational market. Most forensic laboratories do, however, rely on GC-MS for GUS of illicit drugs. GC-MS also enables re-processing of historical data if desired.

6.2 General Approach and Standard Operating Procedure

NFC applies a fairly straight-forward procedure for the investigation of seizures that are suspected to contain narcotics, goods dangerous to health, pharmaceuticals or doping agents. After initial weighing and recording of external characteristics, the samples are subjected to GC-MS screening in which more than 1000 different substances can be identified with certainty. These GC-MS analyses are under strict control and follow the detailed method description in the standard operating

procedure (SOP) of the method. The GC-MS method, which is based upon so called fast GC, has a run time of less than six minutes. A chromatographic separation by GC is a suitable choice as most samples contain volatile and thermally stable compounds. Normally, an alkaline extraction is performed to transfer the analytes to butyl acetate, which is thereafter subjected to GC-MS analysis. If GC-MS fails to determine an absolute identity of the compounds, complementary techniques are applied. For example, isomers and analogues sometimes give rise to indistinguishable EI spectra, and in those situations better results can be achieved with GC-FTIR. In such cases, the sample vial can be moved from the GC-MS to the GC-FTIR and thereafter be analyzed directly without any additional sample preparation, which improves the laboratory throughput.

A combination of experienced-based knowledge and strategic chemical assessments determine to what extent the two complementary techniques should be used and thereby provides the criteria for univocal structure determination. However, the successfulness of the overall screening approach requires that most compound identities can be determined by the GC-MS method alone, which generally proves to be the case. Hence, the number of samples that needs to be reanalyzed by a secondary technique can be kept at a minimum.

6.3 Criteria for Unambiguous Identification

The application of the result of an analytical investigation is connected to the degree of certainty of the chemical identification of a sample under forensic investigation. For forensic applications, the level of confidence must obviously be high as the results in many cases are used as evidence in the court of law. Therefore, it is necessary to have clear regulations regarding the evaluation of the spectral data and for the performance of the applied techniques.[83] Hence, the criteria for unambiguous identification are essential.

The question often concerns the possibility that another compound provide data of high similarity. When such situations are faced, complementary techniques must be applied. This approach is based upon an underlying criterion that defines what additional techniques that are needed to confirm a compound's identity. This is sometimes tacit knowledge.

There are several guidelines describing a good laboratory practice for the identification of compounds. These are usually described based on the ability of different techniques to distinguish between compounds and states a minimum level of confidence for each

of the techniques. For the identification of drugs, the requirement for univocal identification has been central over the past decades.[84]

The chromatographic retention time is useful for identification purposes. However, the retention time criterion can be used in different ways. One common approach is to establish a retention time window of ± 0.1 min (or a maximum of two percent of the compound's absolute retention time) around each analyte retention time. The criterion is fulfilled only when elution occurs within the established time frame. Alternatively, it is also possible to use relative retention times (RRT), where the retention time is compared relatively to a certified reference compound (CRC). In this approach, the criterion is set to a maximum deviation of one percent.[85]

A common approach is to compare e.g. a mass spectrum of an unknown with those included in a spectral library. The spectra in the library must have been recorded using the same experimental conditions in the same laboratory. Hence, a hit with a spectrum in a commercially available library is not good enough.[83]

There are also a number of documents that outline criteria for identification. According to the criteria established in the Commission decision EU 2002/657/EG for the interpretation of EI spectra, there should be at least four ions present at an intensity exceeding 10 percent of the base peak. Moreover, at least four of the ions should be within the tolerances stated in Table 1. Other guidelines include that of the forensic science laboratories that has been established by the International Laboratory Accreditation Cooperation (ILAC-G19:2002).

Table 1. Relative intensities of diagnostic ions and their tolerance compared to a reference spectra.

Relative intensity	Maximum deviation
> 50 %	± 10 %
> 20 % - 50 %	± 15 %
> 10 % - 20 %	± 20 %
< 10 %	± 50 %

6.4 The Need for Reference Compounds

The identification criterion that the reference spectra should be recorded using the same analytical conditions makes the availability of reference substances a central and crucial issue.[86, 87] The escalating number of new substances exemplified the importance of finding these reference materials.[88] To some extent, this demand is satisfied by commercial chemical suppliers. However, it often takes time for standards of new compounds to be available for purchase. Due to this situation, forensic laboratories often rely on in-house structural elucidations. All reference substances that are employed must also be of high purity and be properly characterized.

6.5 Structural Elucidation of Unknowns

For the unambiguous structural elucidation of new substances such as NPS, several spectroscopic techniques are normally used.[89-91] Techniques like NMR and GC-MS are in most cases needed, preferably in combination, to enable a proper identification of structural elements and determination of the chemical structure.[92] Other complementary techniques like QTOF-HRMS[10, 93] and GC-FTIR[91, 94] are useful alternatives that facilitate the identification.

Another, but less common, way of elucidating a chemical structure of a compound is to synthesize it. The synthesized compound can thereafter be used as reference substances in the laboratory. Additionally, seizures containing substances of high purity can be converted to a valuable reference substance for use in the laboratory. This would require rigorous structure elucidation of the compound to validate its use as a standard in the forensic laboratory work.

7 Aim

The aim of the work described in this thesis was to assess the ability of different analytical techniques to differentiate between structurally related NPS, applying a systematic approach where synthesized analogues are compared to already known substances.

Specific objectives were: firstly, to synthesize twelve new synthetic cannabinoids and cathinones in an attempt to predict future NPS. Characterization should be accomplished by using common analytical techniques. This would include GC-MS, GC-FTIR, UHPLC-QTOF-MS and NMR to produce a set of spectroscopic data. Secondly, to evaluate the capability of these techniques to distinguish between isomers and homologous series among the synthetic cannabinoids and cathinones. GC-MS and GC-FTIR should be given higher attention, because these are applied as primary and secondary drug screening methods in routine work at NFC.

Knowledge on the performance of the two techniques expected to give valuable support for laboratories whose mandate is to select analytical techniques and establish a screening strategies.

8 Synthetic Strategies

Since 2006 about 500 mass spectra of unidentified substances (subsequently identified) found in seizures have been registered in an in-house GC-MS library at NFC. This information, in combination with that registered in the laboratory's information management system (LIMS), has enabled study of trends regarding the NPS evolution. Further, this gives an overview of the historical emergence of new substances, which include the occurrence of different structural elements over time. In the following section, the selection and synthesis of compounds are briefly outlined, reflecting the main pathways and approaches.

