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Pyrolytic Fate of Synthetic Cannabinoids

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ABSTRACT

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Corey Andrew Nida

Since the introduction of herbal incense products into the illicit drug market, one of the most concerning factors has been the uncertainty regarding their health effects. Side effects such as anxiety/agitation, increased heartbeat, hallucinations, and suicidal tendencies are commonly reported with the use of products containing synthetic cannabinoid. However, a largely unknown toxicity and pharmacology is still associated with synthetic cannabinoids and long-term health effects have yet to be discussed.

Prior scientific studies have not focused on the big-picture in terms of the pyrolysis of traditional drugs of abuse that are smoked or the relatively new synthetic cannabinoids. Numerous agencies and statistics have reported the number of health-related incidents regarding the use of synthetic cannabinoids, but there has yet to be peer-reviewed reports seeking to understand what caused these health effects. Therefore, the purpose of this research was to investigate the pyrolytic fate of JWH-018, JWH-030, JWH-081, and UR-144 and the smaller components which comprise these synthetic cannabinoids.

Studying a number of the most common components that comprise a large number of the synthetic cannabinoids allows for a broad-ranging, cost-effective dissemination of results. A comprehensive approach was taken for identifying the pyrolytic products observed with a series of indole and naphthalene containing compounds. In doing so, a baseline of pyrolytic products that can form was established and it was found that a number of polyaromatic hydrocarbons, heterocyclic amines, and other hazardous or potentially hazardous compounds were generated.

Analysis of the synthetic cannabinoids in this study showed that known carcinogenic compounds and potentially harmful pyrolytic products, such as carbazole, naphthalene, and benz[a]anthracene, are generated during smoking. The synthetic cannabinoids JWH-071 and JWH-018 were also, respectively, identified as pyrolytic products of JWH-018 and JWH-081. Furthermore, a number of compounds that were not identified have been reported which may, like the additionally generated synthetic cannabinoids, also retain activity at the cannabinoid receptors.

DEDICATION

This work is dedicated to my two very important younger siblings, Brooke and Blaine.

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Graduate school is not a journey that you can make it through alone and as such there are a number of acknowledgements that need to be made.

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°C	Degrees Celsius
Δ^9-THC	Delta-9-tetrahydrocannabinol
$\Delta T_{25\ sec}$	Greatest change in temperature per 25 second timeframe
µL	Microliter
µm	Micrometer
11-OH-Δ^9-THC	11-Hydroxy-delta-9-tetrahydrocannabinol
2-AG	2-arachidonoylglycerol
5F-PB-22	1-(5-fluoropentyl)-8-quinolinyl ester-1H-indole-3-carboxylic acid
AAI	Aminoalkylindole
AAPCC	American Association of Poison Control Centers
AB-CHMINACA	N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1- (cyclohexylmethyl)-1H-indazole-3-carboxamide
AB-FUBINACA	N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide
AB-PINACA	N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide
ADB-PINACA	N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide
AEA	N-arachidonoylethanolamine
AKB-48	1-pentyl-N-(tricyclo[3.3.1.13,7]dec-1-yl)-1H-indazole-3-carboxamide
amu	Atomic mass unit
ATSDR	Agency for Toxic Substances and Disease Registry
CB_1	Cannabinoid Receptor Type-1
CB_2	Cannabinoid Receptor Type-2
Cm	Centimeter
CNS	Central Nervous System
CP-47,497	2-[(1R,3S)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol
CP-47,497 C8 Homolog	2-[(1S,2R)-3-hydroxycyclohexyl]-5-(2-methylnonan-2-yl)phenol

CP-55,244	(2S,4S,4aS,6R,8aR)-6-(HydroxyMethyl)-4-[2-hydroxy-4-(2-Methyl-2-octanyl)phenyl]decahydro-2-naphthalenol
CP-55,940	5-(1,1-dimethylheptyl)-2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol
CSA	Controlled Substances Act
D	Bond dissociation energy
DEA	Drug Enforcement Administration
DHHS	Department of Health and Human Services
eV	Electron volts
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FDASIA	Food and Drug Administration Safety and Innovation Act
GC-MS	Gas Chromatography – Mass Spectrometry
GHB	gamma-Hydroxybutyric acid
HCA	Heterocyclic amine
HHC	9-nor-9ß-hydroxyhexahydrocannabinol
HPHC	Harmful and Potentially Harmful Constituents
HU-210	3-(1,1'-dimethylheptyl)-6aR,7,10,10aR-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
JWH-018	naphthalen-1-yl(1-pentyl-1H-indol-3-yl)methanone
JWH-022	1-naphthalenyl[1-(4-penten-1-yl)-1H-indol-3-yl]-methanone
JWH-030	1-naphthalenyl(1-pentyl-1H-pyrrol-3-yl)-methanone
JWH-071	(1-ethyl-1H-indol-3-yl)-1-naphthalenyl-methanone
JWH-073	1-butyl-3-(1-naphthoyl)indole
JWH-081	4-methoxy-1-naphthalenyl(1-pentyl-1H-indol-3-yl)-methanone
JWH-200	1-[2-(4-morpholinyl)ethyl]-3-(1-naphthoyl)indole
JWH-398	(4-chloronaphthalen-1-yl)(1-pentylindolin-3-yl)-methanone
K2	Herbal Incense Brand Name
kJ	Kilojoule

Levonantradol (CP-50,556-1)	[6S-[3S,6a,6aa,9a,10a]]-5,6,6a,7,8,9,10,10aoctahydro-6-methyl-3-(1 -methyl-4-phenylbutoxy)-1,9-phenanthridinediol 1-acetate
LSD	Lysergic acid diethylamide
m/z	Mass-to-charge ratio
mL	Milliliter
Nantradol (CP-44,011-1)	5,6,6a,7,8,9,10,10a-octahydro-6-methyl-3-(1 -methyl-4-phenylbutoxy)-1,9-phenanthridinediol-1-acetate
NFLIS	National Forensic Laboratory Identification System
NPS	New Psychoactive Substance
NPL	National Priorities List
NSAID	Nonsteroidal Anti-Inflammatory Drug
NTP	National Toxicological Program
PAH	Polyaromatic Hydrocarbon
PB-22	1-pentyl-8-quinolinyl ester-1H-indole-3-carboxylic acid
RoC	Report on Carcinogens
SDAPA	Synthetic Drug Abuse Prevention Act
Sec	Seconds
SMART	Synthetics Monitoring: Analyses, Reporting and Trends
Spice	Herbal Incense Brand Name
SPL	Substance Priority List
THJ-2201	[1-(5-fluoropentyl)-1H-indazol-3-yl](naphthalen-1-yl)methanone
TIC	Total ion chromatogram
UNODC	United Nations Office on Drug and Crime
UR-144	(1-pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone
WHO	World Health Organization
WIN-55,212-2	(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morphinylmethyl)pyrrolo[1,2,3-de]1,4-benzoxazin-6-yl]-1-naphthalenylmethanone
XLR-11	[1-(5-fluoropentyl)-1H-indol-3-yl](2,2,3,3 tetramethylcyclopropyl)methanone

CHAPTER 1: INTRODUCTION

1.1.0 PURPOSE

As a consequence of the sheer number of new synthetic cannabinoids that are continually being introduced into the illicit drug market, most forensic laboratories struggle to keep pace with sample analysis. The unfortunate situation that currently exists in the forensic community has hindered the ability of laboratories to focus on fundamental research in the context of synthetic cannabinoids. As synthetic cannabinoids are usually ingested by smoking, the chemical reactions and pyrolytic products of smoking are of interest. Therefore, the purpose of this research is to better understand the pyrolytic chemistry that occurs upon the burning of a select number of synthetic cannabinoids (JWH-018, JWH-030, JWH-018 and UR-144) under cigarette-like temperatures and conditions.

The value of such research spans many scientific fields. In general, the pyrolysis of drug molecules is poorly understood at best [1]. As such, the experimental research on this subject area can provide benefit to: (1) chemists interested in the mechanistic understanding of organic synthesis and reaction mechanisms present at high temperatures (greater than ~350°C); (2) forensic toxicologists interested in the potentially new markers of exposure/abuse that can be sought in biological specimens for added forensic value; and (3) pharmacologists interested in investigating the cannabinoid receptor activity and competitive binding implications that such pyrolytic products may produce.

1.2.0 BACKGROUND

Cannabinoids can be defined as a diverse group of chemical compounds that exert pharmacological effects via the endocannabinoid system present in our bodies. The word 'group' has been purposefully selected to describe the category of cannabinoids because these compounds do not necessarily conform to a set of rigid structural similarities. There have been various attempts to categorize cannabinoids into given sub-categories, such as phytocannabinoids (plant-derived), endocannabinoids (endogenous) and synthetic cannabinoids. However, when discussing cannabinoids it is more appropriate to use the term "cannabimimetic" (Originating from the *Cannabis* plant and the Greek element *mimetic*, meaning 'to imitate'). Cannabimimetics exert effects similar to the primary psychoactive constituent found in marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC, ***Figure 1***).

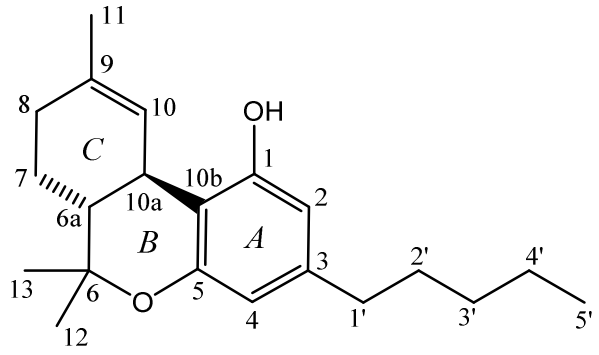


Figure 1: IUPAC numbering system and molecular structure for Δ^9-tetrahydrocannabinol (Δ^9-THC)

1.3.0 BASIC UNDERSTANDING OF THE ENDOCANNABINOID SYSTEM

The endocannabinoid system is an extensive signaling system that consists of receptors (specifically, the cannabinoid receptors), their corresponding endogenous

substrates (known as endocannabinoids), and the enzymes responsible for the production and degradation of these endogenous substrates [2]. Breakthroughs in the late-1980's and early to mid-1990's significantly helped progress the scientific understanding regarding the endocannabinoid system, but substantial work remains to be done.

The discovery of the CB_1 and CB_2 cannabinoid receptors in 1988 and 1993, respectively, led to an immense amount of interest and research that is continuing [3, 4]. These discoveries and the subsequent ability to clone the cannabinoid receptors led researchers to look for the endogenous substrates. In 1992 and 1995, researchers identified two endocannabinoids: N-arachidonoylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG) (***Figure 2***) [5, 6].

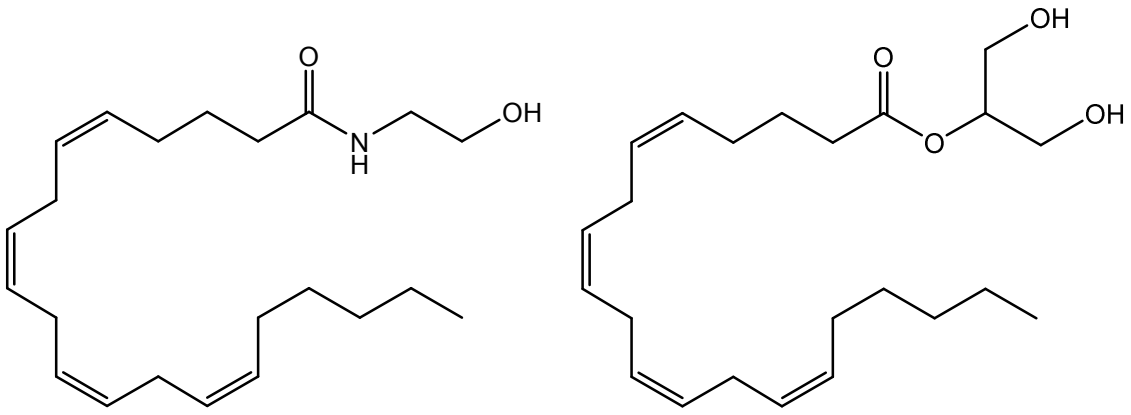


Figure 2: Structures of the N-arachidonoylethanolamine (AEA, left) and 2-arachidonoylglycerol (2-AG, right)

The location of the CB_1 and CB_2 receptors in our bodies are known. The CB_1 receptor is highly abundant in the central nervous system (CNS) but is also found in the peripheral nervous system. Conversely, the CB_2 receptors appear to be localized in the immune system [2, 4, 7, 8]. Given the location of these receptors, there have been many

proposed physiological functions of the endocannabinoid system. Reports indicate that the endocannabinoid system is involved in the body's response to and/or regulation of pain, inflammation, appetite, cognition, and memory and motor function [9 - 17]. Synthesizing potentially lucrative drug molecules aimed at modulating various parts of the endocannabinoid system for specific purposes has resulted in numerous molecular permutations of cannabinoids.

1.4.0 THE DISCOVERY AND EVOLUTION OF CANNABINOIDS: FROM PLANTS TO SYNTHETICS

Before the discovery of the endocannabinoid system and its receptors, a collection of specific behavioral side-effects (including analgesia) were the benchmark for identifying molecules as "cannabinoids". In fact, the structural elucidation of Δ^9-THC, the primary psychoactive component of *Cannabis*, did not occur until the mid-1960's [18]. While it may seem very surprising, given that marijuana has been used in many cultures as a form a medicine for centuries, researchers were hindered by the lack of instrumental and technological tools which ultimately made the discovery possible. Following this revelation, the commercial availability of Δ^9-THC provided researchers with the ability to conduct experiments aimed at understanding the pharmacology, metabolism, and structure-activity relationship of one of the most widely used drugs in the world. Additionally, there was a large amount of interest from the pharmaceutical industry.

Multiple discoveries occurred in the 1970's that served as the basis for interest in creating synthetic cannabinoids. At the beginning of the decade, researchers had identified the major metabolite of Δ^9-THC as 11-hydroxy- Δ^9-tetrahydrocannbinol (11-

OH-Δ^9-THC) (***Figure 3***) [19]. Merely finding this metabolite was not the most interesting part of this discovery. It was later reported that 11-OH-Δ^9-THC was a more potent analgesic in mice than Δ^9-THC (parent compound) [20]. The analgesic capability of 11-OH-Δ^9-THC sparked interest within the scientific community about what other cannabinoid metabolites or analogs might possess similar biological activity.

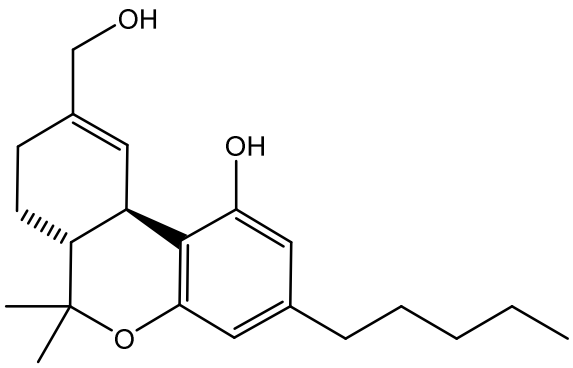


Figure 3: Structure of 11-hydroxy-Δ^9-THC (11-OH- Δ^9-THC), a metabolite of Δ^9-THC

Shortly thereafter, results documenting the behavioral effects and analgesic properties of some of the first synthetic cannabinoids surfaced [21, 22]. This particular group of synthetic cannabinoids, the 9-nor-9-hydroxyhexahydrocannabinols, contained slight modifications to the molecular structures of both Δ^9-THC and 11-OH-Δ^9-THC. Arguably the most significant finding from this work was that 9-nor-9ß-hydroxyhexahydrocannabinol (HHC, ***Figure 4***) showed analgesic potency in mice that was similar to that of 11-OH-Δ^9-THC and morphine (***Figure 5***) [22]. Compared to Δ^9-THC, HHC lacks the double bond between the C-9 and C-10 positions and a hydroxyl replaces the C-9 methyl group.

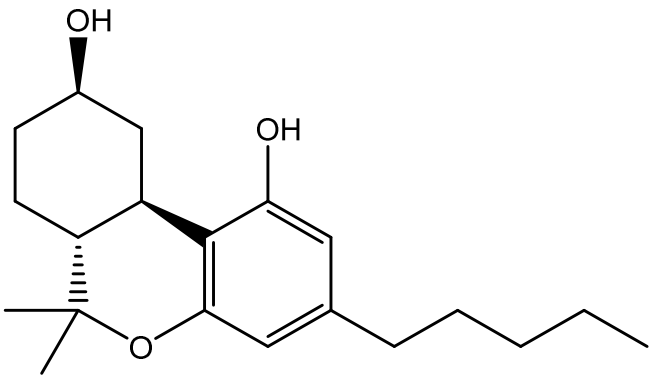


Figure 4: Structure of 9-nor-9ß-hydroxyhexahydrocannabinol (HHC)

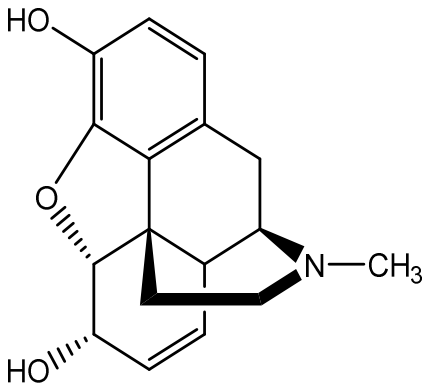


Figure 5: Structure of morphine

While the structural modifications of HHC appear minimal, the importance of these synthetically prepared analogs should not be overlooked. Prior to the Wilson *et. al.* work, much of the cannabinoid research was largely focused on phytocannabinoids and as will be evident in the following sections, a large emphasis was placed on synthetically designing cannabinoids from this point forward [22].

In the 1980's there were many reports of newly created synthetic cannabinoids that differed drastically from the classical structure of phytocannabinoids found in marijuana.