8.1 Selection of Synthetic Cannabinoids (Paper I)

Since the first appearance of synthetic cannabinoids in Sweden 2008, more than 160 analogues have been encountered in Europe.[42] Upon evaluation of the order at which these substances occurred in seizures, it was found that the appearance of various structural elements in these molecules sometimes re-appeared. Utilization of this information to point out possible future analogues was developed and thereafter applied. For the synthetic cannabinoids, a fairly common substituent pattern was recognized; when new cannabinoid subgroups appeared, they had new linkers and attached groups at the indole 3-position, but at the same time the moieties linked to the indole nitrogen were similar to those that previously appeared. Two common cannabinoid subgroups found in Swedish drug seizures in 2012 contained the structures (1*H*-indol-3-yl(2,2,3,3-tetramethylcyclopropyl)methanone (compound **1** Figure16; such as UR-144) and 1*H*-indol-3-yl(adamantan-1-yl)methanone) (compound **2** Figure 16; such as AM-1248 and AB-001) . Further, butyl and ethyl tetrahydropyran occurred as nitrogen substituents. These two nitrogen substituents, together with the 4-fluorobutyl, were considered to be likely structural elements in possible future synthetic cannabinoids, and hence were taken into consideration when selecting the compounds to be synthesized in this work. Other positions susceptible for substitution were considered more difficult to synthesize, which also was a factor when focusing upon the indole nitrogen for substituent motifs in the current study. Series of structures with minor variations compared to existing compounds was selected in order to challenge the analytical methods for their capability of differentiation between structures. Therefore, attention was at the same time paid to select a proper set of already known synthetic cannabinoids with similar motifs, i.e. homologues, for inclusion in the study. The structures of the two scaffolds and the synthetic cannabinoids that were finally selected for synthesis are presented in Figure 16.

In order to avoid synthetic pathways that were too complicated, some retro synthesis schemes were also considered. Further, the syntheses were not aimed at the optimization of high yields. Worth mentioning in this context, is that the synthetic strategy applied in the paper was based on scaffolds. This approach could be useful for forensic laboratories as it would enable fast production of analogues (i.e. reference substances) in few reaction steps.

8.2 Selection of Synthetic Cathinones (Paper II)

The new synthetic cathinones appearing in seizures are structural modifications of previously encountered structures that have been modified. Commonly modified structural elements in the synthetic cathinones are ring substituents, *N* alkylations and alkyl chains. Efforts aiming at predict possible future NPS have been performed by other research groups.[95-97] The synthesis described by Smolianitski *et al*, focuses on the formation of cyclic ketals and thioketals, and resulted in nine new cathinone derivatives. Kavanagh *et al* describe the synthesis of isomers of metylenedioxypyrovalerone (MDPV), butylone and methylone.

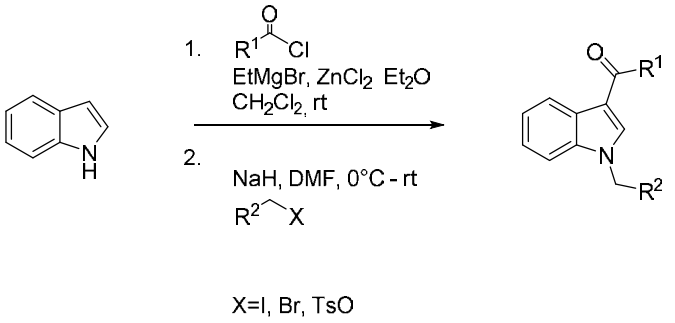
In the work presented in Paper II, six synthetic cathinones were chosen for synthesis. These compounds, which at the time had not appeared on the recreational market, were synthesized using published synthetic pathways. The synthesis of cathinone derivatives is fairly straight forward and well delineated in the literature.[98, 99]

Cathinones synthesized in this work (Paper II) were, in contrast to the synthetic cannabinoids, primarily selected with the aim of complementing those already available as references. The intention of this approach was to provide sets of isomers and homologous that would provide an analytical challenge for the analytical techniques, while at the same time, indicative of synthetic cathinones that could enter the recreational market. Six new synthetic cathinones were finally selected for synthesis. Four additional cathinones were also synthesized. Although they were already known, they needed to be synthesized to provide references of high purity in sufficient amounts.

8.3 Synthetic Aspects

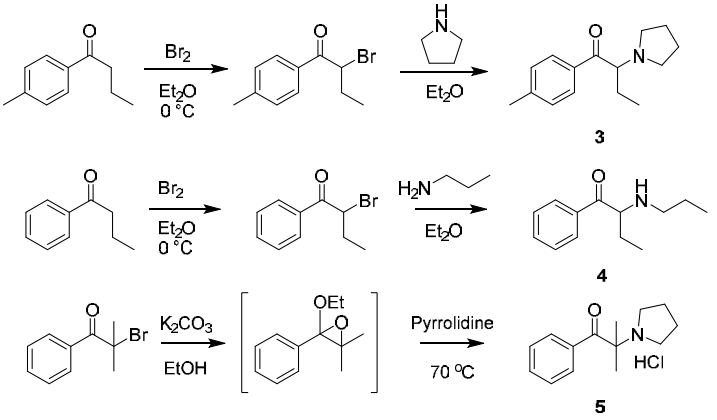
The synthesis of cannabinoids started with acylation of indole using an appropriate acid chloride derivative according to the synthetic routes studied by Frost and co-workers[100]. Scaffold **1** was formed by treatment of 1*H*-indole with ethyl magnesium bromide and thereafter reaction with 2,2,3,3-tetramethylcyclopropanecarbonyl chloride (Scheme 1). Adamantane-1-carboxylic acid was used by the same conditions

resulting in the second scaffold **2** (Scheme 1). Deprotonation was carried out with sodium hydride before alkylation of the indole nitrogen with the corresponding halide or tosylate derivatives.



Scheme 1. General synthesis of the indole derivatives.

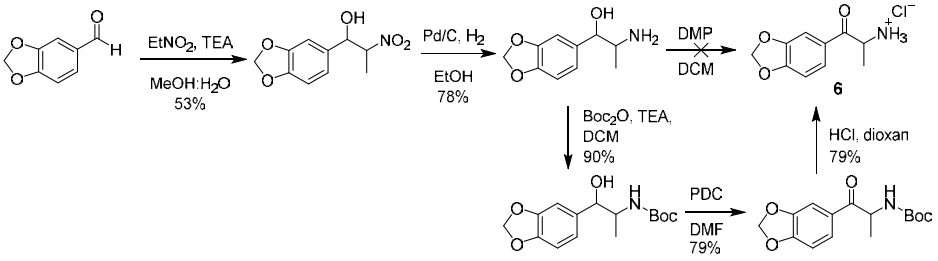
A different strategy was applied for the synthetic cathinones. Generally, amine formation was accomplished with either a bromination (such as compound **3** and **4**), with a subsequent nucleophilic substitution or nitration. Some brominated starting materials were however available and was allowed to react, via an epoxide intermediate, to final product. That was the case for compound **5** (MPP[101], Scheme 2).



Scheme 2. Reagents and conditions used in the synthesis of compounds **3 – 5**.

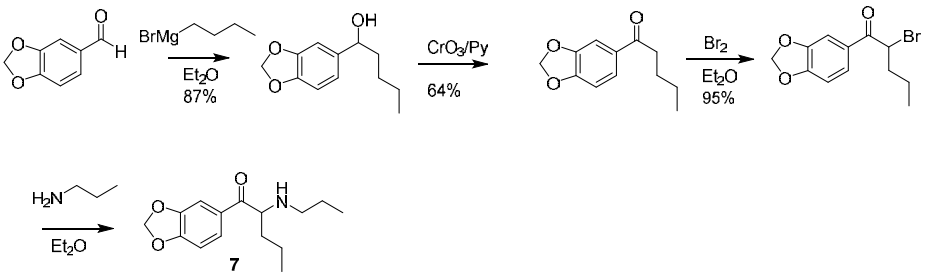
Compound **6** was synthesized from piperonal by producing a nitro group in the first reaction step. After reduction of the nitro group with hydrogen gas on Pd/C, the subsequent oxidation with Dess-Martin periodinane was found to be unsuccessful. Therefore an alternative route had to be used where the amine was protected using

the Boc group prior to oxidation of the alcohol with pyridinium dichromate (PDC). Deprotection of the Boc in HCl yielded compound **6** (Scheme 3).



Scheme 3. Reagents and conditions used in the synthesis of compound **6**.

To avoid the use of protective groups (as used for compound **6**) and thereby the additional reaction steps, a different route was adopted for compound **7**. Instead of nitration, the synthesis started with an alkylation using a Grignard reaction. In this case it was possible to oxidize the alcohol with chromium (VI) oxide to the corresponding ketone. The remaining aldehyde was transformed to the corresponding sulphonic acid and washed away before the bromination step. Finally, amination gave compound **7** (Scheme 4).



Scheme 4. Reagents and conditions used in the synthesis of compound **7**.

8.4 Synthesized and Studied Compounds

This paragraph depicts the chemical structures of both the synthesized and studied compounds in Papers I (Figure 16) and II (Figure 17). The numbering of the structures are based on their occurrence in this thesis and renumbered compared to those in the included papers.

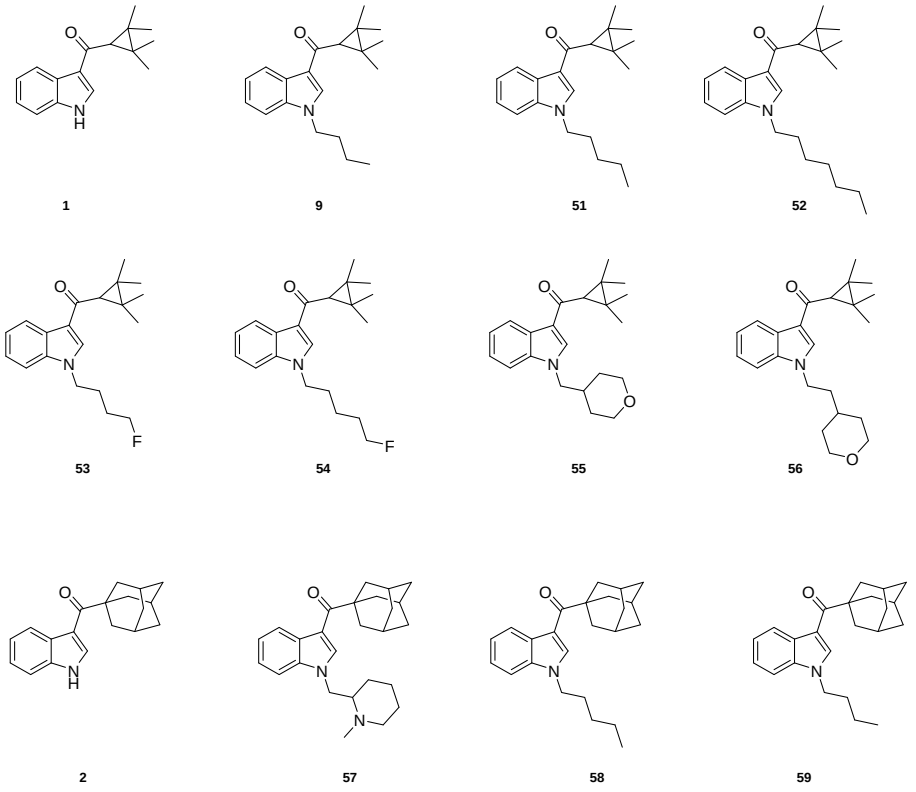


Figure 16. The synthetic cannabinoids studied in this work.

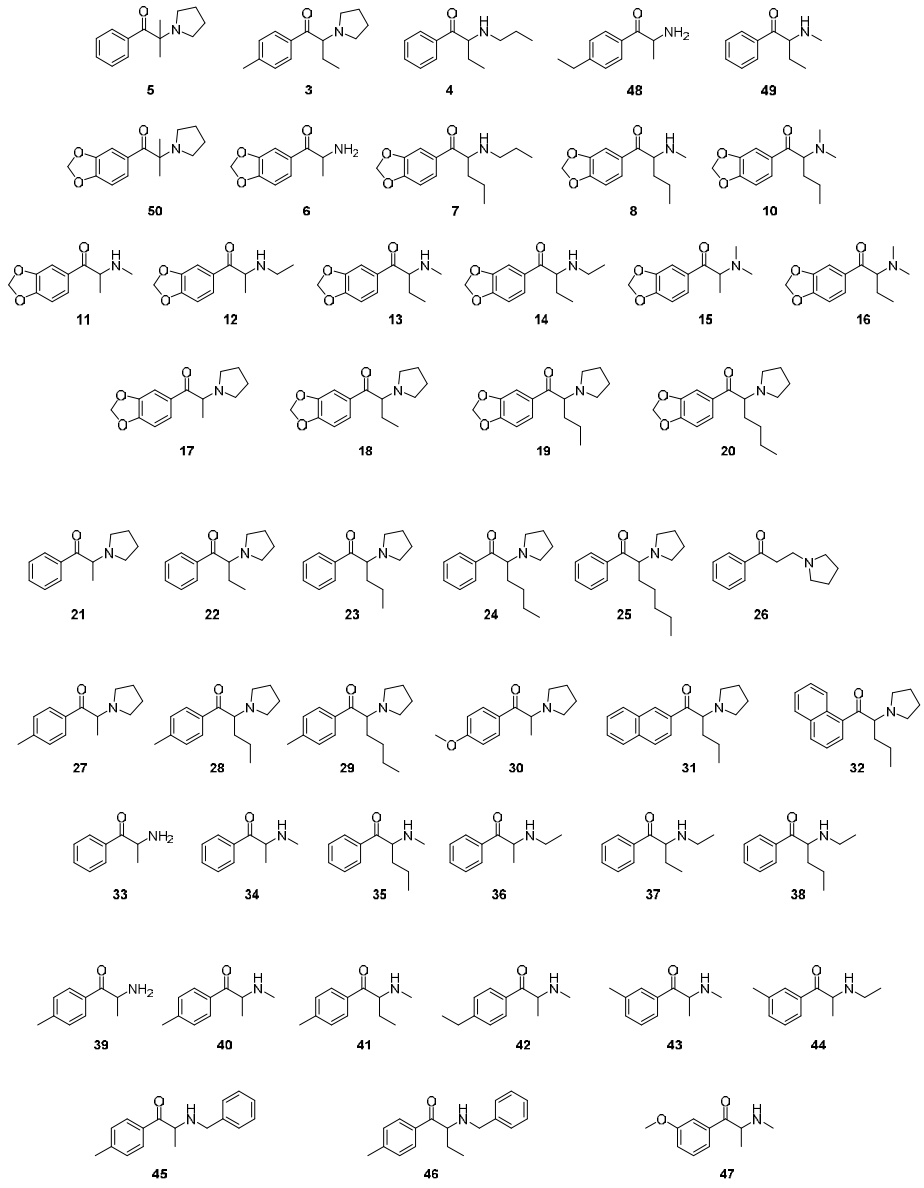


Figure 17. The synthetic cathinones studied in this work.