During 1970's and 1980's, Pfizer-Central Research pioneered efforts to create a newly defined analgesic pharmacophore (necessary structural features of a pharmacological class of molecules) that would be distinctly different from morphine and the other traditional opioid analgesics [23]. This initiative was a direct result of the reported analgesic effects of 11-OH-Δ^9-THC, HHC, and Δ^9-THC. While Pfizer desired to synthesize a compound possessing only the structural features of cannabinoids needed to retain the analgesic properties, but eliminate those causing the psychoactive properties of Δ^9-THC, Pfizer inevitably created a number of compounds which retained those unwanted effects.

In an attempt to identify an analgesic-cannabinoid pharmacophore, the group at Pfizer initially investigated the effects of structural modifications made to the benzopyran, phenolic group, and the lipophilic C-3 of the A-ring, (see ***Figure 1***) for side chain moieties of cannabinoids. All Pfizer-Central Research compounds have a company-associated abbreviation ("CP") that is listed before a series of numbers and is presumably a reference to Charles Pfizer, who was one of the original founders of company. Some of the first reported non-opioid analgesics to come as a result of this work were the compounds Nantradol (also known as CP-44,001-1) [23, 24] and Levonantradol (also known as CP-50,556-1, ***Figure 6***) [25, 26].

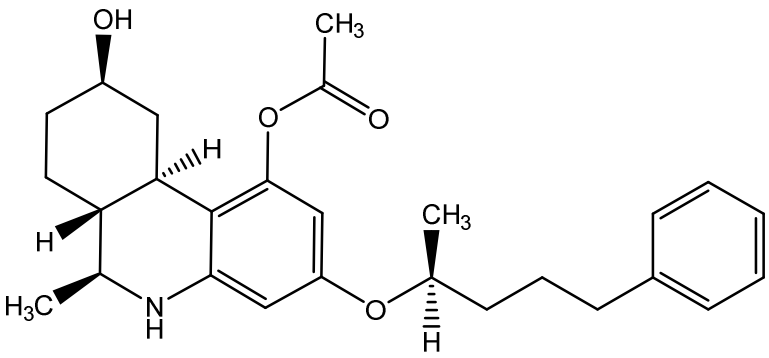


Figure 6: Structure of levonantradol (CP-50,566)

Compared to cannabinoids, which contained the characteristic benzopyran moiety and alkyl C-3 side chain, levonantradol instead contains a benzoquinoline moiety and a larger C-3 side chain that possesses an aromatic ether. While levonantradol is an analgesic derived from the cannabinoid structure, it was actually found to be more potent than morphine. Furthermore, experimental evidence suggests that levonantradol was not acting upon the opioid receptor. Unlike opioid analgesics which were known to be blocked by naloxone (opioid antagonist) the analgesic potency of levonantradol was unaffected by the antagonist. With the distinctly different structural features that levonantradol possessed, in relation to cannabinoids, and the evidence that they were not acting at the opioid receptor, Pfizer then investigated the hypothesis that cannabinoids and opioids were indeed acting at different biological sites.

Further work pursued additional structural modifications that led to a signficant discovery in the science of cannabinoids. The molecule CP-47,497, which lacked a tricyclic-based structure altogether, was proposed to be a molecule possessing the bare-minimum structural features required to retain non-opioid analgesic effects [27, 28, 29].

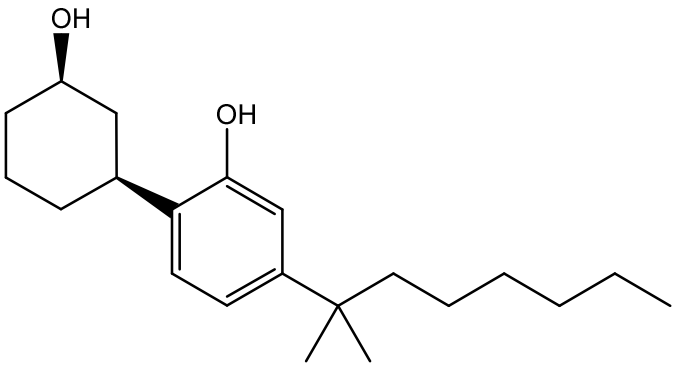


Figure 7: Structure of CP-47,497

Compared to Δ^9-THC, CP-47,497 appears radically different: it contains a slightly larger lipophilic C-3 side chain (1,1-dimethylheptyl moiety), does not contain a pyran moiety that Δ^9-THC was known for and, like HHC, it also lacked both the C-9 methyl substituent and C-9 double bond. Similar to Levonantradol, CP-47,497 was found to be unaffected by naloxone and further supported the belief that the analgesic effects of cannabinoid-based molecules and the opioid analgesics were acting at different biological sites. However, it was also discovered that CP-47,497 was an even more potent cannabimimetic than Δ^9-THC [27]. With the numerous unwanted side-effects that would inevitably accompany the desired analgesic effect of CP-47,497, research continued forward in hopes of finding a new therapeutically-beneficial drug molecule.

In the mid to late-1980's, Pfizer published cannabimimetic effects of two more potent CP molecules: CP-55,244 and CP-55,940 (***Figure 8***) [30]. Both of these molecules exceeded the potency of levonantradol (CP-50,566-1) and continued to challenge the current understanding of what structural features were deemed as necessary to invoke cannabinoid-like effects. CP-55,244 differs slightly from CP-47,497 in that it contains an additional ring structure with a hydroxy methane substituent. CP-55,940 also lacked the

pyran moiety and possessed the 1,1-dimethyheptyl moiety but differed from CP-55,244 in that instead of an additional cyclic component, it contained a propanol functional ground appended to the 'B-ring'. As much as the developments revealed by Pfizer were historically significant in the context of cannabimimetics, not all major revelations were the result of structural modifications to cannabinoid-like molecules.

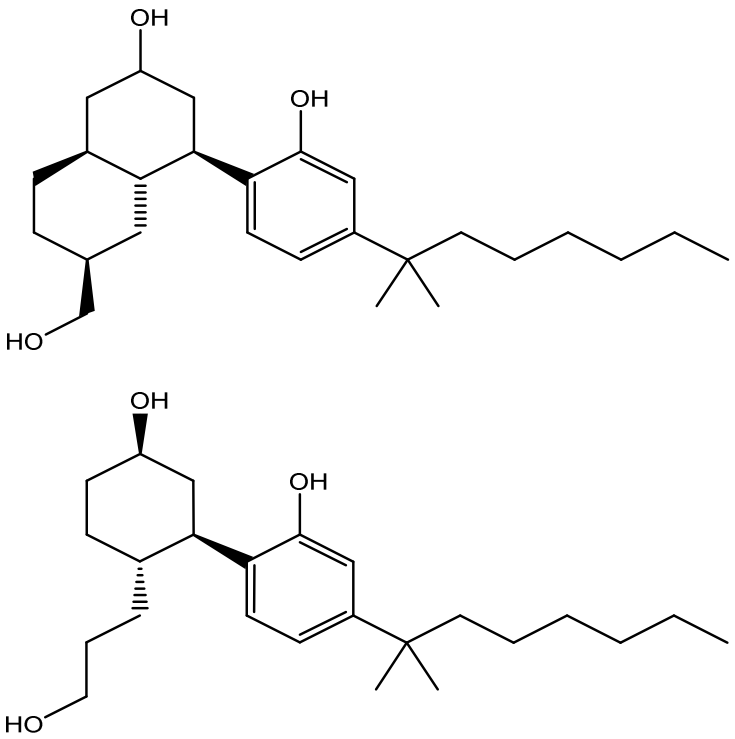


Figure 8: Structures of CP-55,244 (top) and CP-55,940 (bottom)

In the late 1980's and early part of the 1990's, researchers at the pharmaceutical company Sterling Drug (Sterling-Winthrop Research) were also working on the development of a potent non-opioid based analgesic [31]. As was the case with the Pfizer synthesized compounds, compounds beginning with "WIN" and followed by a series of numbers are those that originated in the Sterling-Winthrop Research group. However,

the focus at the time was to create a more potent, non-acidic, non-steroidal anti-inflammatory drug (NSAID). Therefore, instead of deriving their drug compounds from a cannabinoid-based molecule, work at Sterling-Winthrop sought to structurally modify the NSAIDs indomethacin and clometacin (***Figure 9***) [32]. Researchers disclosed that the compound known as pravadoline (WIN-48,098) (***Figure 9***) produced analgesic effects but that when compared to both morphine and NSAIDs, deviations from expected results were observed [33]. Like some of the previously mentioned synthetic cannabinoids, the efficacy of pravadoline was unaffected by the antagonist naloxone: indicating that it was not acting at the opioid receptor like morphine. Additionally, the potency of pravadoline distinguished it from other known NSAIDs. Taken together with the (at the time) current discoveries of the endocannabinoid system, investigations into the cannabinoid receptor activity of pravadoline and its analogs was a natural area of research to explore.

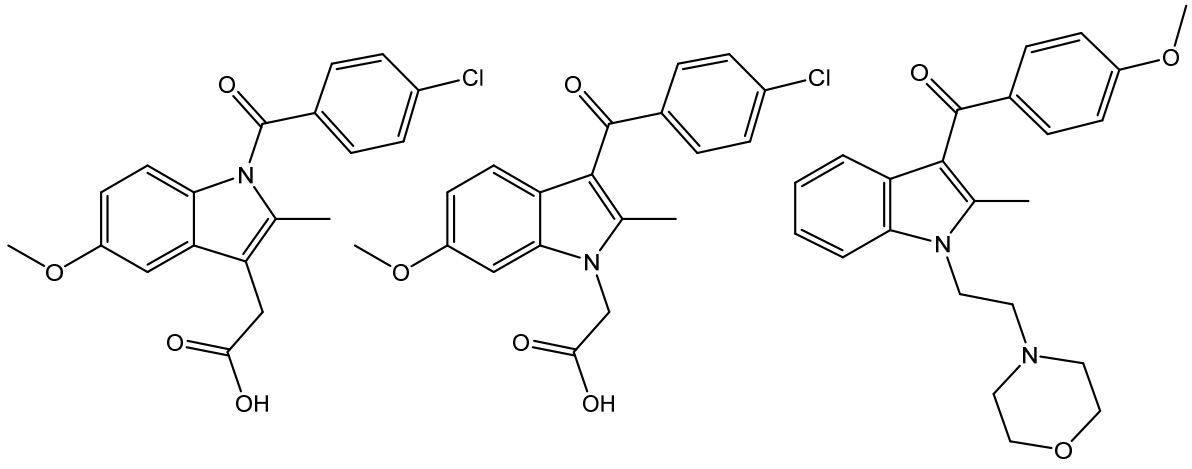


Figure 9: The NSAID molecules, indomethacin (left) and clometacin (center), from which Sterling-Winthrop Research ultimately developed pravadoline (WIN-48,098; right)

Structurally, pravadoline contains an indole-core with an N-morpholinoethyl moiety, a 2-methyl substituent, and a methoxybenzene bridged to the indole at the C-3 position by a carbonyl group. A series of analogs, based upon the features of pravadoline, were synthesized to investigate the existence of a new cannabimimetic pharmacophore and can be found in ***Figure 10*** [32]. One of the most potent cannabinoids ever created, WIN-55,212-2 (***Figure 11***), came as a result of this research and became the prototypical compound for a new class of cannabimimetics known as amminoalkylindoles (AAI). The Sterling-Winthrop Research group proposed that the indole nucleus, N-aminoalkyl side chain, and C-3 aromatic moiety appended to the indole by a carbonyl group were essential features of this AAI class of cannabinoids.

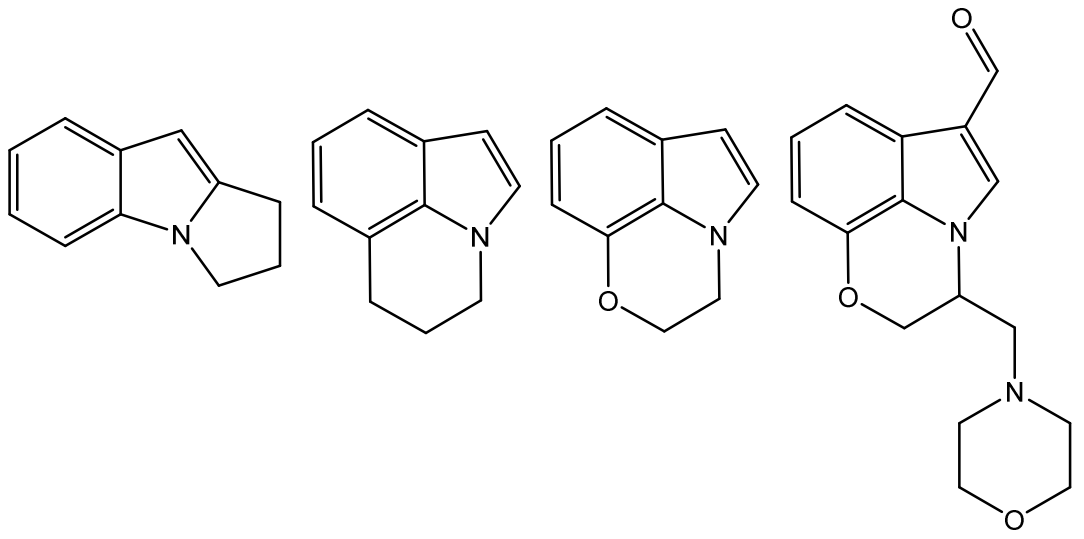


Figure 10: Examples of core structures used by Sterling-Winthrop to create numerous aminoalkylindole synthetic cannabinoids

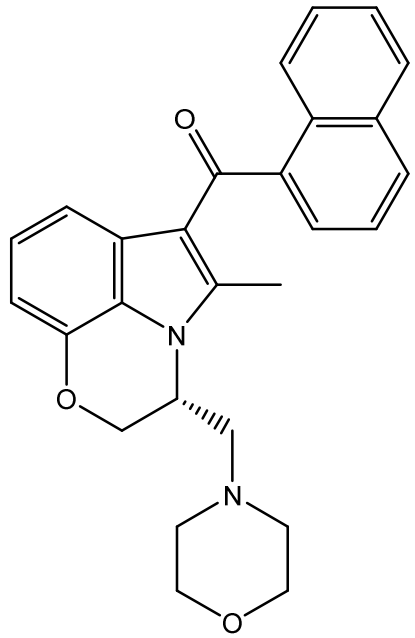


Figure 11: Structure of WIN-55,212

Throughout the 1990's, there were hundreds of cannabinoid receptor agonists (both CB_1 and CB_2) synthesized by lead researcher John W. Huffman of Clemson University. All synthetic cannabinoids made by this research group have the prefix "JWH" before the corresponding numerical code. Huffman's research was heavily focused on using molecular modeling capabilities that allowed the research group to compare the three-dimensional/conformational similarities that existed between more traditionally derived cannabinoids, such as Δ^9-THC and CP-55,490, and AAIs, including WIN-55,212. It was found that the aminoalkyl portion of the recently reported AAI class of cannabinoids was not essential and instead a four to six membered alkyl side chain maximized cannabinoid receptor affinity [34, 35].

Additional cannabinoid derivatives based upon pyrrole and indene cores led to hypotheses about the receptor-binding mechanisms of cannabinoid receptors. For instance, the proposition that certain molecular side-groups enhanced binding affinity through hydrogen bonding interaction with the receptors was challenged with the development of high-affinity indene molecules. Instead of hydrogen bonding interactions, Huffman proposed that agonist affinity was more directly related to aromatic stacking between the cannabinoid molecule and corresponding receptor [36 - 38].

There are too many JWH compounds (not all of which structurally resemble one another) to discuss in detail. For simplicity purposes, the molecular cores for which many common JWH compounds were based upon are illustrated in **Figure 12** and the JWH synthetic cannabinoids studied in this research project are shown in ***Figure 13***. It is worth noting that the hundreds of JWH compounds were largely synthesized for the purpose of better understanding the cannabinoid receptors [39]. The multiple molecular combinations of cannabimimetics that have been investigated by Huffman's group had significantly advanced the understanding of structure activity relationships pertaining to both cannabinoid receptors.