9 NPS Analogue Differentiation

An increased knowledge on how minor structural differences affects analytical properties may strengthen identification of and differentiation between compounds. In the following section the capabilities of various applied analytical techniques to distinguish between compounds are discussed and demonstrated with illustrative examples. It covers the work presented in Papers I and II as well as some additional complementary findings. The compounds were characterized using GC-MS, GC-FTIR, LC-HRMS and NMR. Most attention is paid to GC-MS and GC-FTIR, because these techniques constitute the primary and secondary drug screening methods applied at NFC.

The usefulness of the applied techniques depends on the analytical challenge in question and they all have their pros and cons regarding their ability to determine a univocal identification. Even though they are cutting edge techniques that provide advanced features, no method alone is able to cope with the mission of identifying all compounds. Instead, a combination of techniques is needed. In the following, the abilities of the evaluated methods are compared for different analytical problems. The compounds to which techniques have been applied have been divided into groups based on their type of structural similarity. These groups are (i) homologous compounds and (ii) isomeric compounds. Other examples that cannot be grouped under these two headings are discussed in the paragraph *Other Findings*.

9.1 Spectral Comparison of Homologous Compounds

The investigated compounds were homologues with different *N*-alkyl groups or different alkylation at other positions in the molecule. The compounds within these homologous series exhibited similar gas phase FTIR spectra and were, consequently, difficult to distinguish using GC-FTIR. As expected, GC-MS was more successful for these compounds. The alkyl homologues generated clearly different mass spectra that mainly depended on the increased mass in the homologous series. These finding were true for both synthetic cannabinoids and cathinones, which is exemplified by the synthetic cannabinoid compounds **52** and **53** in Figures 18 and 19. The two compounds differ only in the *N*-alkylation; compound **52** has a heptyl group, while there is a fluorobutyl group in compound **53**. The 3-carbonylindole ion (*m/z* 144) is present in the spectra of both compounds and gives rise to fragment ions at *m/z* 116 and 130 after further fragmentation comprising a fingerprint for its presence. The differences are, however, obvious in other parts of the spectra. In fact, all ions (including the

molecular ions) that still contain the *N*-alkyl group differ in the two spectra. Hence, GC-MS can easily distinguish between the two compounds.

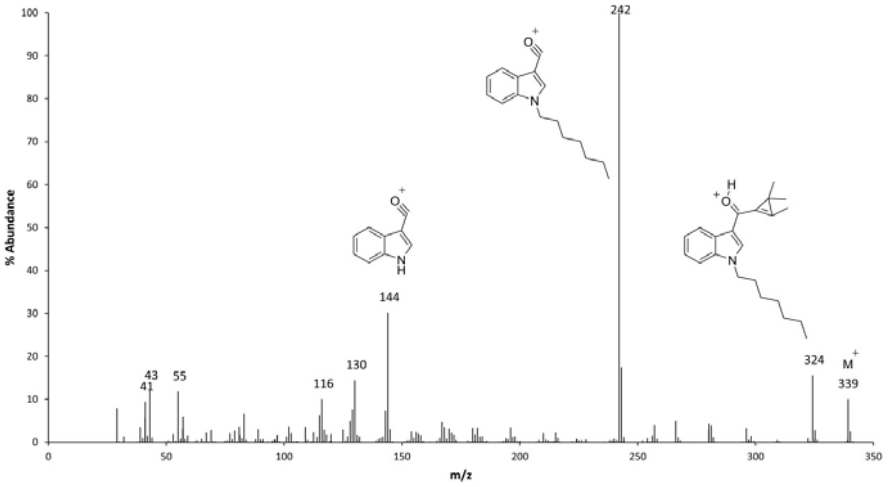


Figure 18. Mass spectra of compound **52**.

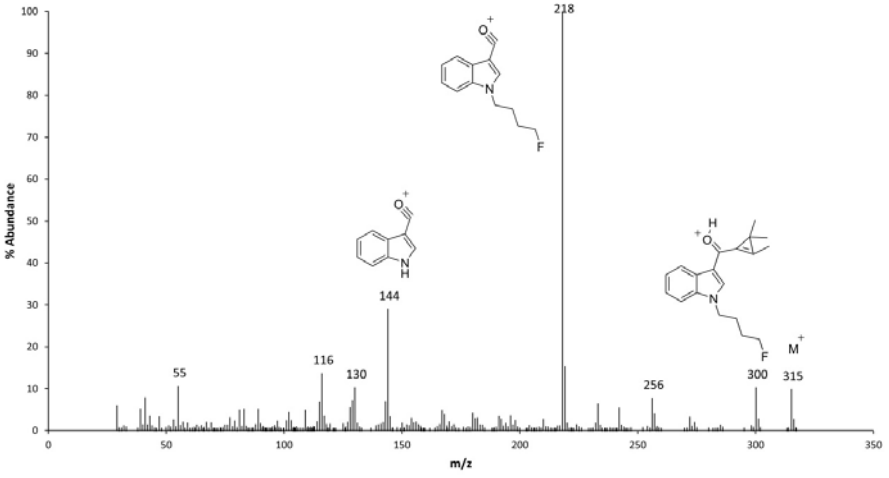


Figure 19. Mass spectra of compound **53**.

GC-FTIR was, as mentioned, less successful for these compounds. The mass differences within the homologous series do not affect the spectra much and it is therefore difficult to differentiate these compounds from each other using GC-FTIR. This is illustrated in Figure 20, which shows the FTIR spectra of compounds **9** (*N*-butyl), **51** (*N*-pentyl), **52** (*N*-heptyl), **53** (*N*-fluorobutyl) and **54** (*N*-fluoropenyl). As can be seen, this set of compounds generates very similar spectra. Hence, it may be difficult to identify a compound solely based on its FTIR spectra in gas phase.

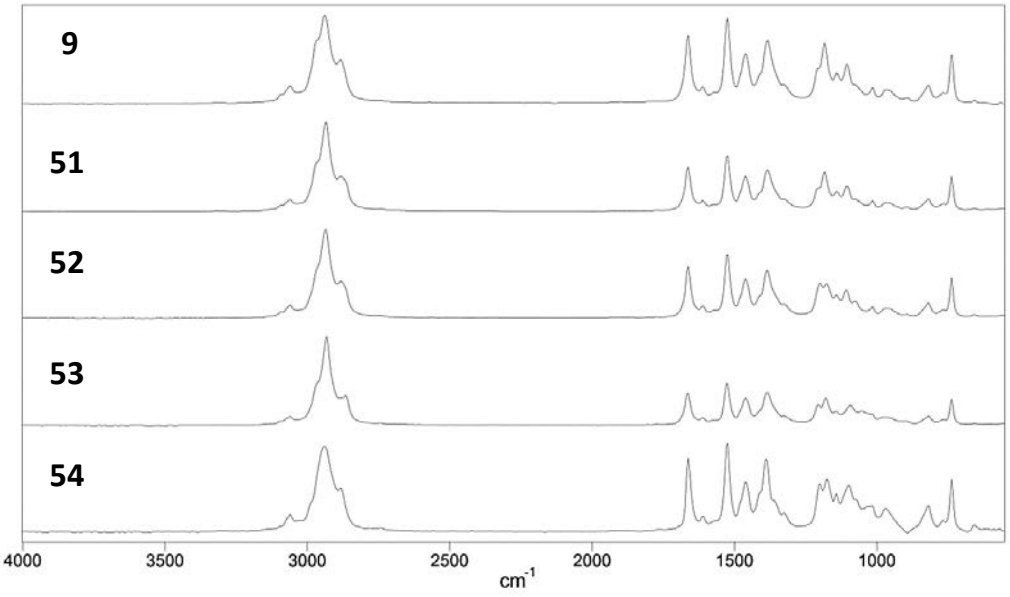


Figure 20. Comparison of GC-FTIR spectra of compounds **9** and **51-54**.

It is sometimes useful to calculate the difference between the molecular ion and the base peak. This provides an indication of the major neutral loss and may help in the assignment of an appurtenant subgroup when working with an unknown spectrum. This was quite clear with the synthetic cannabinoids as shown in Table 2, where two major groups containing fragments at *m/z* 97 and 135, respectively, could be formed.

Table 2. Differences between the molecular ions and the base peaks of the synthetic cannabinoids.

Compound	M^+	Base Peak	Δ (M^+-Base peak)
1	241	144	97
9	297	200	97
51	311	214	97
52	339	242	97
53	315	218	97
54	329	232	97
55	339	242	97
56	353	256	97
2	279	144	135
58	349	214	135
59	335	200	135
57	390	98	292

The easy loss of the indole *N*-alkyl substituents resulted in spectra with high similarity. This might complicate the interpretation, especially in cases where the molecular ion and other fragments that contain the *N*-alkyl substituent are absent or present at low intensities. Moreover, when both the molecular ion and the base peak contain structural information regarding the *N*-alkylation, the smaller fragments have two different sources, which further complicate the construing of the spectra.

In general, the synthetic cannabinoid spectra contained relatively few specific fragments in their EI mass spectra. This could make compound identification difficult, although it in some cases could be advantageous that a compound group exhibit characteristic spectra. Nevertheless, this situation often demands that other complementary analytical techniques are employed to enable proper identification.

Also the *N*-alkyl homologues of synthetic cathinones showed spectral differences in their EI mass spectra. This can be illustrated by the mass spectra of the synthesized *N*-propyl analogue **7** and its methyl analogue **8** (Figures 21 and 22). The different base peaks at *m/*z 86 and 114, respectively, obviously makes the two mass spectra different and thereby compound identification, as well as their differentiation, possible. However, the molecular ions (*m/z* 235 and 263, respectively) were small in both cases (still visible at a level of sub 0.1 percent), which aggravates the interpretation.

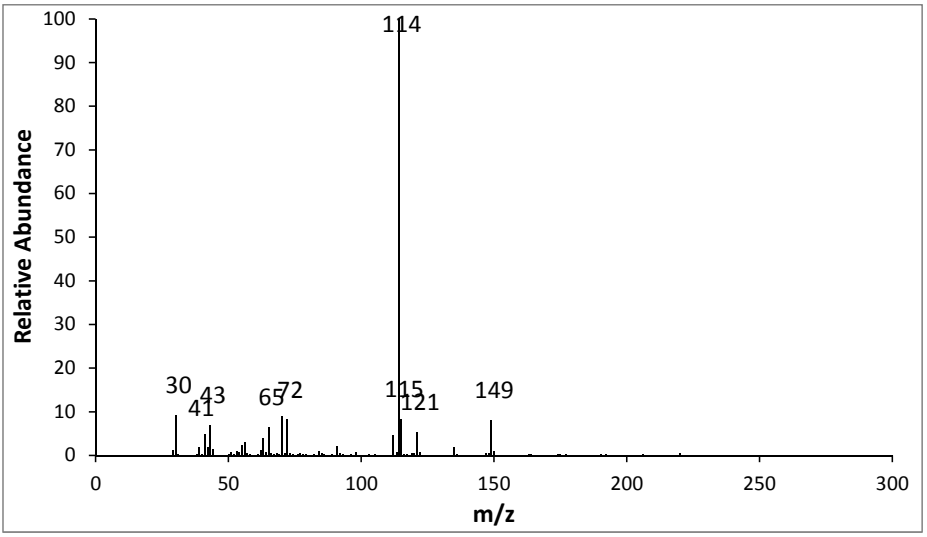


Figure 21. Mass spectrum of compound **7**.

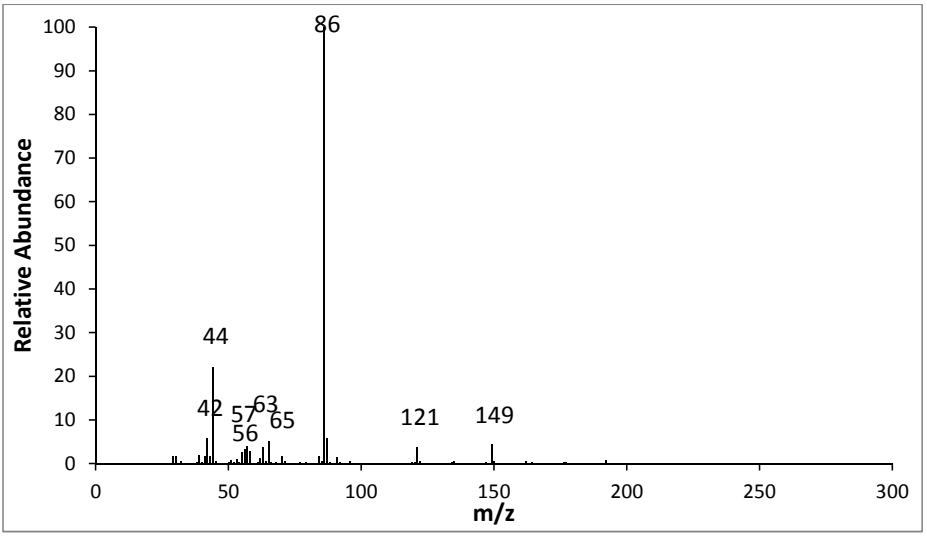


Figure 22. Mass spectrum of compound **8**.

The gas phase FTIR spectra of the same two compounds (**7** and **8**) are similar, but distinguishable as there are minor differences in the region just below 3000 cm^{-1} (C-H stretching, Figure 23). If compound **7**, is instead compared to the *N*-dimethyl substituted dipentylone **10**, the differences are more significant also in the lower region about 1000 cm^{-1} (Figure 24). Hence, it can be concluded that FTIR spectra

provided by GC-FTIR is less successful for this type of compounds than is the EI spectra obtained by GC-MS analysis. The added value of GC-FTIR as a secondary technique for these compounds is therefore limited and is in general not needed as the EI spectra, produced by a primary GC-MS screening method, properly can do the job.