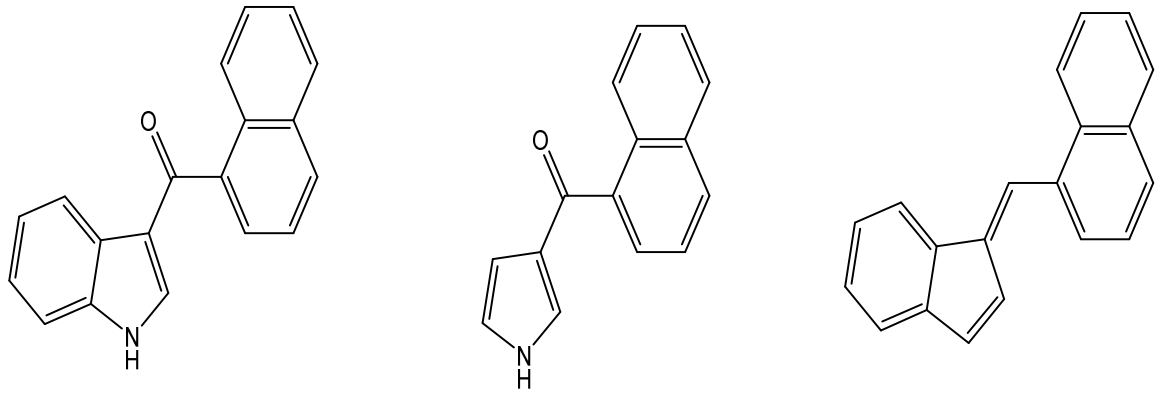


Figure 12: Examples of core structures used by Huffman to create numerous synthetic cannabinoids; naphthoyl indole (left), naphthoyl pyrrole (center), and naphthyl indene (right)

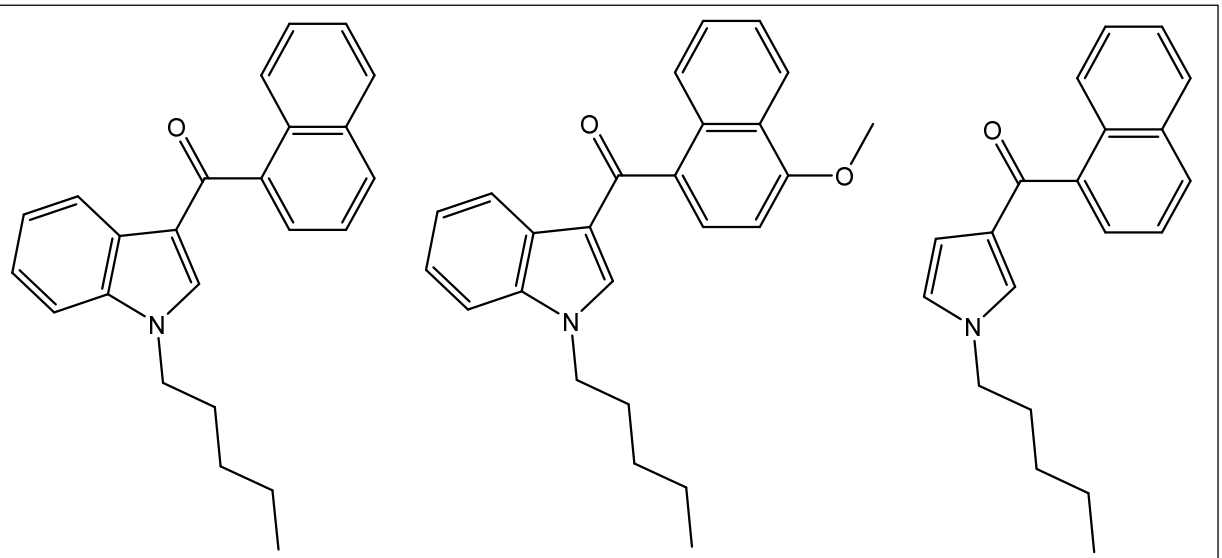


Figure 13: Structure of JWH-018 (left), JWH-081 (center), and JWH-030 (right)

Synthesis of cannabinoids has continued into the 21st century and unique structural modifications continue to be reported and observed. Researchers at Abbott Laboratories created a series of analogs (***Figure 14***) to investigate the cannabimimetic potential of various cycloalkyl and side-chain modifications to the indole and ketone moieties of the indolyl-core stemming from earlier studies out of the Sterling-Winthrop

and Huffman research laboratories [40, 41]. One of the most potent molecules from this class of compounds was the synthetic cannabinoid UR-144 (***Figure 15***) [42]. Due to both its popularity and structural similarity to the JWH compounds being investigated, UR-144 was included as the fourth and final synthetic cannabinoid of this project.

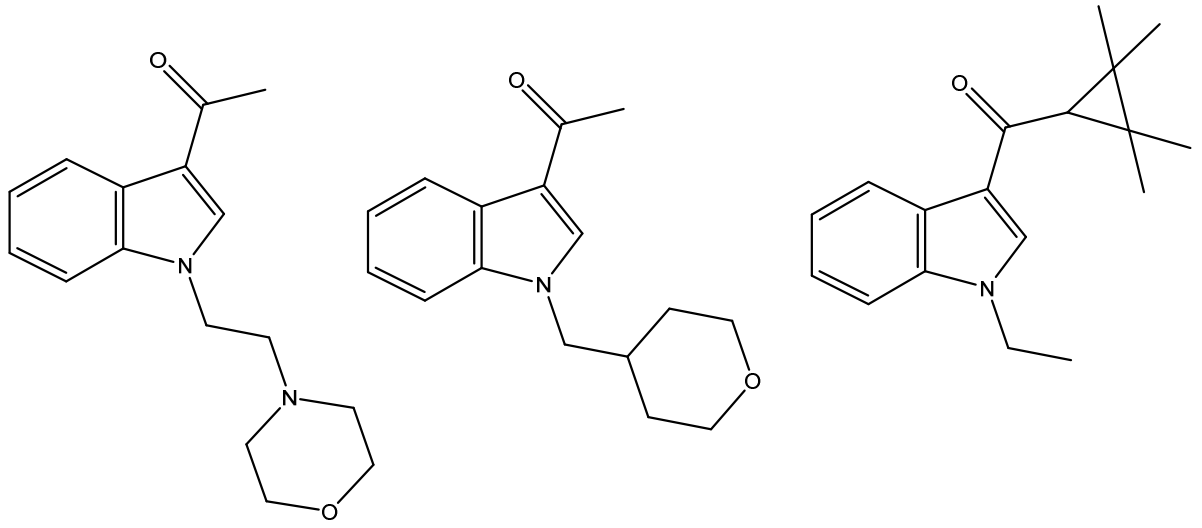


Figure 14: Examples of core structures used by Abbott Laboratories to create numerous Indol-3-yl synthetic cannabinoids

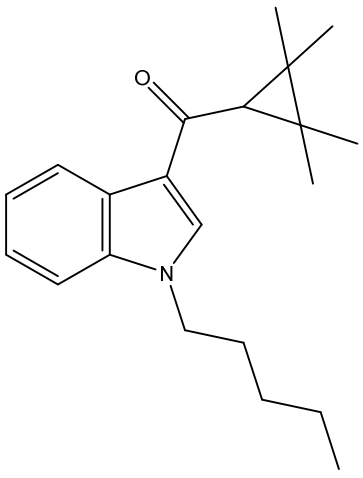


Figure 15: Structure of UR-144

1.5.0 GLOBAL IMPACT AND LEGAL STATUS OF SYNTHETIC CANNABINOIDS

Synthetic cannabinoids under the brand-names “Spice” and “K2” were first reported in Europe in the mid-2000’s. Manufacturers of K2 and Spice initially sold the majority of their products via online retailers, advertising them as incense or an ‘herbal incense’ (mixture of natural herbs) that was “not intended for human consumption” [43 - 46]. However, in 2008, German and Austrian laboratories analyzed a set of seized Spice products and detected the presence of JWH-018, leading them to conclude that the ingredients listed on the package did not accurately describe the contents. Subsequent investigations by authorities in Germany, the United States, the United Kingdom, Denmark, Finland, and the Netherlands also indicated the presence of CP-47,497, the CP-47,497 C8 homolog, HU-210, JWH-073, and JWH-398 in various seized Spice products all of which were not listed as ingredients on the packaging [44].

Soon after the report detailing the true active ingredients of K2 and Spice was published, an influx of products containing synthetic cannabinoids appeared on the market [45, 47]. The availability of such products and their popularity resulted in increased reports by poison control centers and hospitals within the U.S. of individuals believed to have consumed synthetic cannabinoids. Prior to March 2010, there were an estimated 112 reports to the American Association of Poison Control Centers (AAPCC) involving synthetic cannabinoids [48]. By the end of 2010, a total of 2,906 suspected cases were reported nationwide and in 2011, this number further increased to 6,956 incidences [49]. There have been no fewer than 2,600 reports involving synthetic cannabinoid per calendar year to the AAPCC since 2010. **Table 1** shows the reported number of incidents per year for the past five years. This escalation of reported calls was not

isolated within the U.S. as a study conducted in the United Kingdom in 2011 showed that banned synthetic cannabinoids continued to be used in various products within the country [50].

Table 1: American Association of Poison Control Center statistics on reported incidents attributed to synthetic cannabinoid use [48]

Calendar Year	Number of Reported Incidents
2010	2,906
2011	6,968
2012	5,230
2013	2,668
2014	3,680
2015	4,109 *through June 24, 2015*

The primary route of ingestion for synthetic cannabinoids is inhalation. Inhalation is accomplished by rolling the dried leaf matter and smoking as a user would smoke marijuana or a cigarette. In comparison, oral consumption typically results in lesser effects, potentially due to rapid and extensive first-pass metabolism which decreases the effective concentration in the blood. On the other hand, inhalation of the smoked drug results in rapid absorption into the bloodstream via the lungs. The rapid bioavailability that ensues from smoking synthetic cannabinoids coupled with their high affinity for CB_1 receptors can result in a greater likelihood of overdoses in users, particularly first time users. The enhanced affinity for the CB_1 receptors that synthetic cannabinoids possess also presents its own pharmacological effects.

Although synthetic cannabinoids bind to the same cannabinoid receptors as Δ^9-THC, these designer drugs appear to have significant risks to users that are not observed with the use of marijuana [43, 44, 51 - 54]. Likely a consequence of the enhanced binding affinity that synthetic cannabinoids possess, adverse symptoms including but not limited to seizures, convulsions, increased and irregular heartbeat, depression, extreme anxiety leading to suicide, and the onset of psychotic episodes have been reported by emergency personnel [43, 48, 55 - 58].

Due to the increased rate at which synthetic cannabinoid incidents were being reported to hospitals, emergency rooms and poison control centers, legislative action was required. On March 1, 2011 the United States Drug Enforcement Administration (DEA) exercised its emergency capability to temporarily regulate the most commonly reported synthetic cannabinoids. In doing so, the DEA temporarily placed five synthetic cannabinoids (JWH-018, JWH-073, JWH-200, CP-47,497 and the C8 homolog of CP-47,497) into the Schedule I category under the Controlled Substances Act (CSA), finding it necessary to "avoid an imminent hazard to the public safety" [43, 48, 59, 60].

While placing these compounds into Schedule I was a temporary provision, its legal implications and benefit to society should not be overlooked. Schedule I drugs are classified as substances which "have a high potential for abuse, no currently accepted medical use in treatment in the United States, and a lack of accepted safety for use of the drug under medical supervision". Such substances include heroin, lysergic acid diethylamide (LSD), and gamma-hydroxybutyric acid (GHB). Schedule I status also allows law enforcement and the DEA to fully impose criminal, civil, and administrative penalties, sanctions, and regulatory controls on the manufacture, distribution,

possession, importation, and exportation of these synthetic substances [48, 61]. Additionally, the emergency action provided the DEA and Department of Health and Human Services (HHS) time to gather more scientific evidence and approval that would ultimately be used to construct a permanent legislative decision regarding synthetic cannabinoids.

On July 10, 2012 President Obama signed into law Senate Bill 3187 which is formally entitled the Food and Drug Administration Safety and Innovation Act (FDASIA). Though it is commonly referred to as the "Synthetic Drug Abuse Prevention Act of 2012" (SDAPA), this ruling permanently placed 26 designer drugs into Schedule I of the CSA [62]. Among the 26 designer drugs listed, fifteen are synthetic cannabinoids, including JWH-018 and JWH-081. Additionally, the term cannabimimetic agent was specifically defined in a legal context as "any substance that is a cannabinoid receptor type 1 (CB_1 receptor) agonist as demonstrated by binding studies and functional assays within (given) structural classes…" [63]. This ruling sought to address the growing concern regarding the recent popularity of various herbal incense products marketed as "legal highs" and combat the possession, sale, distribution, and use of such products. However, illicit synthetic chemists continue to manufacture new 'generations' of synthetic cannabinoids that are not specifically included in this legislative action.

Many of the newer 'generations' of synthetic cannabinoids structurally differ enough from those listed in the SDAPA that new legislative actions need to continue to be taken to control the influx of new synthetic cannabinoids. For example, the synthetic cannabinoid UR-144 (which is being studied in this project) was temporarily placed into Schedule I status (along with XLR-11 and AKB-48) on May 16, 2013 [64]. Four more

synthetic cannabinoids (PB-22, 5-fluoro-PB-22, AB-FUBINACA and ADB-PINACA) were temporarily placed into Schedule I status on January 10, 2014; followed by another three (AB-CHMINICA, AB-PINACA and THJ-2201) on December 19, 2014 [65, 66]. ***Figure 16*** helps illustrate some of the deliberate structural modifications that have been made to avoid legislative control.

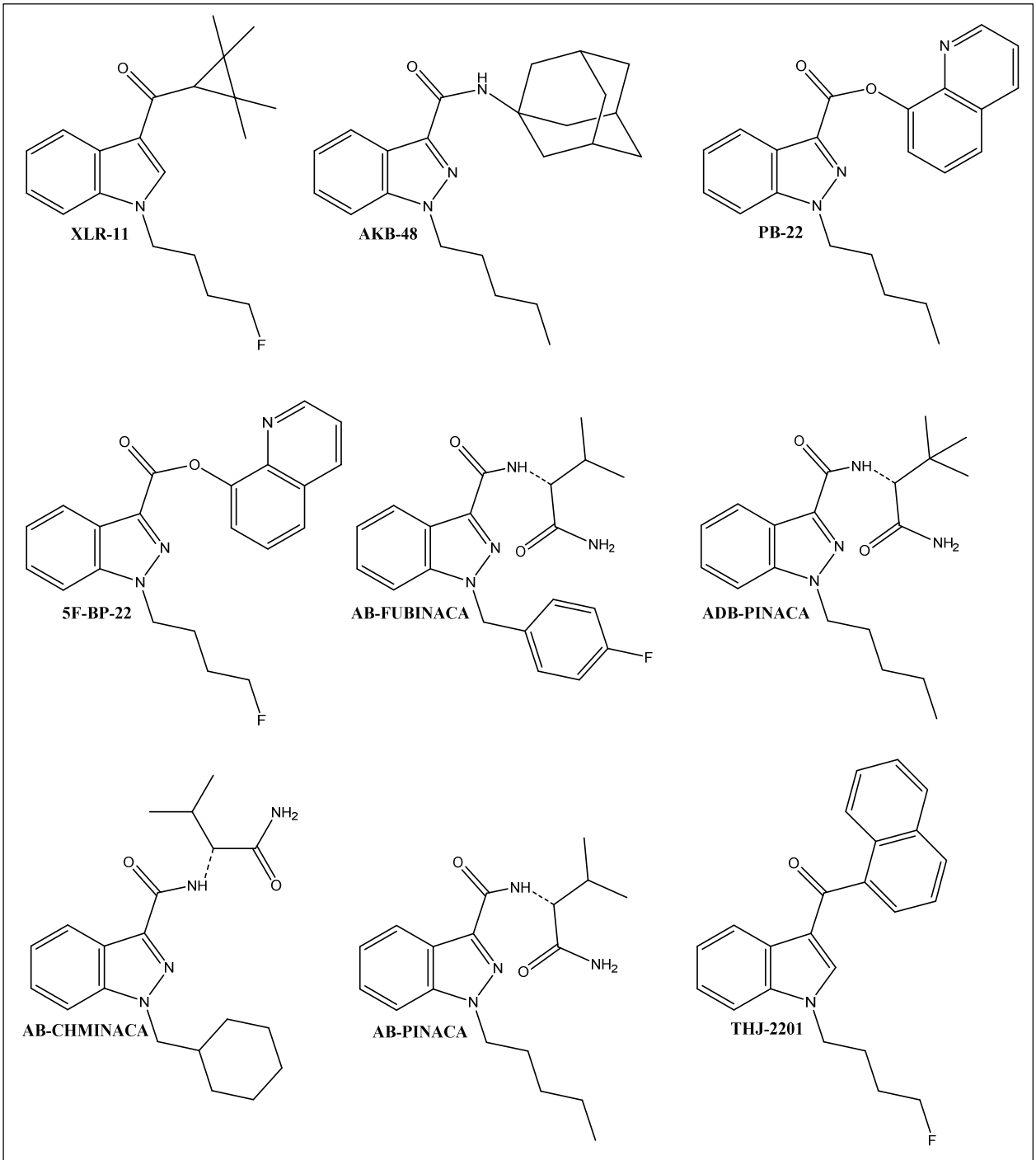


Figure 16: Structures of the synthetic cannabinoids specifically listed in Schedule I of the CSA through DEA temporary emergency scheduling in 2013 and 2014

Naming of many of these compounds is not as clear as the CP, WIN and JWH series. It has been suggested that the APICA, APINACA, CHMINACA abbreviations stem from the IUPAC naming of the chemical. On the other hand, AKB-48 is the name of a popular Japanese girl band that has been compared to the British girl band the Spice Girls and thus appears to be a comedic reference to the original herbal incense brand 'Spice'. However, XLR-11 is the first liquid-fuel rocket engine developed in the US and its name could be a nod to the "high" user's experience [67].

It is important to note that these are just some of the synthetic cannabinoids which have been identified in recent years and caught the attention of law enforcement agencies. According to the National Forensic Laboratory Information System (NFLIS), a reporting system comprised of over 300 state and local laboratories, 33,096 synthetic cannabinoid drug reports were documented in 2012 and 35,101 in the year 2013 [68, 69]. Additionally, the United Nations Office on Drug and Crime (UNODC) Global Synthetics Monitoring: Analyses, Reporting and Trends (SMART) Programme, which provides statistics on all types of new psychoactive substances (NPS), has indicated an increase in the number of synthetic cannabinoids appearing on the illicit drug market since 2009 [70]. ***Figure 17*** has been reproduced with permission from the UNODC Global SMART Programme and depicts the total number of NPS as well as the significant number of new synthetic cannabinoids that continue to be identified per year.