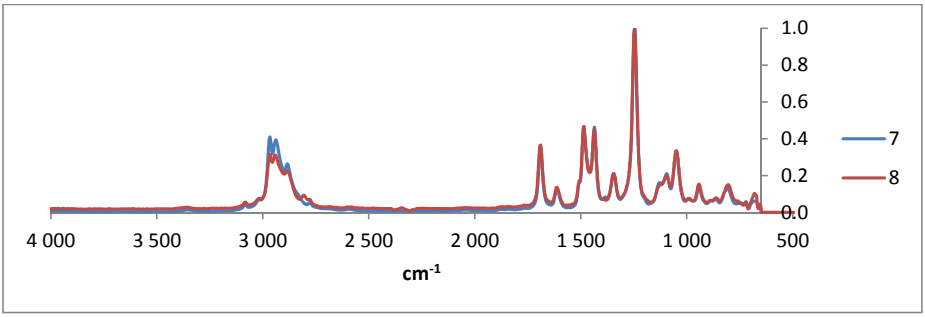


Figure 23. GC-FTIR spectra of the two homologues **7** (synthesized) and **8** (pentylone).

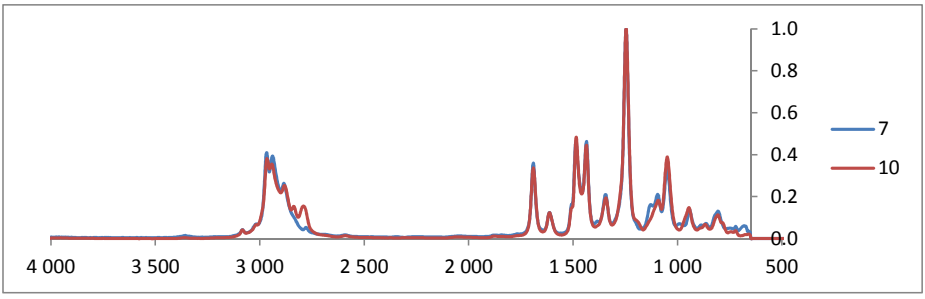


Figure 24. GC-FTIR spectra of compound **7** (synthesized) and **10** (dipentylone).

9.2 Spectral Comparison of Isomeric Compounds

Isomeric compounds have the same molecular formula, and therefore the only difference in the molecule is the different connectivities within the respective molecules. Hence, they have the same exact mass and cannot be differentiated only based on their measured accurate mass. Only when a MS^2 technique is applied, the derived fragment ions can be used to determine the structure. None of the compounds synthesized in this work exhibited HRMS fragment spectra with a degree of similarity that made them indistinguishable from each other. In contrast, the GC-MS spectra were in some cases insufficient for discriminating between isomeric cathinone derivatives as well as isomeric cannabinoid derivatives. This can be exemplified by comparing the EI spectra of compound **18** (one of four available pyrrolidene

substituted MDMA analogues) and compound **50**, which is an isomer synthesized in this study (Figures 25 and 26). As can be seen, the two spectra possess a high degree of similarity with only small differences in the lower mass region. Even though there are minor differences at lower masses, this is a typical example of a situation in which confident identification is only achieved with the use of complementary techniques.

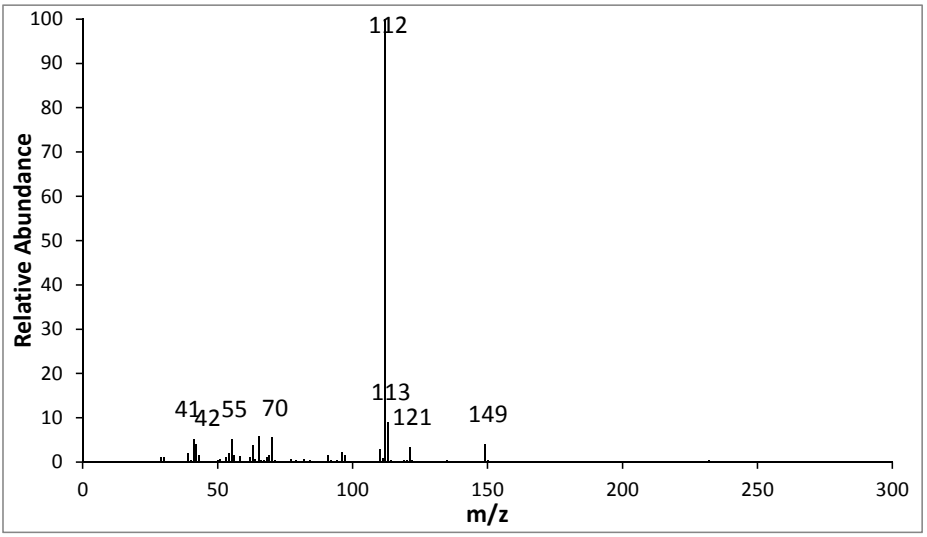


Figure 25. Mass spectrum of compound **18**.

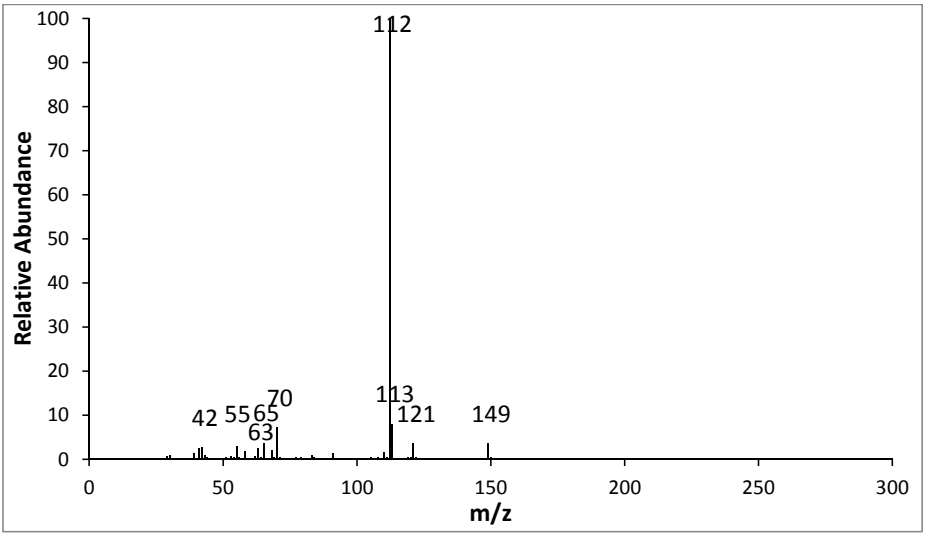


Figure 26. Mass spectrum of compound **50**.

As opposed to GC-MS, GC-FTIR was more successful for the isomeric compounds. The FTIR spectra of compounds **18** and **50** showed indeed significant differences as is illustrated in Figure 27. Hence, this pair of cathinone isomers is an example where the application of GC-FTIR as a secondary screening method is complementary and therefore highly valuable.

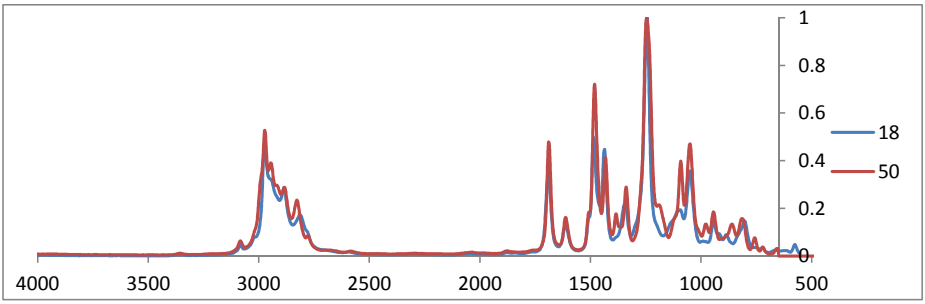


Figure 27. GC-FTIR spectrum of compounds **18** and **50**.