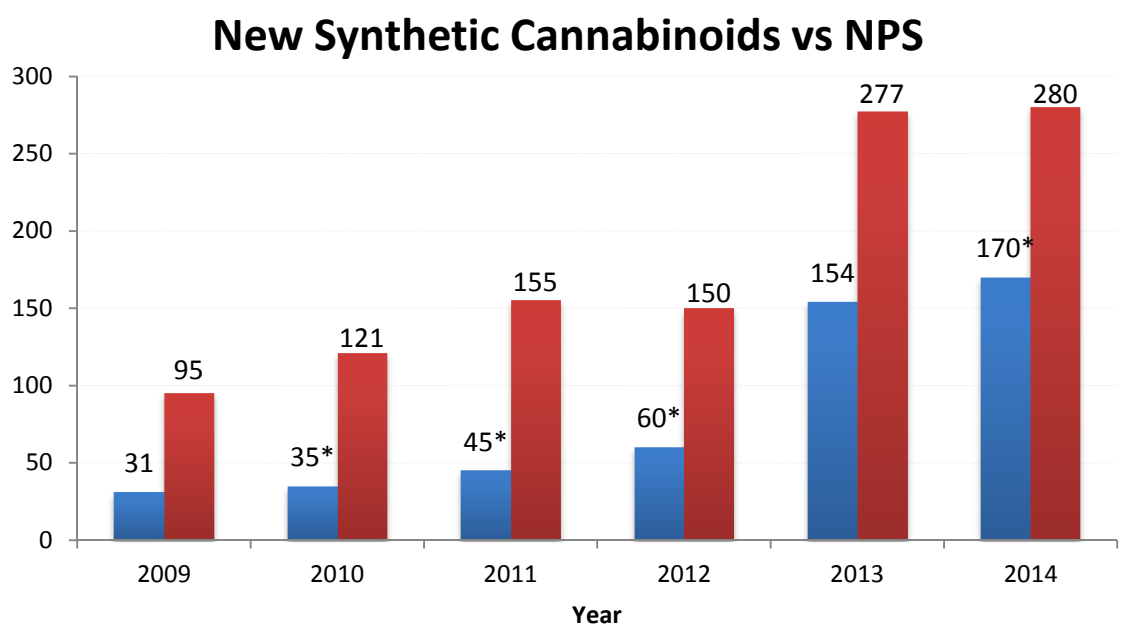


Figure 17: Overall number of new psychoactive substances (red) and synthetic cannabinoids (blue)

**values are estimated based upon the UNODC Global SMART Update 2015 [70]*

According to these global statistics, synthetic cannabinoids represented nearly 38% of the compounds in this highly dangerous category of drug in 2014; a 13% increase from five years prior, in 2009. This trend is particularly troublesome because as targeted legislation has been enacted to control such substances, more and more continue to appear on the illicit drug market.

1.6.0 CONCLUSION

With the structural elucidation of Δ^9-THC in the mid 1960's came a wave of interest regarding the potentially therapeutic benefits of it and other cannabinoids found in marijuana. The desire to develop a new non-addictive analgesic drug molecule that could replace morphine ultimately led to the creation of some of the first reported

synthetic cannabinoids. Furthermore, the discovery of the CB_1 and CB_2 receptors of the endocannabinoid system in the early 1990's led to the development of many synthetic cannabinoids.

Parallel to the pursuit of the therapeutic benefits of the endocannabinoid system, synthetic cannabinoids have emerged as one of the most dangerous categories of designer drugs. While a number of these compounds are discarded pharmaceutical drug candidates, others appear to come from illicit synthetic chemists. Due to the large number of compounds being identified on an annual basis, the primary focus regarding synthetic cannabinoids has largely revolved around developing methods to detect and legally regulate a continually changing class of chemicals. Combined with the essentially unknown long-term health effects of abusing cannabinoid receptor agonists and antagonists, synthetic cannabinoids are firmly regarded as one of the largest forensic problems.

CHAPTER 2: PYROLYSIS AND EXPERIMENTAL DESIGN

2.1.0 INTRODUCTION

According to the International Union of Pure and Applied Chemistry (IUPAC), pyrolysis is defined as "a chemical degradation reaction that is caused by thermal energy" [71]. Pyrolysis is routinely used in the processing of oil and coal, the disposal of wastes, and the production of various solvents and polymers [69]. Two major concerns regarding pyrolysis revolve around the health and environmental impact unwanted by-products may pose. While it will be discussed in greater detail later, it is worth noting that many polyaromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs) can be generated via pyrolysis. These include some of the most well documented harmful and potentially harmful constituents (HPHCs) and carcinogens. The Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and the Agency for Toxic Substances and Disease Registry (ATSDR) have each highlighted a number of PAHs as chemicals that are reason for major concern to the environment and human health [72 - 74].

Pyrolysis is often applied in forensic chemistry to analyze paints, polymers, fibers, and controlled substances [1, 76]. While inhalation is a common route of administration for many controlled substances, including synthetic cannabinoids, few peer-reviewed publications have fully investigated all the pyrolytic products that are formed via smoking. A handful of publications have studied the impact of heat on synthetic cannabinoids as it pertains to thermal degradation in the injection port of a gas chromatograph [77, 78]. Other work has sought to identify the presence of a given parent molecule in biological fluids after smoking a product containing one or more synthetic

cannabinoids [79, 80]. At the time of this work, however, there have not been any reports detailing a “big-picture” of what happens during the smoking of synthetic cannabinoids. Given the rate at which synthetic cannabinoids are being detected by laboratories and reported by emergency and law enforcement personnel, it is important that scientific insight be provided regarding the reality of smoking these substances.

Underlying questions of what occurs during smoking of these compounds helped drive the work of this research project and attracted interest from the Drug Enforcement Administration and other researchers. While it has been documented that a number of synthetic cannabinoids possess greater affinity for the CB_1 receptor than Δ^9-THC, scientific results regarding the effects of pyrolytic products have not been established. Curiosity as to what, if any, structurally similar pyrolytic products may be created upon smoking drove this research. Isomeric variations, analogs, and molecular cores of the given parent molecules themselves could be biologically active at CB_1 receptors. If this were found to be true, consequences of mixture toxicity would be at stake and of great importance to those interested in the public health aspects of this research. Additionally, some of these products may be detrimental compounds such as HPHCs that are known to be formed via cigarette smoking [74].

Interest in identifying pyromarkers was another motivation behind this research. Screening assays for synthetic cannabinoids are in constant need of adaption to stay relevant. Depending upon the uniqueness of the pyromarkers, certain compounds may warrant incorporation into the screening assays for the synthetic cannabinoids being examined. The ability to expand screening assays would ultimately lead to better means of detection when it comes to synthetic cannabinoids.

2.2.0 THE CHEMISTRY OF PYROLYSIS: BONDS, RADICALS AND REACTIONS

Since organic compounds have limited thermal stability, heating of an organic substance beyond a certain temperature induces thermal changes to the molecular structure. At the time of its inception, pyrolysis was used primarily to describe such reactions occurring in an inert atmosphere (i.e. absence of oxygen, etc.). However, now the term is commonly used to describe reactions occurring above approximately 300°C - 350°C, in either an inert or non-inert environment, while the term thermal degradation is used to describe processes below this temperature range. As such, the work being conducted in this project is best categorized as pyrolysis.

Although pyrolysis comes from the Greek elements ‘pyro-’, meaning fire, and ‘-lysis’, meaning loosening, separating, or breaking down, there is a great deal of chemical complexity in the process that is not directly inferred from this definition. The literal interpretation of pyrolysis might suggest that only smaller components are generated from a parent molecule (i.e. degradation). However, compounds with a higher molecular weight than the parent(s) are often created during the process.

The generation of complex molecular species can be attributed to subsequent interaction(s) between the fragments resulting from decomposition of the initial compound, usually via radical reaction mechanisms. Some of the radical fragments that appear to play a significant role in the formation of PAHs and HCAs (both of which are of specific interest) are acetylene, vinylacetylene, butadiene, cyclopentadiene, methyl cyclopentadiene, and indene. These particular fragments are capable of interacting with larger fragments or products and thereby creating larger radical species. These larger radicals can undergo a termination reaction, which effectively creates a large molecular

weight product, or can undergo further reactions and ultimately create even larger molecular weight products [81].

2.2.1 BOND DISSOCIATION ENERGY

Bond dissociation energy (D) is the amount of energy required to break a given bond and is typically reported in kilojoules (kJ). The greater the bond dissociation energy, the stronger the bond is and therefore the less likely it is to break. Tabulated values of bond strengths (like those listed in ***Table 2***) which only list the atoms that make up the bond itself can serve as a general guide to understanding the relative strength of the bonds in a molecule. The true strength of a bond is influenced by other factors such as the neighboring atoms and the presence of conjugation within the molecule. Nonetheless, when evaluating a molecular structure, bond strengths can give an indication of where a radical mechanism may originate based upon which bonds are more likely to break first (weakest bonds). From ***Table 2***, we can see that the carbon – nitrogen single bond (C – N) is relatively weak when compared to some of the more common bonds present in organic molecules. As such, this implies that C – N bonds would be more likely to break first. However, such bonds would not be the only ones to break and it is helpful to avoid falling into that misconception. With the amount of thermal energy that is present in pyrolysis, a large number of bonds could be susceptible to breaking.

Table 2: Bond energy values for common organic constitutents [82]

Constituents of Bond	Average Bond Dissociation Energy (kJ/mol)	Constituents of Bond	Average Bond Dissociation Energy (kJ/mol)
C – N	293	H – H	436
C – C	348	C = C	614
C – H	413	C = O	743

2.2.2 RADICAL REACTIONS

Radical (chain) reactions readily occur during pyrolysis and as with all radical-based reactions, the concepts of initiation, propagation, and termination are important for understanding how such a mechanism occurs (see ***Figure 18***). An initiation step in the chain reaction is where a radical product is generated from a non-radical containing reactant(s). In organic compounds, radicals can be easily generated during pyrolysis via homolytic bond cleavage (radical splitting), particularly hydrogen abstraction and/or scission reactions [81]. A propagation step in the reaction is one in which a radical reactant generates a radical product, while termination involves two radical reactants producing a non-radical containing product.

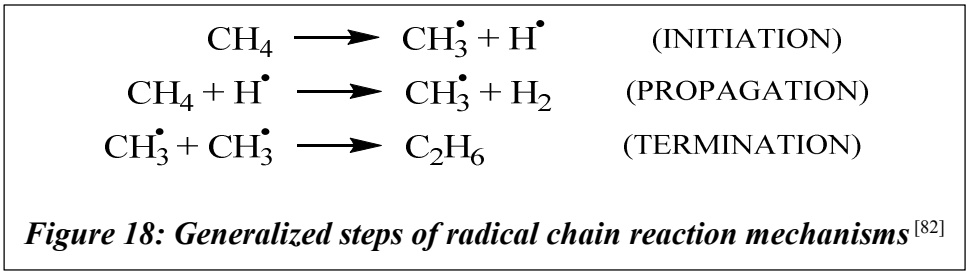


Figure 18: Generalized steps of radical chain reaction mechanisms [82]

Generally speaking, the products formed as a result of a termination step tend to rely heavily upon the ability of the propagation steps to create radicals. In pyrolysis though, initiation steps can have a more pronounced effect on the products formed because the high temperature enables more radicals to be generated via initiation than other reactions. This increased role of initiation can consequently impact the products formed via termination and therefore add to the overall mixture of products formed. Only the products formed during termination that do not undergo any further reaction(s) will be observed as pyrolytic products [83].

2.2.2.1 ELIMINATION REACTIONS

Elimination reactions are very common in pyrolysis and as the name indicates, these reactions are characterized by the loss of an atom or atoms. Although it is perceived that noticeably smaller products are generated via elimination reactions, radical mechanisms in the presence of elevated temperatures have been found to result in products that have an increased number of aromatic rings. Two examples of how such a mechanism can ultimately lead to ring expansion are illustrated in ***Figure 19*** and ***Figure 20***. In these figures, both radical elimination pathways proceed via the same steps. The loss (elimination) of a methyl radical from the most substituted carbon atom is then followed by an attack on the adjacent benzene atom. In doing so, it provides a

means for the respective five membered rings to expand to their six membered forms [84].

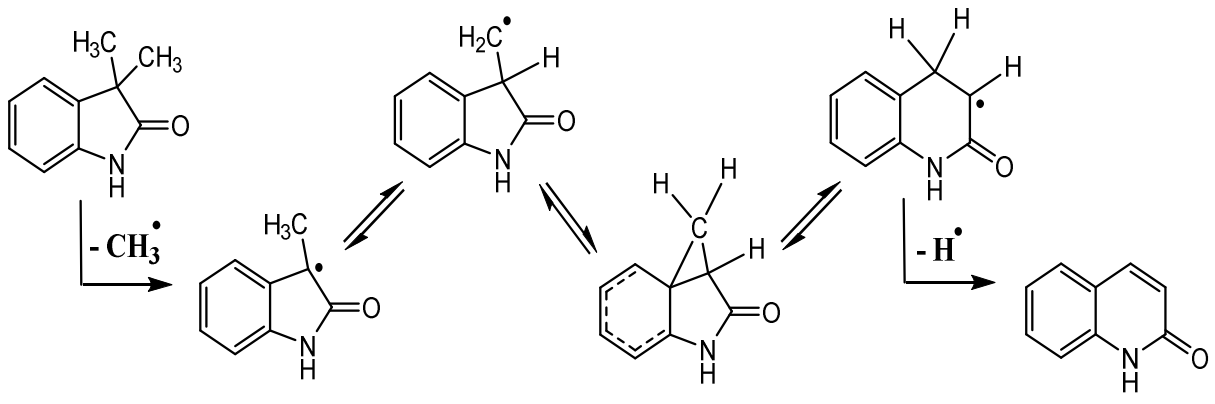


Figure 19: Example of an elimination reaction leading to heterocyclic ring expansion [84]

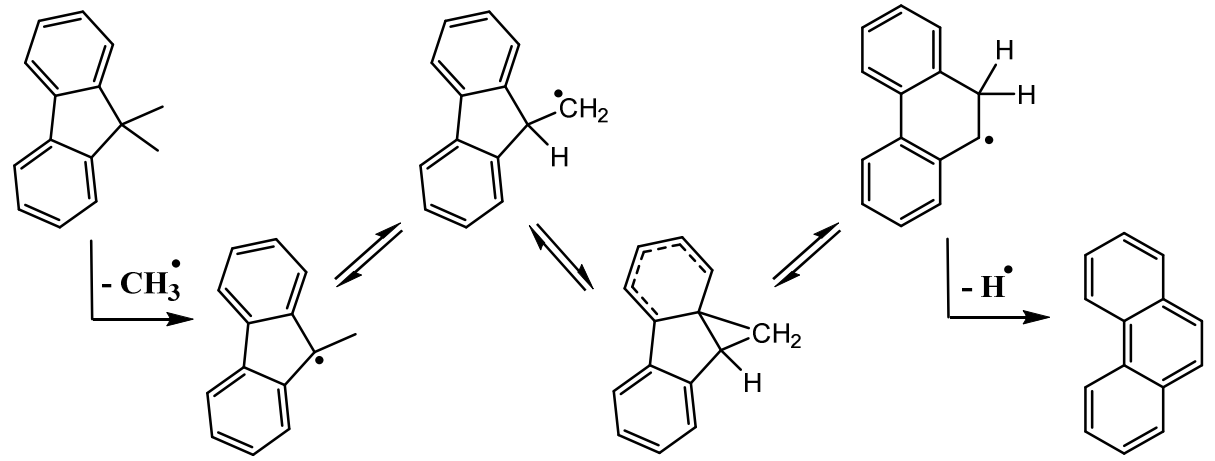


Figure 20: Example of an elimination reaction leading to homocylcic ring expansion [84]

2.2.2.2 SUBSTITUTION REACTIONS

Substitution reactions are characterized by a replacement or 'substitution' of an atom or molecular feature and a handful of relevant examples have been included. In each of

the examples shown, a hydrogen radical is generated and replaced by a larger molecular species which leads to the formation of polycyclic species or condensed PAH. ***Figure 21*** provides a plausible mechanism by which a bicyclic product is generated from benzene molecules. In this representation, the benzene radicals interact with one another through radical coupling to produce a product that is larger than the initial benzene molecule [83].

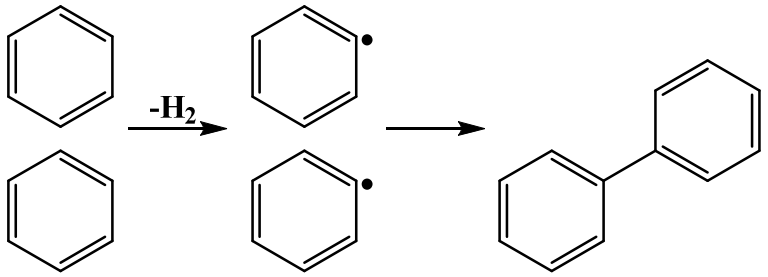


Figure 21: Example of a substitution reaction leading to the formation of a bicyclic compound [83]

Another example of how a product with a molecular weight greater than the parent can be formed is given in ***Figure 22***. Here the relatively small toluene molecules interact after the formation of hydrogen radicals and undergo a coupling. In this instance, the pathway also shows how the substituted bicyclic product can undergo further elimination to yield a fused tricyclic compound that is the PAH phenanthrene [83].

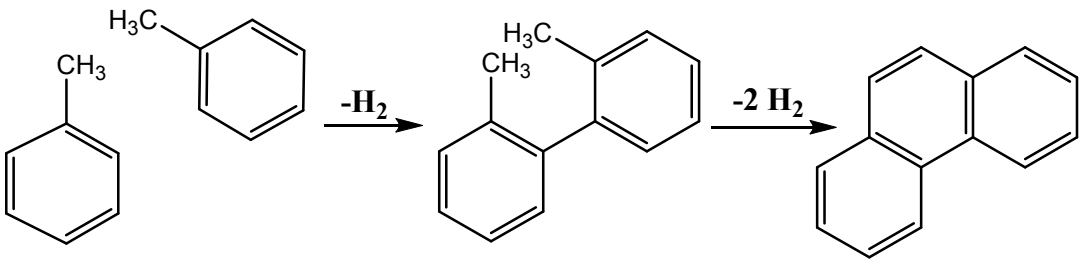


Figure 22: Example of a substitution reaction leading to the formation of a polyaromatic compound [83]

Figure 23 further exemplifies how products of even greater molecular weight can be generated. In this pathway, the naphthalene radicals couple together to form a dimer complex (binaphthyl species).Similar to the previous figure, this polyclic molecule can undergo an elimination to yield yet another well-known PAH, benzo[k]fluoranthene [81].