Another aspect to the certainty of an identity of a compound, apart from usual identification criteria, is the probability that another compound has similar spectra. The number of compounds that theoretically have the same molecular mass is therefore of interest. Continuing the discussion based on compound **50**, the unit mass resolution GC-MS library consisting of more than 1000 entries, had six other compounds with the same molecular weight (261.2 amu). They were prodine, properidine, propylphenidate, isopropylphenidate, 4-MeO-alpha-PVP and MDPBP. For compound **48** ($C_{11}H_{15}NO$) the number of compounds that had the same unit mass in the GC-MS library was seventeen. The obvious solution to this problem should then be to move over to a HRMS technique. Surprisingly, there are still ten compounds possessing the same exact mass to four decimal places as compound **48** (177.1154)! However, from a chemical view it is logical since many compounds have occurred that are isomers. The compounds were bufedrone, ethyl cathinone, phenmetrazine, 5-APDB, 4-APDB, 6-APDB, 2-methyl methcathinone, 3-methyl metcathinone, mephedrone and 7-APDB. In such cases, retention times (see below) or the power of NMR provides a definitive answer. In light of the analysis, the forensic chemist needs to be aware of the possible compounds that could be present in the sample. This emphasize that not only technological aspects, but also strategies and instrumental techniques are required.

9.3 Other Findings

Comparing the GC-FTIR measurements of the synthetic cathinones, some details were found. Firstly, plotting the relative intensity of the carbonyl stretching band around 1700 cm^{-1}, the intensities was significantly weaker for the synthetic cathinones bearing a metylenedioxy motif (compounds **6**-**8**, **10**-**20** and **50**, Figure 28) compared to others. Compound **50** showed the highest intensity among them and may be regarded differently. One explanation is that this is the only of the methylenedioxy compound that contains a geminal methyl groups adjacent to the carbonyl, which clearly will influence the carbonyl absorption. Also the intensity for the pyrrolidine containing compounds was affected. However, there was more diversity within them. This enables a type of classification into subgroups based on the measured intensity of the carbonyl stretching band (Figure 28).

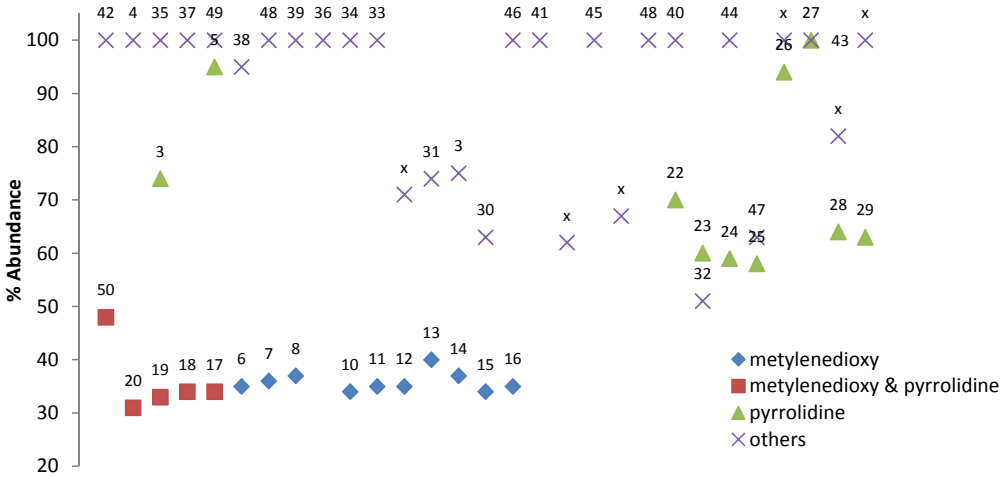


Figure 28. Relative intensities for the carbonyl stretching band 1700 cm^{-1} with regard to structural element for the synthetic cathinones studied. Numbers indicate compounds.

The influence of *N*-alkylation and *N*-β-alkylation seems also to affect the carbonyl stretching band value. These observations may imply that a substance with a high degree of substitution lowers FTIR shifts (wavenumber from 1697 to 1690). A representation for the shifts of the carbonyl stretching band for all the analyzed compounds are presented in Figure 29.

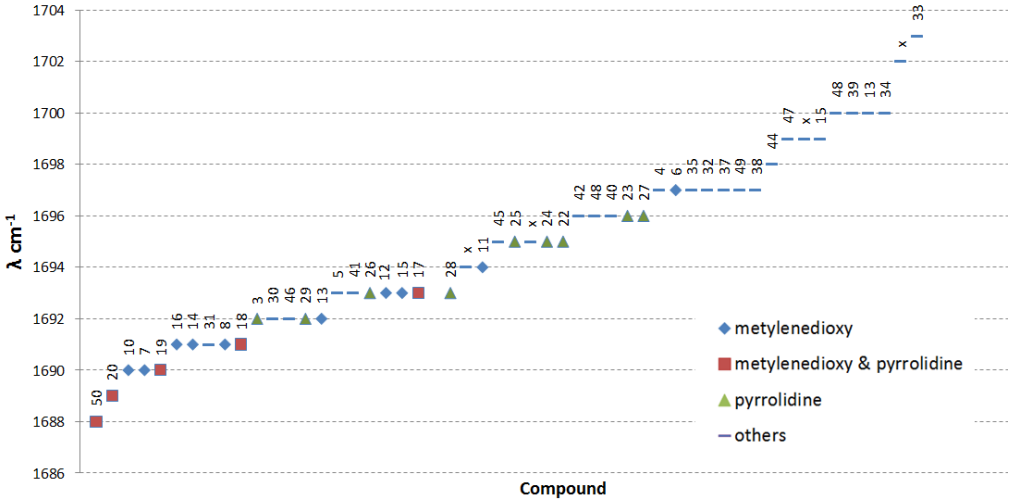


Figure 29. Shifts of the carbonyl stretching band for the synthetic cathinones. Compounds indicated by numbers.

Co-elution is one limitation of identifying compounds based upon their gas chromatographic retention time. Therefore, hyphenated techniques, where chromatographic separation is combined with a selective technique, is needed. Despite its limitations, the retention time is often a required identification criterion used in guidelines orthogonal to spectroscopic data. Especially useful is the retention time when it comes to the differentiation of compounds producing similar spectra.

A commonly applied retention time windows is ±0.1 minutes. The synthetic cathinones with a methylenedioxy motif (compounds **6**-**8**, **10**-**20** and **50)** had overlapping retention times. For each retention time windows, there were at least two compounds (Figure 30).