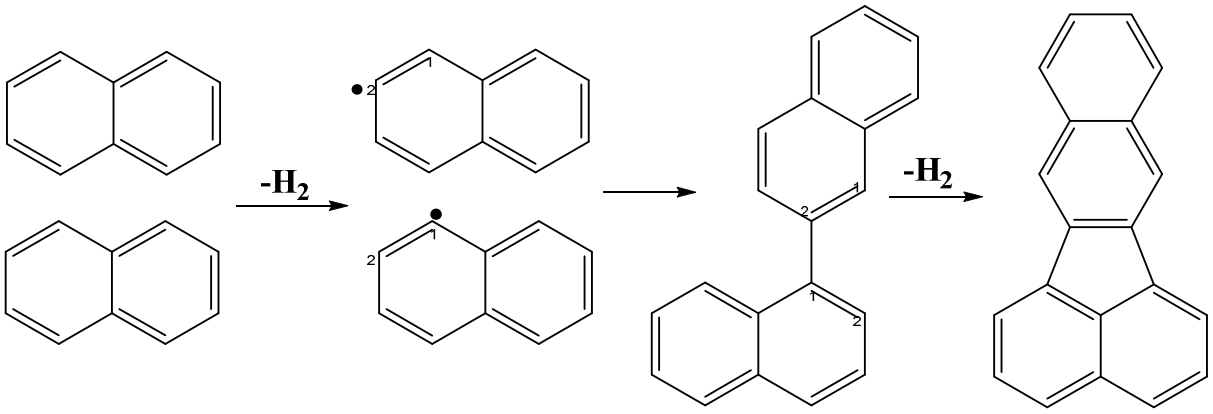


Figure 23: Example of a substitution reaction leading to the formation of a condensed polyaromatic compound [81]

2.2.2.3 MOLECULAR REARRANGEMENT

The last types of reaction that will be specifically highlighted are molecular rearrangement reaction. In this type of reaction, a molecular feature is shifted or 'rearranged' on the structure and therefore results in a structurally similar product. Often

times a positional isomer can be formed as a result of molecular rearrangements but other types of molecular rearrangements can also occur.

Listed below, in ***Figure 24*****,** is an example of how the alkyl substituent of a heteroatomic molecule can undergo rearrangement during pyrolysis. This particular mechanism is termed a 1,5-sigmatropic rearrangement and is routinely observed with the shift of a hydrogen atom. Due to the high temperatures in pyrolysis though, this type of rearrangement has also been found to occur with alkyl substituents even larger than methyl groups [83].

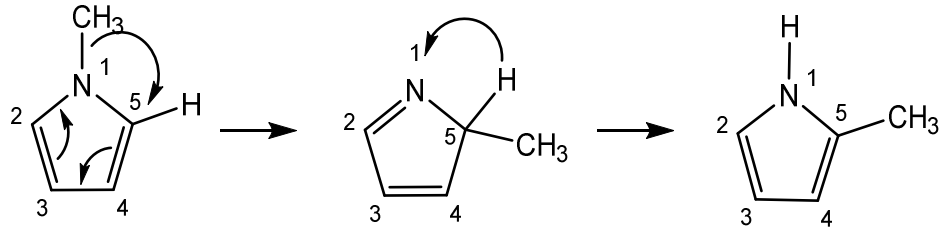


Figure 24: Example of a molecular rearrangement leading to a positional isomer [83]

Not all rearrangement reactions produce a positional isomer though, and a separate example of a rearrangement reaction that can be observed during pyrolysis is illustrated in ***Figure 25***. Driven by the stability of an enlarged aromatic system, this particular type of reaction highlights the formation of a more stable PAH species from a conjugation-rich parent [81].

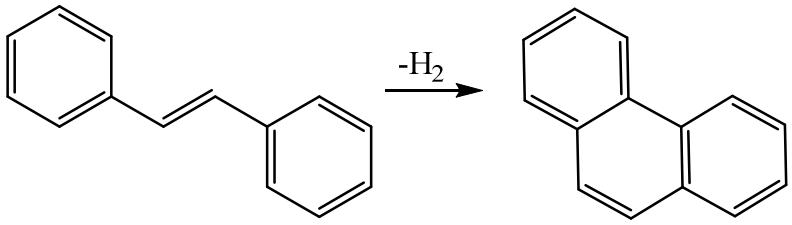


Figure 25: Example of a rearrangement reaction driven by the stability of aromatic systems [80]

2.3.0 EXPERIMENTAL

To investigate the formation of various pyrolytic products associated with the smoking of synthetic cannabinoids and relevant small molecules of interest, an in-house smoking apparatus was used over the course of this study (see ***Figure 26***). The apparatus was set up using common laboratory glassware and equipment such that it can be performed in many laboratories.

A plug of quartz wool was placed in the top of a standard five and three-quarter inch glass pipette. Depending upon the chemical, between five and ten milligrams of the substance was directly weighed onto the plug of wool inside of the glass pipette. Another plug of wool was then placed on top of the solid. This quartz wool 'sandwich' served to hold the chemical in place so that heat could be directly applied to the substance. Each chemical substance was analyzed individually with new glass pipettes and quartz wool for each trial.

Once the solid and wool were physically loaded into the pipette, the pipette was inserted through a small circular opening in a rubber stopper. The rubber stopper was securely fitted into the opening of the Erlenmeyer filter-flask which contained 50 milliliters (mL) of chloroform. The filter-flask was connected to a vacuum source and the

depth of the glass pipette was adjusted such that when the vacuum was applied, mild "bubbling" of the solvent could be observed. Heating of the controlled and non-controlled substances was carried out using a commercially available handheld blowtorch. The torch was held in place with a separate ring stand within a ventilated hood so that the distance and orientation of the flame were held constant between runs. A more thorough discussion and description of the temperature ranges and length of heating will be discussed in the coming sections.

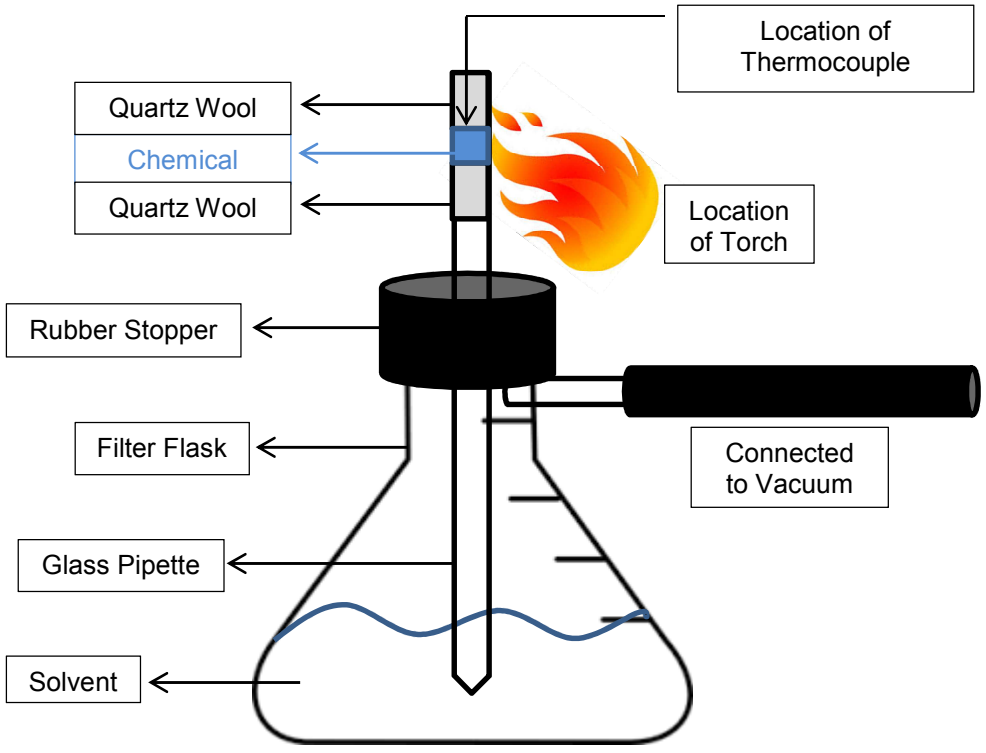


Figure 26: Diagram of the smoking apparatus used in our experiments

2.3.1 SAMPLE PREPARATION

Using the above apparatus (***Figure 26***), a total of three separate extracts were collected for future analysis: (1) the quartz wool, (2) the interior of the glass pipette, and (3) the solvent from the flask. The system was allowed to cool to room temperature before the extraction of any constituents. All of the wool was first removed from the pipette and placed in a 2 mL plastic syringe to analyze for any remaining solid or products that may have been collected. The plastic syringe was fitted with a 0.45 micrometer (μm) syringe filter and 1 mL of solvent was used to extract the wool directly into a 2 mL glass vial. The resulting liquid was then evaporated with air, reconstituted in 100 microliters (μL) of solvent (90% chloroform: 10% methanol), vortexed for 15 seconds, and placed into a 200 μL glass insert for GC-MS analysis. All subsequent extracts underwent the same preparatory steps (evaporation, reconstitution, and transfer).

The interior of the glass pipette was rinsed with 1 mL of solvent and the resulting liquid was directly collected into a 2 mL plastic syringe (also fitted with a 0.45 μm syringe filter) for evaluation of the second extract. As for the third fraction, the solvent from the bottom of the filter flask was directly evaporated with the previously mentioned reconstitution and transfer steps.

2.3.2 TEMPERATURE MEASUREMENT

In this experimental design, a conventional thermometer would not have been suitable for measuring the extreme temperatures occurring during pyrolysis. A thermoelectric couple (thermocouple) is ideally suited for such applications and was therefore used to monitor the temperature of our experiments. Thermocouples operate

on the thermoelectric principle known as the Seebeck Effect. German physicist Thomas Seebeck is credited with discovering that when two dissimilar metals (wires) are joined at both ends and a temperature difference (T_1 and T_2) exists between these two ends, a voltage is generated [85]. Therefore, by measuring the voltage output with a voltmeter (not shown in ***Figure 27***), an accurate temperature reading is provided to the user.

The point at which the two dissimilar metals are joined is known as a junction point. This union of the wires is exposed so it can be directly placed at the site where a measurement is needed. The opposite ends of the wires are integrated into an isothermal block so that they are held at roughly the same temperature (T_2). The metal prongs protruding from the isothermal block are plugged into the voltmeter component of the device which has various controls as well as a digital display screen for the temperature reading. A schematic diagram of the relevant components of a thermocouple, like the Omega HH506RA Thermocouple by Omega Engineering, Inc. (Stanford, CT) used in this study, is shown in ***Figure 27*** [86].

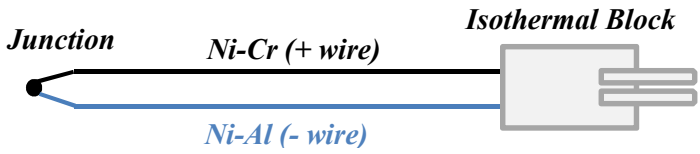


Figure 27: Schematic illustrating the primary components of a thermocouple

Note: The distance between the two wires has been grossly exaggerated and the length of the actual wire has been significantly shortened for display purposes.

In the above thermocouple schematic, there are two dissimilar metal wires that are labeled according to the metals that make up their composition. Many types of metals and alloys have been calibrated for use in thermocouples and the variations in metallic composition allow these devices to be used in a wide range of applications and over a wide range of temperatures. A “Type K” thermocouple which is appropriate for measuring temperatures between -200°C and 1250°C was used in the experiments described within this work. Such a thermocouple is composed of a nickel-chromium alloy for the positive lead and a nickel-aluminum alloy for the negative lead. Above 0°C, the limit of error for a Type K thermocouple is the greater of 2.2°C or 0.75%, which was deemed sufficient for our purposes [87, 87].

2.3.3 HEATING CONDITIONS

Since the intent of this research was to provide useful information about the reactions occurring when a synthetic cannabinoid is smoked by an individual, it was appropriate to begin by identifying a relevant temperature condition for smoking experiments. To simulate the temperature ranges that would be expected with smoking plant material laced with synthetic cannabinoids, commercially available cigarettes were used to model the heating conditions.

Marlboro Gold Special Blend (formerly Marlboro Light) cigarettes were used to establish the temperature conditions of this study. Two distinctly different thermal conditions occur with cigarettes: a “smoldering” condition when the cigarette is lit but not actively being smoked and an “inhalation” condition when an individual takes a ‘puff’ from the cigarette. To capture the temperature profile of both conditions that can exist,

we performed separate replicates with either a low, constant flow through the cigarette or with a pulsed, high flow through the cigarette.

2.3.4 CIGARETTE STUDY

The total length of the cigarettes used was 8 centimeters (cm). The filter portion (tan color, ***Figure 28***) of the cigarette measured 3 cm while the remaining 5 cm of the cigarette was the actual body (white color) of the cigarette. The thermocouple junction was directly inserted through the side of the cigarette at a point that was 1 cm away from the lit end of the cigarette. The end of the cigarette that would be put in one's mouth (tan, filter end) was connected to rubber tubing that was attached to a vacuum source. A ring stand and clamp were used to hold the cigarette in place within the fume hood to avoid exposure to the smoke. In both temperature experiments, the thermocouple continuously measured the temperature until the burning front completely surpassed the location of the thermocouple wires.

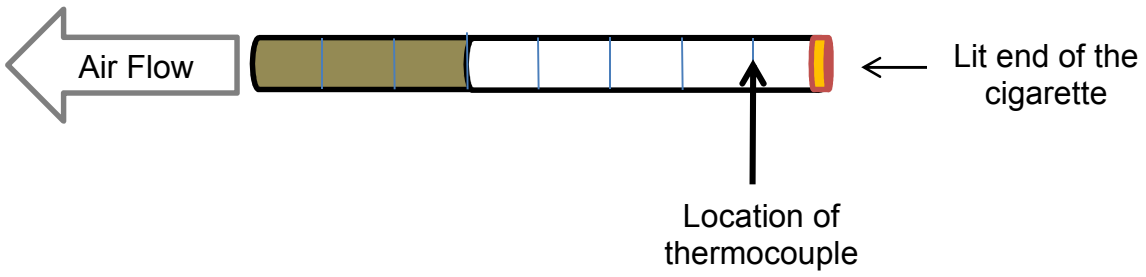


Figure 28: Diagram showing placement of thermocouple in cigarette study

1 centimeter markings represented with thin blue vertical lines have been added for guidance

In the first part of the investigation of cigarette temperatures, the cigarette was lit and the vacuum line was maintained in a slightly opened position such that a minimal

amount of air was continuously drawn from the lit end of the cigarette through the length of the cigarette. Again, this scenario was used to experimentally simulate the conditions of a cigarette that was not actively being 'puffed' by the user ("smoldering"). The smoldering temperature was continuously recorded until the burning front of the cigarette completely passed the location of the thermocouple.

For the second portion of the cigarette temperature investigation, a different flow was used. Instead of using a constant, minimal flow of air from the vacuum line, the air flow was sharply increased as the burning front reached the thermocouple. This sudden pulse of air flow was intended to simulate the conditions that would occur during the inhalation aspect of smoking a cigarette.

2.4.0 RESULTS

Thirteen replicates for each smoking condition were conducted with the experimental design that was previously discussed in **Section 2.3.0**. A new cigarette was not necessarily used for each replicate unless an insufficient amount of the cigarette remained; if more than 3 cm of the body (white color) of the cigarette remained from the previous run, then the cigarette was re-used. For example, if after an initial trial, the burning front of the cigarette was stopped between 3.0 and 3.5 cm from the start of the filter end, then the remaining portion of the cigarette body was used for the following trial.

2.4.1 SMOLDERING CONDITIONS

From the data listed in ***Table 3***, the average minimum temperature of the cigarette, which corresponded to the starting temperature of the trial, was found to be between

22°C and 29°C (average of 25°C, standard deviation of 2°C). The highest recorded temperature for the smoldering scenario was shown to range between 598°C and 736°C (average temperature of 671°C, standard deviation of 44°C). Overall, the heating of the cigarette resulted in an average increase in temperature of 647°C with a standard deviation of 44°C.

In an effort to show how quickly the temperature could elevate in a smoldering cigarette, the greatest change in temperature over any 25 second span ($\Delta T_{25\ sec}$) of the entire trial was calculated (***Table 3***). Evaluating all trials, it was found that the $\Delta T_{25\ sec}$ varied between 174°C and 391°C, with an average of 288°C and a standard deviation of 72°C.

Table 3: Temperature data for 'smoldering' cigarette conditions

Replicate	Minimum Temperature (°C)	Maximum Temperature (°C)	Overall Change in Temperature (°C)	$\Delta T_{25\ sec}$ (°C) for Smoldering Scenario
1	22	736	714	211
2	25	631	607	174
3	25	707	682	391
4	29	629	600	211
5	23	631	608	266
6	24	623	599	276
7	24	689	665	280
8	23	598	575	317
9	29	713	685	296
10	23	665	642	220
11	26	699	674	345
12	26	698	672	367
13	25	707	682	391
Average	24	671	646	288
Standard Deviation	2	44	44	72

2.4.2 INHALATION CONDITIONS

From the data listed in ***Table 4***, the average and range of temperatures were calculated. The initial (minimum) temperature ranged between 21°C and 40°C, with an average temperature of 27°C and standard deviation of 5°C. The maximum temperature detected by the thermocouple was found to range between 637°C and 762°C, with an average of 693°C and a standard deviation of 34°C. Overall, the average increase in temperature of the cigarettes was 665°C with a standard deviation of 36°C. The $\Delta T_{25\ sec}$ for the inhalation trials was found to range between 385°C and 706°C with an average change of 576°C (standard deviation of 73°C).

Table 4: Temperature data for 'inhalation' cigarette conditions

Replicate	Minimum Temperature (°C)	Maximum Temperature (°C)	Overall Change in Temperature (°C)	$\Delta T_{25\ sec}$ (°C) for Inhalation Scenario
1	21	682	661	655
2	21	762	741	683
3	29	723	694	584
4	22	707	685	500
5	25	733	708	706
6	27	660	633	512
7	35	660	625	538
8	25	664	639	495
9	25	690	665	602
10	25	637	612	557
11	30	708	677	623
12	24	693	669	545
13	40	681	642	494
Average	27	692	665	576
Standard Deviation	6	34	36	73

2.5.0 Results and Discussion From Cigarette Study

Comparing the mean of the two different temperature profiles, one can see that the minimum temperature recorded was similar for both scenarios, differing by only 2°C. This would be expected, but since not all trials began with an unused cigarette, it was important to make note of the starting temperatures so that it can be taken into account when evaluating the change in temperature. The average maximum temperature for the inhalation condition and average overall change in temperature for the inhalation conditions was 21°C and 19°C greater, respectively, than that of the smoldering condition. The findings of both the maximum temperature achieved was similar to reported temperatures of tobacco [89].

There was, however, a significant difference between the 'greatest change in temperature over a 25 second span' ($\Delta T_{25\ sec}$) for the two conditions. The average $\Delta T_{25\ sec}$ observed for the data representing the simulated inhalation scenario (576°C) was twice as high as the temperatures observed for the simulated smoldering scenario (288°C). These findings helped provide insight into what temperature range these studies with synthetic cannabinoids should be conducted at in order to provide data that would be as relatable as possible to a real life situation.

2.6.0 Investigation into Proper Heat Source

Once the temperature conditions that occur were experimentally established, an adequate heat source for the experimental setup was needed. First and foremost, these studies required a heat source that was capable of providing temperatures in excess of 750°C (coinciding with the maximum temperatures observed in our cigarette studies). However, one that was capable of supplying sufficient energy to heat the apparatus at

least 550°C in 25 seconds (coinciding with the largest change in temperature observed) was most ideal.

2.6.1 BUTANE HEAT SOURCE

The first type of heat source that was investigated was a common household butane lighter (Zippo Multi-Purpose Lighter with premium isobutane fuel; Bradford, PA, USA). To evaluate the heating capability of such a lighter, an experimental apparatus with no chemical or controlled substance present was set up. The flame from the butane lighter was directed at the location where the chemical substance would be located and the temperature was measured for a total of 60 seconds. The temperature versus time plot of five blank trials are is displayed in ***Figure 29*** while the overall average plot is given in ***Figure 30*** . The initial, final, and change in temperature that was observed over the 60 second run time were extracted from these figures and can be found in ***Table 5***.

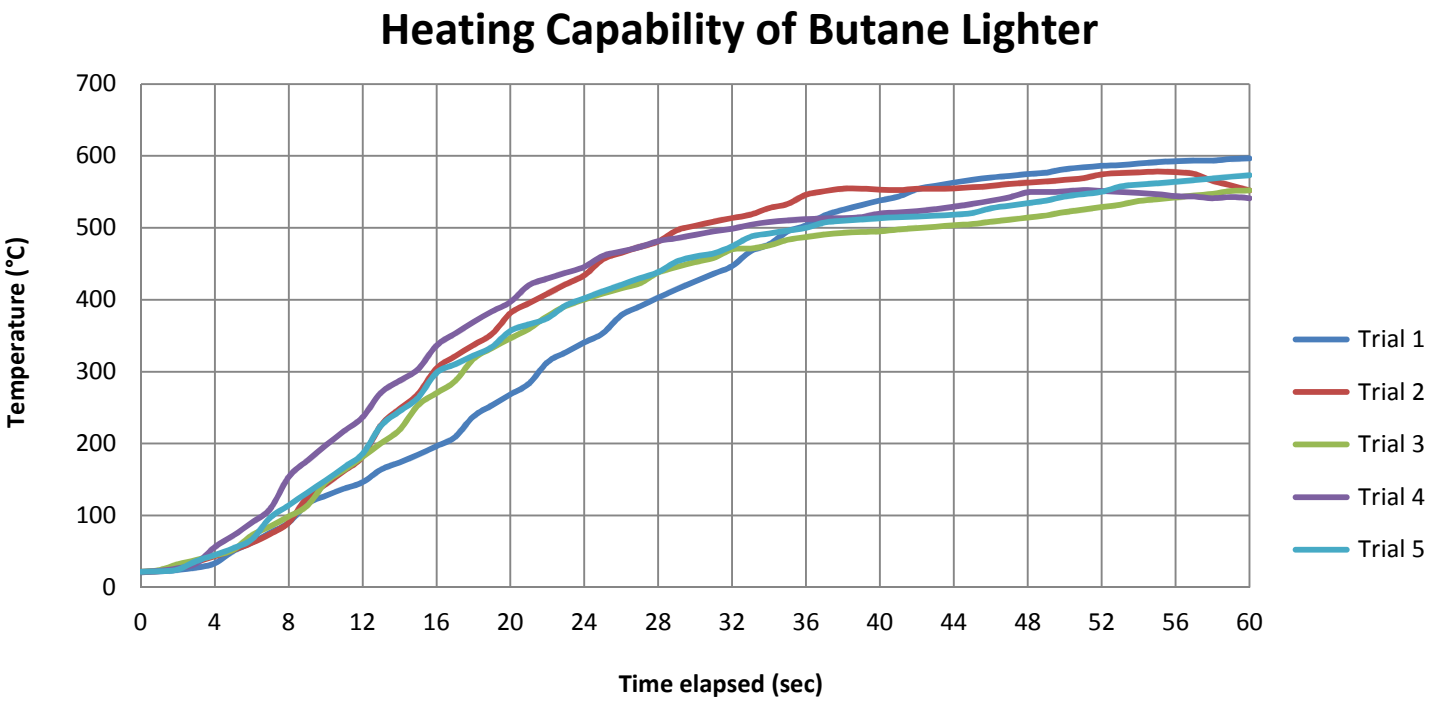


Figure 29: Temperature profile for butane lighter

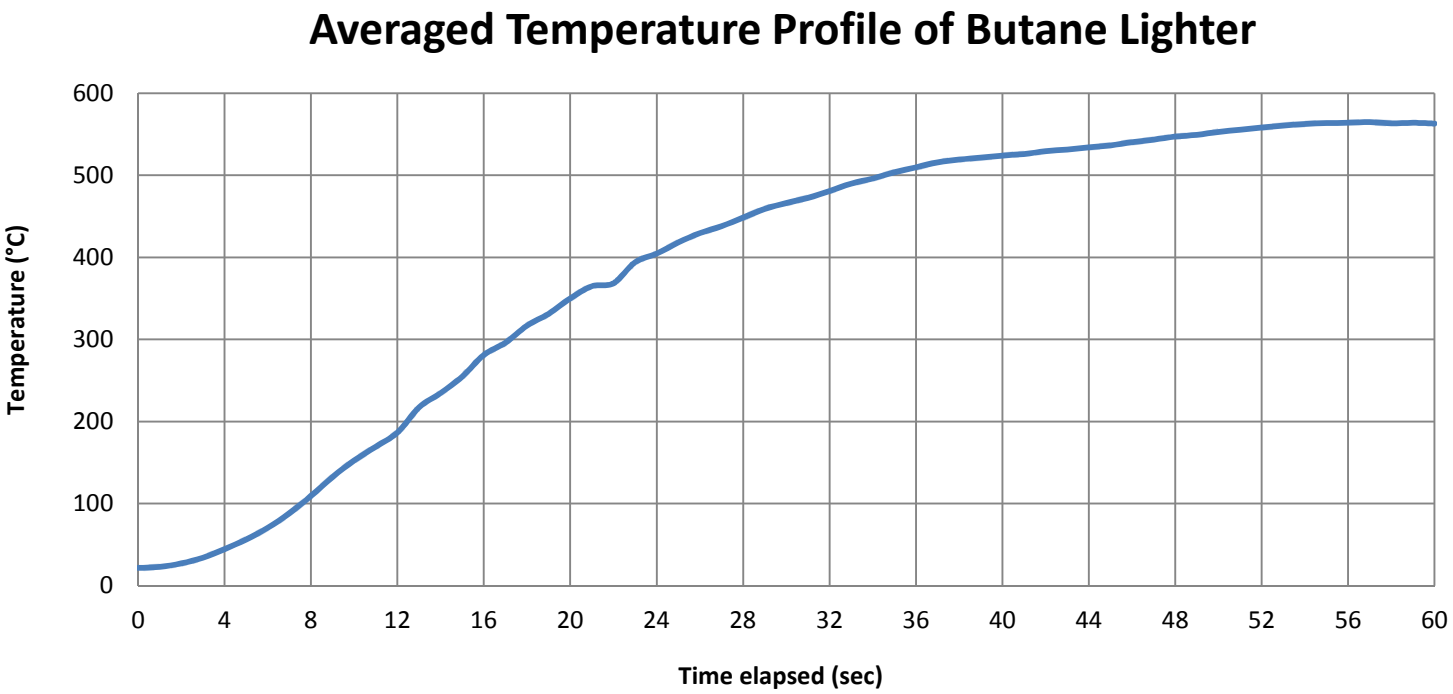


Figure 30: Average temperature profile for butane lighter

Table 5: Temperature ranges observed with butane lighter

Trial	Temperature (°C) at 0 seconds	Temperature (°C) at 60 seconds	Overall Increase in Temperature (°C) over 60 seconds
1	21	597	576
2	22	552	530
3	22	552	530
4	22	541	519
5	22	573	552
Average	22	563	541
Standard Deviation	0	22	23

Using the information in ***Table 5*** it was calculated that the average initial, final, and overall change in temperature were 22°C, 563°C, and 541°C respectively. Even over the course of 60 seconds, the butane lighter did not reach the desired maximum temperature of 750°C and was not able to heat the experimental apparatus fast enough to match the rapid change in temperature that was observed in the simulated cigarette scenarios. Since the temperature capability of the butane lighter did not generate ideal output conditions, an alternative heat source that could better match the maximum and rate of change observed during the cigarette studies was sought.

2.6.2 SUITABILITY OF PROPANE HEATING

Since the majority of lighters use a butane fuel source, a standard handheld propane blowtorch (BernzOmatic Propane Torch; Columbus, OH, USA) was investigated for the desired heat output. Replicate trials (n=5) were conducted and the 'blank' experimental apparatus was set up to monitor the temperature capability of the propane torch. ***Figure 31*** and ***Figure 32***, respectively, provide the individual and average temperature versus time plots observed with this particular propane torch. The tabulated results are provided in ***Table 6*** for each of the five trials. From this table, it was determined that the average starting temperature was 26°C and the average final temperature was 904°C. The average overall change in temperature was found to 879°C per minute.

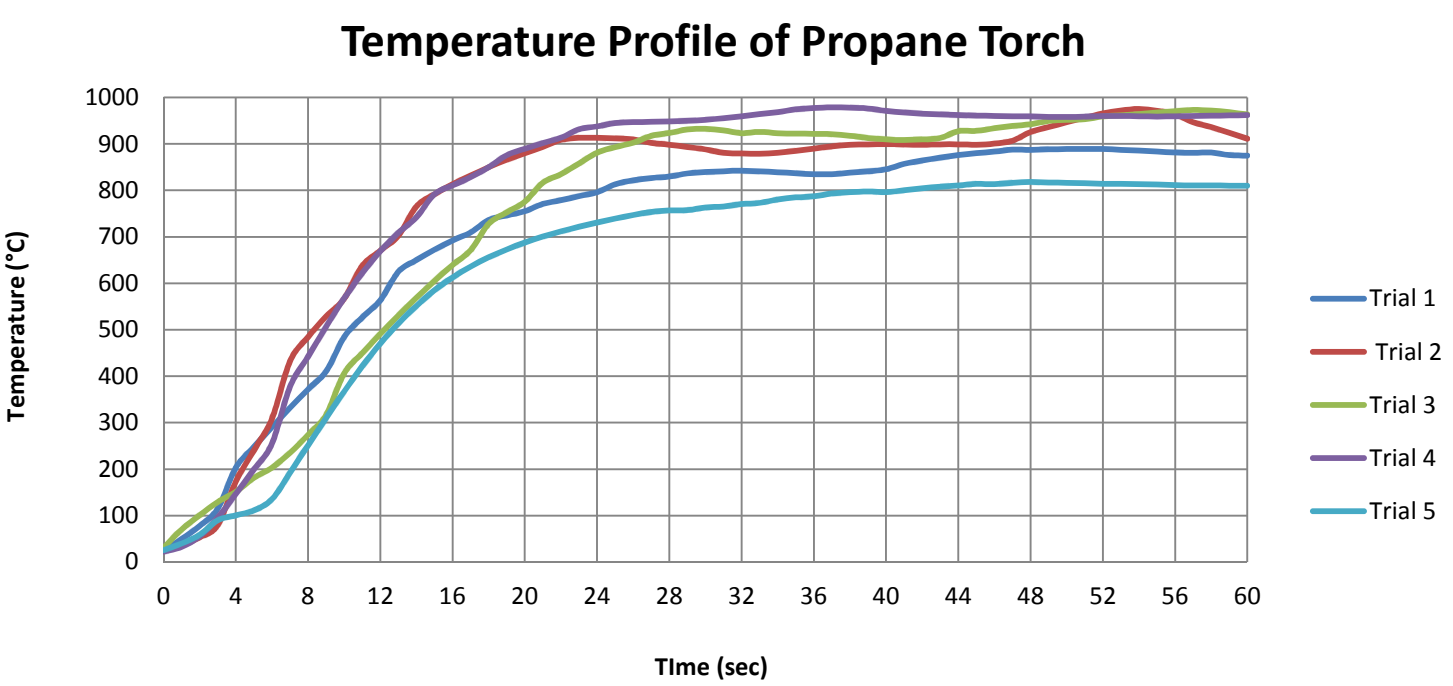


Figure 31: Temperature profile for propane torch

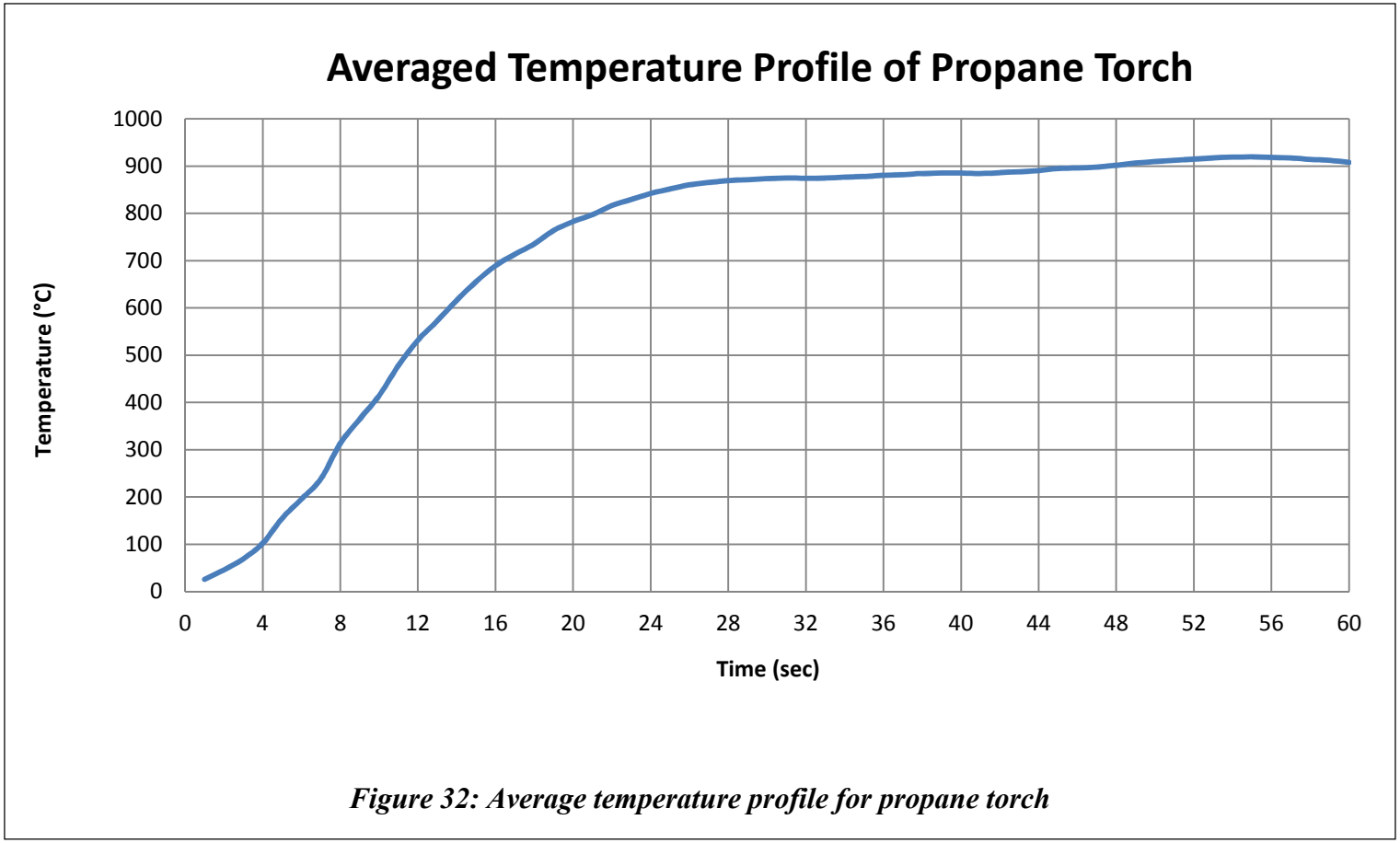


Figure 32: Average temperature profile for propane torch

Table 6: Temperature ranges observed with propane torch

Trial	Starting Temperature (°C) at 0 Seconds	Ending Temperature (°C) at 60 Seconds	Overall Increase in Temperature (°C) in 60 Seconds
1	24	875	851
2	26	911	885
3	31	963	923
4	22	963	940
5	25	810	785
Average	26	904	877
Standard Deviation	3	65	62

2.6.3 RESULTS AND DISCUSSIONS: HEATING SOURCES INVESTIGATED

In deciding upon an appropriate heat source for these experiments, it is important to remember that cigarette temperatures were found to elevate upwards of 700°C in a 25 second span and approach a maximum temperature of 800°C overall. Using the propane torch, the average temperature of the system reached approximately 800°C within 20 seconds (see ***Figure 29***) but the butane lighter was unable to surpass 600°C in a 60 second time period (see ***Figure 30***). Given the nature by which drugs are typically smoked, we sought a heat source that could cause a rapid elevation of temperature. After examining the heating profiles, it was determined that the propane torch would be more suitable for our particular experimental applications.