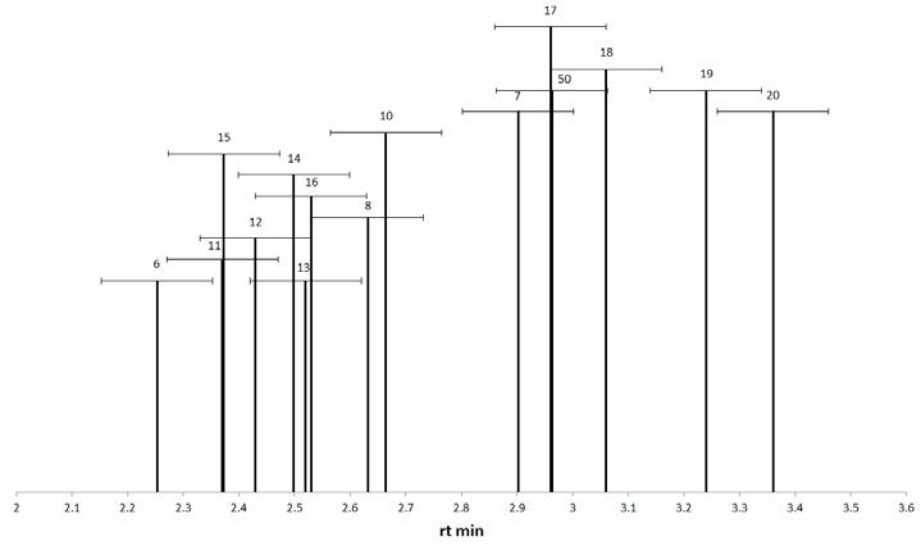


Figure 30. Schematic representation of retention times of compounds 6-20 including retention time windows of ±0.1 minutes.

Taking into account retention times of the standard screening method at NFC, there were between 29 and 48 other compounds (probably distinguishable by mass spectra) that were within the cathinones retention time window. Further, if all different retention times in the method were plotted, a type of density description of retention times are produced (Figure 31). This can be used as guidance in method development in evaluating different chromatographic properties, for achieving more diverse retention times.

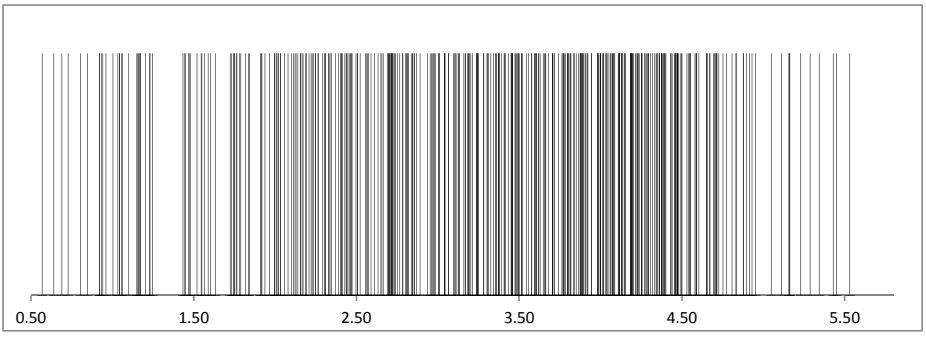


Figure 31. Schematic description of the densities for the retention times of the NFC screening method consisting of more than 450 compounds.

10 Conclusions

This work provides spectroscopic data for a number of new synthetic cannabinoids and cathinones that potentially could reach the recreational drug market in the future. Further, it is concluded that GC-MS and GC-FTIR complements each other well in their ability to differentiate between the investigated analogues from these two groups.

GC-MS analysis of isomeric synthetic cathinones revealed that such compounds may exhibit similar EI mass spectra. Therefore, these types of compounds could be difficult to distinguish based solely upon their mass spectra. GC-FTIR, on the other hand, was more successful for this analytical challenge as the gas phase FTIR spectra of these isomers were significantly different. The synthetic cannabinoids showed that the use of HRMS data was proven to be useful in the differentiation of compounds with similar molecular ions in the GC-MS spectra.

For homologous series of both cannabinoids and cathinones the outcome was reversed. These homologues, of which most were alkyl substituents, did in general provide EI spectra that could easily be differentiated. This was mainly due to their different molecular weights that affected the mass of the molecular ions and larger fragments containing the alkyl substituents. However, there were exceptions. The molecular ion was often unstable and underwent further fragmentation. In some cases, the intensities of these important fragments were so low that spectra became difficult to distinguish.

The results of this work have demonstrated that neither GC-MS nor GC-FTIR *alone* can successfully distinguish between all synthetic cathinones and cannabinoids. Hence, there is a need for complementary techniques or methods. At NFC, drug screening is accomplished using GC-MS as the primary method and GC-FTIR as a secondary method. In this approach, all samples are analyzed by GC-MS and for samples where this method cannot make an unambiguous identification, GC-FTIR is used as a complementary technique. The findings of this work does, in some ways, confirm the ideas behind the screening approach applied at NFC, and that it is indeed a good approach to apply GC-MS as a primary screening methodology with back up by a GC-FTIR method.

The work described in Papers I and II gives a wide perspective of the utilization of the different applied techniques analyzing synthetic cannabinoids and cathinones, although generalizations are not possible. However, some features are common for

groups of compounds, like isomers and homologues described above. By comparing the spectral data of both the synthetic cannabinoids and cathinones, it was evident that small structural changes may result in pronounced spectral changes. Further, it was confirmed that a proper evaluation of a technique's ability to differentiate between analogues only can be performed when a reference compound is at hand.

Through structured working procedures, this work also showed that there are other types of criteria that can be used as a complement to already established ones, such as analysis of the GC-FTIR carbonyl stretching band. The determination of criteria needed in the identification of a substance can be regarded as a hypothesis testing. A broad knowledge of the capabilities and limitations of the different techniques is needed in order to determine the scope of this hypothesis. Eventually, the question *"Is certain good enough?"* is the essence of identification.

To summarize, the results presented in this thesis show that a successful unambiguous determination of NPS is dependent on well characterized reference substances, the use of complementary techniques, developed strategic chemical assessments and experienced-based "chemical intelligence".

11 Future Perspectives

To gain additional understanding of capabilities of various analytical techniques to differentiate NPS, the work presented in this thesis displays spectroscopic data of structural analogues and pinpoints some areas where chemical awareness is required. Since the evolvement of upcoming NPS will not end within a foreseeable future, there are still many questions that initiate the quest for further answers.

The method applied in Paper I regarding future occurring structural elements has elaboration potential. The application of more sophisticated methods, also applying statistical tools, may lead to a higher degree of preparedness when used together with intelligence analysis.

Further, the utilization of scaffold based syntheses can be used to create libraries of NPS. Even though a large set of compounds may eventually facilitate the structural elucidation process, it would seem however that the costs outweigh the benefits, since the currently adopted process is fast and efficient. Further, in a community context, the time of structural elucidation is only a fraction of the whole time-span of the classification process. Yet, such an approach should, in my opinion, be most beneficial regarding the utilized identification criteria and knowledge about spectroscopic limitations.

Another area not evaluated in this work is the analysis of more complex samples, such as mixtures or at low concentrations, which could lead to the inability to use some of the identification criteria. Under these conditions, both strategies for method development and sample preparation (i.e. purification) as well as synthesis as a proof of identity, can be investigated.

Finally, there are also unresolved questions regarding the analysis of distinctive spectroscopic data from an overall standpoint regarding the structural diversity of NPS. Derived data also for other NPS subgroups in combination with those described in this work may be used for making conclusions regarding univocal structural identity.

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