2.7.0 CONCLUSION

The temperature profile of a burning cigarette is known to be highly variable and difficult to accurately describe. The conditions for the apparatus being used in this study were experimentally determined by monitoring the temperature of cigarettes in both a smoldering and inhaled state. An increase in temperature of the burning zone by more than 500 degrees, with overall temperatures in excess of 700°C, was commonly observed with the cigarettes.

When organic molecules are subjected to such extreme heating conditions, the pyrolytic reactions that take place can result in the generation of a number of products. While it is expected that pyrolytic products of smaller molecular weight are generated via simple bond cleavage, it is not uncommon to find species having a molecular weight greater than that of the parent molecule. While the benefits and consequences of controlled pyrolysis for industrial and municipal purposes is known, the generation of both small and large pyrolytic products through the smoking process can unfortunately pose unforeseen health hazards to a given user.

CHAPTER 3: PYROLYSIS OF NON-CONTROLLED SUBSTANCES

3.1.0 INTRODUCTION

Given the limited reports regarding the pyrolytic products generated during the smoking of synthetic cannabinoids, a comprehensive approach was taken with this research. New synthetic cannabinoids are routinely detected by forensic laboratories, but the core components which make up the synthetic cannabinoids are not as quick to change. Therefore, a select number of chemical moieties that comprise the synthetic cannabinoids JWH-018, JWH-030, JWH-081 and UR-144 were experimentally studied to better understand the type of pyrolytic products that are likely to form and the subsequent health hazards that these compounds may pose.

As indicated in the previous section, polyaromatic hydrocarbons and heterocyclic amines can be formed through various pyrolytic reactions. The toxic and/or carcinogenic potential of PAHs and HCAs is a major health-related concern that has been well-documented by a number of government agencies and international organizations. The most common sources of PAH exposure stem from cigarette smoke, automobile exhaust, coal processing, and incineration of wastes [74]. As such, individuals are most likely to be exposed to a mixture of PAHs (as opposed to a single PAH) and it is common to see the health effects of PAHs collectively, rather than individually, described.

The Report on Carcinogens (RoC), provided by the National Toxicological Program (NTP), has a number of PAHs, HCAs, and compounds of interest categorized as either “known to be human carcinogens” or “reasonably anticipated to be human carcinogens” [90, 91]. The Substance Priority List (SPL), which is supplied by the ATSDR and provides

information on the most commonly found hazardous chemical substances at sites designated by the National Priorities List (NPL), also includes a number of PAHs and compounds of interest to this research [75]. For the purpose of this study, the World Health Organization (WHO) International Agency for Research on Cancer (IARC) monographs on the evaluation of carcinogenic risks to humans were used to classify the severity of the pyrolytic products identified [92, 93]. Four distinct carcinogenic categories have been established by IARC and the inclusion criteria have been summarized below in ***Table 7***.

Table 7: Criteria used by the International Agency for Research on Cancer (IARC) to classify carcinogenic pyrolytic products [92]

IARC Carcinogenic Classification	Definition	Justification Criteria
Group 1	Carcinogenic to humans	(a) Sufficient evidence of carcinogenicity in humans; OR (b) Less than sufficient evidence of carcinogenicity in humans AND sufficient evidence of carcinogenicity in experimental animals
Group 2A	Probably carcinogenic to humans	(a) Limited evidence of carcinogenicity in humans AND sufficient evidence of carcinogenicity in experimental animals; OR (b) Inadequate evidence of carcinogenicity in humans AND sufficient evidence of carcinogenicity in experimental animals
Group 2B	Possibly carcinogenic to humans	(a) Limited evidence of carcinogenicity in humans AND less than sufficient evidence of carcinogenicity in experimental animals; OR (b) Inadequate evidence of carcinogenicity in humans AND sufficient evidence of carcinogenicity in experimental animals
Group 3	Not classifiable as to its carcinogenicity to humans	(a) Inadequate evidence of carcinogenicity in humans AND inadequate or limited evidence of carcinogenicity in experimental animals
Group 4	Probably not carcinogenic to humans	(a) Evidence suggesting a lack of carcinogenicity in humans and experimental animals

3.2.0 MATERIALS AND METHODS

The chemicals evaluated for this study were selected because they are smaller components that make up the larger molecular structures of JWH-018, JWH-030, JWH-081, and UR-144. With the exception of 3-naphthoylindole, these non-controlled substances have been generically divided into 'naphthalene-containing compounds' or an 'indole-containing compounds' for the purpose of this project. The weight of each particular substance that was pyrolyzed can be found in **Appendix A**.

3.2.1 NAPHTHALENE-CONTAINING COMPOUNDS

The naphthalene-containing compounds that were pyrolyzed originated from Sigma-Aldrich (Saint Louis, Missouri, USA). The four naphthalene-containing compounds included in our analysis were: Naphthalene (solid, purity ≥ 99%, CAS No: 91-20-3), 1-methylnaphthalene (liquid, density of 1.001 g/mL, purity ≥ 95%, CAS No: 90-12-0), 1-methoxynaphthalene (liquid, density of 1.094 g/mL, purity ≥ 98%, CAS No: 2216-69-5), and 1-ethylnaphthalene (liquid, density of 1.008 g/mL, purity ≥ 97%, CAS No: 1127-76-0). The molecular structures, chemical formula, and molecular weight for these specific compounds are provided in ***Figure 33***.

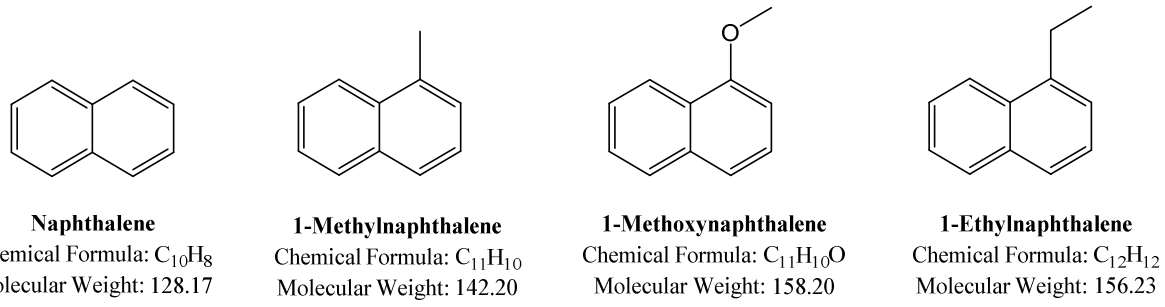


Figure 33: The naphthalene-containing compounds investigated in the pyrolysis study

3.2.2 INDOLE-CONTAINING COMPOUNDS

Four indole-containing compounds were also included in this study. Two of these compounds originated from Sigma-Aldrich: Indole (solid, purity ≥ 99%, CAS No: 120-72-9) and 3-methylindole (solid, purity ≥ 98%, CAS No: 83-34-1). Two indole-containing compounds were synthesized internally and provided for inclusion in this study: 1-pentylindole (liquid, purity not provided, CAS No: 59529-21-4) and Indole-3-carboxaldehyde (solid, purity not provided, CAS No: 487-89-8). The total ion chromatogram (TIC) of both in-house synthesized analytes are provided in **Appendix B**. The molecular structure, chemical formula, and molecular weight for each indole-containing compound is provided in ***Figure 34***.

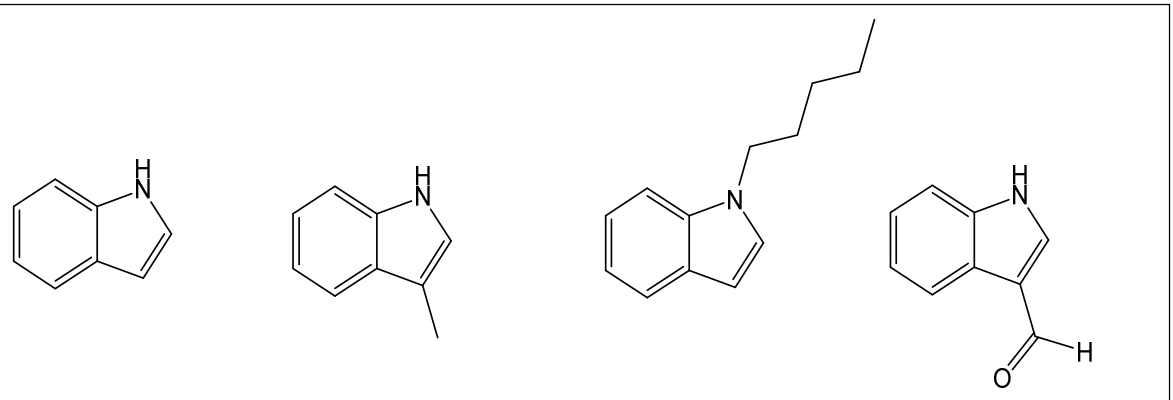
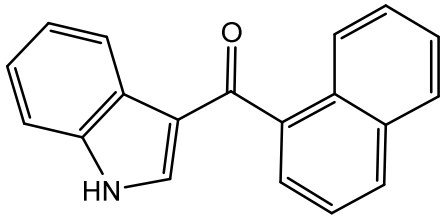


Figure 34: The indole-containing compounds investigated in this pyrolysis study

3.2.3 3-NAPHTHOYLINDOLE

The largest non-controlled substance that was included in this study was 3-naphthoylindole (solid, ≥90%, CAS No: 109555-87-5). This compound, which

represents the core of JWH-018 and JWH-081, was included as an intermediate between the smaller naphthalene and indole based components and the much larger synthetic cannabinoids. ***Figure 35*** provides the molecular structure, chemical formula, and molecular weight of 3-naphthoylindole while **Appendix B** contains the TIC.



3-Naphthoylindole
Chemical Formula: $C_{19}H_{13}NO$
Molecular Weight: 271.32

Figure 35: The dual-containing compound investigated in this pyrolysis study

3.2.4 INSTRUMENTATION

Gas chromatography – mass spectrometry (GC-MS) was extensively used throughout this research project for analysis of the unknown pyrolytic products. The technique couples the separation power of a gas chromatograph with the detection capabilities of a mass spectrometer and has long been referred to as the gold standard in forensic laboratories. It is also ideal for volatile and semi-volatile substances such as drug molecules.

Ideally, the analytes are individually separated in the gas chromatograph, prior to reaching the mass spectrometer, so that the detection is not obscured by simultaneous elution of compounds; such was not always achievable in this study. The basic components of a gas chromatograph are the carrier gas, injection port, oven, and

capillary column. When an analyte is injected into the heated GC injection port, the sample is simultaneously volatilized and passed onto the column. These volatile and semi-volatile analytes are separated as they partition between the mobile phase and stationary phase. The mobile phase (carrier gas) is a high purity gas that will not chemically interact with the analytes. The stationary phase, located inside of the polyimide coated fused silica capillary, provides the partitioning mechanism that ultimately separates the analytes as they travel through the length of the column. Capillary columns commonly used in forensic analysis are made of polysiloxane with a varying degree of phenyl composition (i.e. 1% or 5%). The capillary column is housed in a temperature controlled oven that facilitates the separation of the analytes before they enter the mass spectrometer.

The basic components of a mass spectrometer are the vacuum system, ionization source, mass analyzer, and detector. The entire MS process is carried out under extremely low pressure (~10^{-8} atmospheres) so that the generation of ions and their corresponding flight path can be precisely controlled throughout the mass spectrometer. To achieve the low pressure needed, a dual-stage vacuum pumping system (roughing pump and turbo molecular pump) is used to create a vacuum within the system.

After an analyte has been injected into the GC and subsequently eluted from the column, it enters the ionization source of the mass spectrometer. A standard electron ionization source (70 eV) was used to ionize the gas phase molecules so they could be separated based upon their mass-to-charge (m/z) ratio by the mass analyzer.

A significant amount of fragmentation often occurs in electron ionization because of the excess energy absorbed by the parent compound. Therefore, the molecular ion and

resulting molecular fragments need to be separated and detected by a mass analyzer and detector. A single quadrupole mass analyzer was used in this study and an electron multiplier was used to amplify and detect the separated ions as they reached the end of the mass spectrometer system.

Through the use of GC-MS, both a chromatogram and mass spectrum are generated and used for the data analysis of a given sample. Coupling retention time and the results of the mass spectrum enables analysts to identify a given compound. For best practices, a reference standard should be run on the same system to verify that the retention time and mass spectrum unequivocally match one another. However, if a standard does not exist or cannot be feasibly obtained, analysts often turn to commercially available mass spectral databases ("libraries") for assistance. In such an instance, the mass spectrum obtained by a user can be electronically searched against a comprehensive spectral library for tentative identification of the analyte. Due to the significant number of pyrolytic products being analyzed in this project, commercially available mass spectral databases and in-house libraries were heavily relied upon for tentative identification when standards were not accessible.

3.2.5 METHOD PARAMETERS

All samples were analyzed on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer using a J&W DB-1ms column (30m; 250µm x 0.25µm). 1.0 µL samples were injected into a 280°C injection port using a 10:1 split ratio. Helium at a constant flow of 1 mL/min was used as the carrier gas with a solvent delay of 2.20 minutes. The temperature program for the gas chromatograph was initially held for 2 minutes at 90°C and then increased 14°C per min to a final temperature of

300°C, with a 7 minute hold (34 minute total run time). The transfer line temperature was set at 280°C with an ion source temperature of 230°C and quadrupole temperature of 150°C. Mass spectra were acquired using a scan range of 30 to 550 atomic mass units (amu) with Agilent ChemStation software.

3.2.6 DATA PROCESSING

Three individual pyrolysis trials were conducted for each non-controlled substance and a condensed version of the data processing steps is illustrated in ***Figure 36***. For each of these trials, extracts were collected from the wool, pipette, and solvent layer: resulting in a total of nine fractions (i.e. Wool-1, Pipette-1, Solvent Layer-1, Wool-2, Pipette-2, etc.) per analyte. Each extract was run in duplicate (Wool-1a, Wool-1b, Pipette-1a, etc.) using the GC-MS and method previously described, with solvent blanks between injections. The two resulting chromatograms were overlayed using Agilent ChemStation software and visually inspected for any discrepancies.

The analytes that were observed in both replicates, at the same retention time (±0.05 min), were further investigated using the instrument software. For a given compound, the mass spectrum was searched against the NIST 2014 Mass Spectral database and an in-house library for potential matches. A tentative identification was made with compounds that, after background subtraction, had a quality score of at least 70%.

When a tentative identification could be made for a given analyte it was included in a spreadsheet for later analysis. The spreadsheet was used to manage and compare the retention time, compound name, and most intense ions observed across the extracts of each analyte. Once the data for each chromatogram were input, the list of compounds

was further narrowed to include only those that had similar mass spectral profiles and library matches. Only the pyrolytic products that appeared in at least two of the three extracts for a given compound (i.e. a pyrolytic product identified in two of the three pipette extracts for compound ‘X’) were focused on in this report.

When possible, confirmation of a tentatively identified pyrolytic product was verified with a reference standard material and has been noted. Not all the pyrolytic products were distinguishable from one another as a result of co-elution and/or low abundance levels. Additionally, the mass spectrum of some products did not register a positive match in the database being used. Therefore, the results contain only those compounds which met the previously mentioned criteria and do not include every observable peak found in a particular pyrolytic chromatogram (pyrogram).

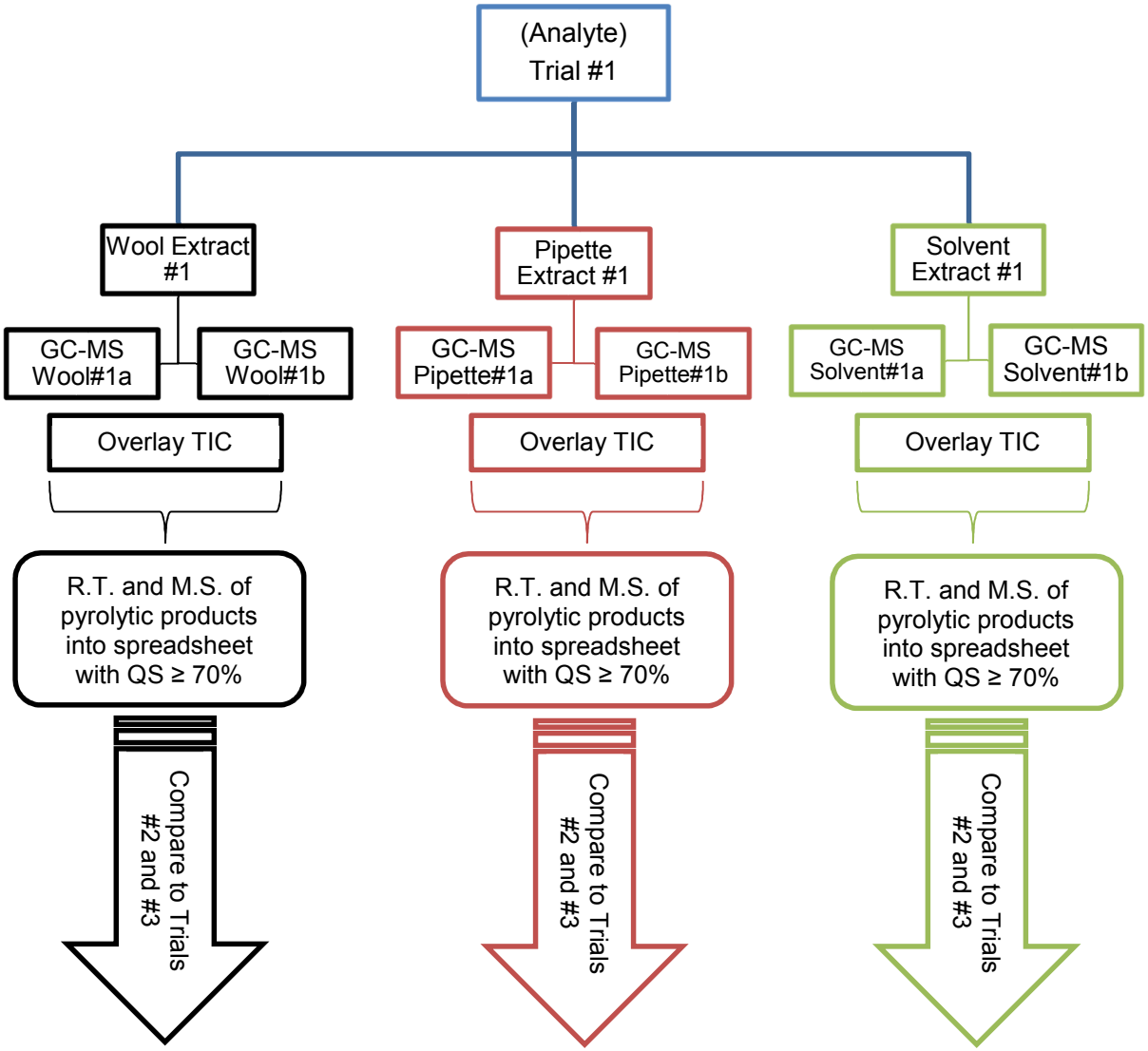


Figure 36: Condensed schematic of data processing steps

3.3.0 RESULTS AND DISCUSSION

The pyrolytic products that were reproducibly identified in extracts from each of the non-controlled substances analyzed are provided in both tabulated and structure formats. The majority of the compounds were tentatively identified (T) but when accessible, a reference material was used to verify (V) the identity of a given pyrolytic product. Where appropriate, the IARC carcinogenic group that the pyrolytic product belongs to (Group 1, 2A, 2B, 3, or 4) is also provided and discussed. Since extracts from the quartz wool (W), pipette (P), and solvent (S) were independently analyzed, the fraction from which the product was identified is also included.

3.3.1 MATRIX BLANKS

Quartz wool (Costech Analytical Technologies, Valencia, California, USA) was selected as the substrate for the experimental apparatus due to its relatively inert nature and suitability for high temperature conditions. To identify the compounds inherently generated as a result of the experimental apparatus being used, matrix blanks were performed and extracted using the same previously discussed steps. A pyrogram which is representative of the matrix components commonly observed can be found in ***Figure 37***.

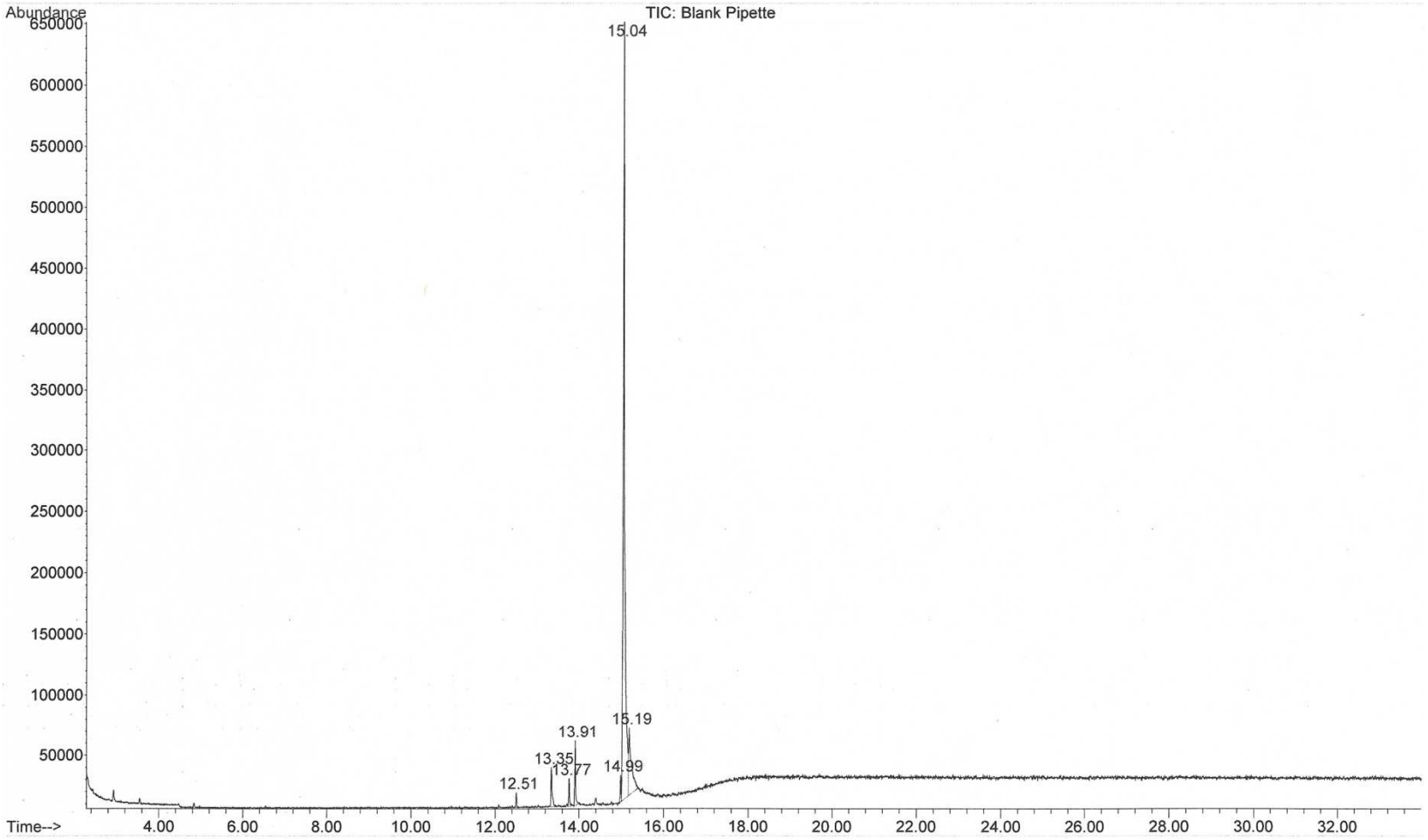


Figure 37: Pyrogram representative of the matrix components

Although tentative identification was not possible for each of the matrix components, the retention time was still noted. However, major components that were identified from the matrix blanks were as follows: dodecanamide (12.51 min), oleanitrile (13.35 min), tetradecanamide (13.77), hexadecanamide (13.91 min), and 9-octadecenamide (15.04 min). No further investigation of these compounds was pursued and as such these particular components were not considered as pyrolytic products when examining the pyrograms for the non-controlled and controlled substances. Nearly identical pyrograms representative of the wool and solvent layer can be found in **Appendix C**.

3.3.2 NAPHTHALENE-CONTAINING COMPOUNDS

The pyrolysis of the naphthalene-containing compounds resulted in the detection of 122 total pyrolytic products (see ***Table 8*** and ***Figure 38***). The greatest number of identifiable products were observed with 1-methoxynaphthalene (73), followed by 1-ethylnaphthalene (63), naphthalene (38), and 1-methylnaphthalene (36). Overall, the majority of the pyrolytic products observed were substituted and unsubstituted PAHs. Nine products were observed in at least one extract from each of the four of the analytes pyrolyzed: naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2-ethenylnaphthalene, biphenyl, anthracene, 9-(phenylmethylene)-9H-fluorene, 1,2'-binaphthalene, and 2,2'-binaphthalene. These were, therefore, considered to be non-specific.

While three, four, and five membered ring systems were identified as products across all the naphthalene-containing compounds, it was also found that certain pyrolytic products were only observed in the extracts of certain analytes. Sixty-five of the 122 pyrolytic products (53%) were only identified in extracts from one of the four

analytes, compared to the nine previously mention compounds were identified in extracts from all analytes. ***Figure 39*** shows the distribution of the 122 identified pyrolytic products among the four analytes pyrolyzed and provides insight into the 'uniqueness' of the pyrolytic products.

As mentioned previously, it is not uncommon for PAHs to be collectively categorized with a given health-hazard. However, seventeen of the chemicals that were identified as a result of the pyrolysis of these naphthalene-containing compounds have been individually listed by the IARC. Six of these compounds were identified in at least three of the four analytes studied: the Group 2B carcinogens naphthalene, benzo[k]fluorene, and benz[a]anthracene, as well as the Group 3 carcinogens anthracene, fluoranthene, perylene. The other eleven compounds were identified in one or two of the analytes: Group 2B carcinogens – styrene, benzofuran, benzo[c]phenanthrene; Group 3 carcinogens – phenol, coumarin, acenaphthene, fluorene, phenanthrene, pyrene, 11H-benzo[b]fluorene, and dibenz[a,h]anthracene.

Table 8: Pyrolytic products identified from investigation of the naphthalene-containing compounds

Retention Time (min)	Compound	ID	CG	Naphth.	1-CH_3 Naphth.	1-OCH_3 Naphth.	1-C_2H_5 Naphth.
2.68	Phenylacetylene	T	-	P			
2.82	Styrene	T	2B	P			
3.29	Benzaldehyde	T	-	P			
3.41	Phenol	T	3	P			
3.75	Benzofuran	T	2B	P			
4.20	3-Methylphenol	T	-	P			
4.27	Indene	T	-	P	P	P	
5.44	2-Methylindene	T	-	P		P	
5.48	Azulene	T	-		P	P, S	
5.78	Naphthalene	**V**	2B	P	P	P, S	P
5.96	2,3-Dimethylindene	T	-			P	
6.27	Quinoline	**V**	-	P	P	S	
6.53	2-Ethylindene	T	-			P	
6.58	1-Methylindole	**V**	-			S	
6.62	1-Indone	T	-	P			
6.67	Benzocylcoheptatriene	T	-			P, S	
6.73	Indole	T	-		P	P	
6.82	Phthalic anhydride	T	-			S	
6.99	1-Methylnaphthalene	**V**	-	P	P	P	P
7.02	2-Methylquinoline	T	-		S		
7.14	2-Methylnaphthalene	T	-	P	S	P	W, P, S
7.20	3-Phenylfuran	T	-			S	
7.24	1-Ethylidene-indene	T	-			P	
7.47	3-Methylquinoline	**V**	-			P	
7.62	3,4-Dihydro-1(2H)-naphthalenone	T	-				P
7.71	2-Methylindole	**V**	-	P			
7.82	Coumarin	T	3	P		P	
7.88	1,4-Naphthalendione	T	-	P		S	
7.99	1-Ethylnaphthalene	**V**	-		P, S	P	W, P, S
8.12	2-Ethenylnaphthalene	T	-	P	P	P	W, P, S

8.23	2,3-Dimethylnaphthalene	T	-		P	P	P
8.29	Biphenyl	T	-	P	P	P	W, P
8.38	1-Methoxynaphthalene	**V**	-		S	W, P, S	W, S
8.43	1,4-Dimethylnaphthalene	T	-		P		P
8.45	Acenaphthylene	T	-	P	P		W, P, S
8.54	1,2-Dimethylnaphthelene	T	-		P	P	P
8.58	2-(1-Methylethyl)naphthalene	T	-				P
8.61	1-(1-Methylethyl)naphthalene	T	-				P
8.80	Acenaphthene	**V**	3		P		W, P, S
8.84	1-Naphthalenecarboxaldehyde	T	-		P		
8.86	1-Naphthalenol	T	-	P		P, S	S
8.95	2-Naphthalenol	T	-	P			P
8.99	2,4-Di-t-butylphenol	T	-			W	W, P
9.00	Menadione	T	-			P, S	
9.11	1-Isopropenylnaphthalene	T	-				P
9.14	1,4,6-Trimethylnaphthalene	T	-				P
9.16	4-Hydroxy-4-methyl-naphthalen-1-one	T	-			P, S	P
9.29	1(2H)-Acenaphthylenone	T	-	P		P, S	
9.43	4-Methyl-1,1'-biphenyl	T	-				P
9.47	1-Naphthalenemethanol	T	-		P, S		S
9.52	1-(1-Naphthalenyl)-ethanone	T	-				W, S
9.54	6-Phenyl-3,5-hexadien-2-one	T	-			P, S	
9.63	1H-Phenalene	T	-				P
9.69	Fluorene	**V**	3	P			P
9.74	α-Methyl-2-naphthalenemethanol	T	-				W, P, S
9.78	1-(2-Propenyl)naphthalene	T	-			P	P
9.89	1-Dimethoxymethylnaphthalene	T	-		P, S	P	W, P, S
9.93	2-Naphthalenecarboxylic acid methyl ester	T	-				S
9.98	2-Methyl-1-naphthalenol	T	-			P	
10.19	Dibenzofuran	T	-			S	P, S
10.21	1-Acenaphthenol	T	-				P
10.30	[1,1'-Biphenyl]-4-carboxaldehyde	T	-			P, S	
10.34	1-Methyl-9H-fluorene	T	-				P
10.40	2-Hydroxyfluorene	T	-			P, S	

10.44	5,7-Dimethyl-1-naphthol	T	-			P, S	
10.56	3-Hydroxybiphenyl	T	-			S	
10.61	2-Ethyl-3-methylene-indan-1-one	T	-			P, S	
10.71	[1,1'-Biphenyl]-2,5-diol	T	-			S	
10.85	N,N-Dimethyl-1-naphthaleneamine	T	-				P
10.86	9H-Fluoren-9-one	T	-			P, S	
10.93	2-Hydroxy-1-naphthoic acid methyl ester	T	-			S	
11.06	1-[1,1'-Biphenyl]-4-yl-ethanone	T	-			P, S	
11.26	Anthrancene	**V**	3	P, S	P	P, S	P, S
11.36	Phenanthrene	T	3	P			P
11.51	1-(1-Naphthyl)-1,2-ethanediol	T	-			S	
11.56	Anthrone	T	-			S	
11.83	1-(Phenylmethylene)indene	T	-	P	P	P, S	
11.87	2-Phenyl-1H-indene	T	-			S	
12.03	1H-Phenalen-1-one	**V**	-			S	P, S
12.05	4-Hydroxy-1-naphthaldehyde	T	-			S	
12.10	1H,3H-Naphtho[1,8-cd]pyran-1-one	T	-				P
12.30	4H-Cyclopenta[def]phenanthrene	T	-				S
12.32	1-Methylanthracene	T	-			S	
12.64	1-Phenylnaphthalene	T	-	P		P, S	
12.88	Naphthalic anhydride	T	-				P, S
13.05	6,6-Diphenylfulvene	T	-	P			
13.20	8-Formyl-1-naphthalenecarboxylic acid	T	-				S
13.30	Fluoranthene	**V**	3	P		P, S	P, S
13.44	2-Styrylnaphthalene	T	-	P			
13.61	9-Phenanthrol	T	-			S	
13.64	Pyrene	T	-			S	P, S
13.71	Benzo[b]naptho[2,3-d]furan	T	-	P		S	
13.80	p-Terphenyl	T	-			S	
13.99	1-Phenyl-2-naphthalenol	T	-			S	
14.26	11H-Benzo[b]fluorene	T	-			P, S	
14.39	7H-Benzo[c]fluorene	T	-	P		P, S	
15.07	9-(Phenylmethylene)-9H-fluorene	T	-	P, S	S	S	P, S

15.35	Benzo[c]phenanthrene	T	2B	P		P, S	
15.42	11H-Benzo[a]fluorene-11-one	T	-			S	
15.53	9-Phenyl-9H-fluorene	T	-			S	
15.69	Benz[a]anthrancene	**V**	2B	P		P, S	P
15.75	Naphthacene	T	-	S		P, S	P
15.81	1,2'-Binaphthalene	T	-	P	P, S	P, S	P, S
15.96	3-Methylcholanthrene	T	-		S		P, S
16.40	1-(2-Naphthalenylmethyl) naphthalene	T	-		P, S		P, S
16.46	9,10-Dihydro-8-methylbenzo[a]pyrene	T	-		P, S		P, S
16.51	1,1'-Methylenebis-naphthalene	T	-				P, S
16.55	7-Methyl-benz[a]anthracene	T	-			S	
16.61	2-Methylcholanthrene	T	-		P, S		P, S
16.66	2,2'-Binaphthalene	T	-	P, S	P, S	P, S	P, S
16.75	1,1'-Binaphthalene	T	-		P, S	P, S	S
17.15	3,3'-Dimethyl-1,1'-binaphthalene	T	-		P, S		P, S
17.23	8,8'-Dimethyl-1,1'-binaphthalene	T	-		P, S		S
17.33	1-Phenylpyrene	T	-				P, S
17.37	4,4'-Dimethyl-1,1'-binaphthalene	T	-		S		S
17.46	Benzo[k]fluoranthene	T	2B		S	P, S	P, S
17.54	Dinaphtho[1,2-b:1',2'-d]furan	T	-			P, S	
17.91	Benzo(a)pyren-7-ol	T	-			P, S	
18.01	2,2-Dimethyl-1,1'-binaphthalene	T	-		P, S		S
18.10	Perylene	**V**	3		P, S	S	P
18.48	2,3-Diphenyl-inden-1-one	T	-			P, S	P, S
19.99	Dibenz[a,h]anthracene	T	3			P, S	P, S

Identification (ID) refers to whether the pyrolytic product was tentatively identified, T, or verified with a reference material, **V**. The carcinogenic group (CG) indicates the IARC classification to which the analyte belongs [1, 2A, 2B, 3, or 4]. The extract in which the pyrolytic product is indicated with a single capital letter representative of each of the three extracts: W (wool), P (pipette), and S (solvent layer).

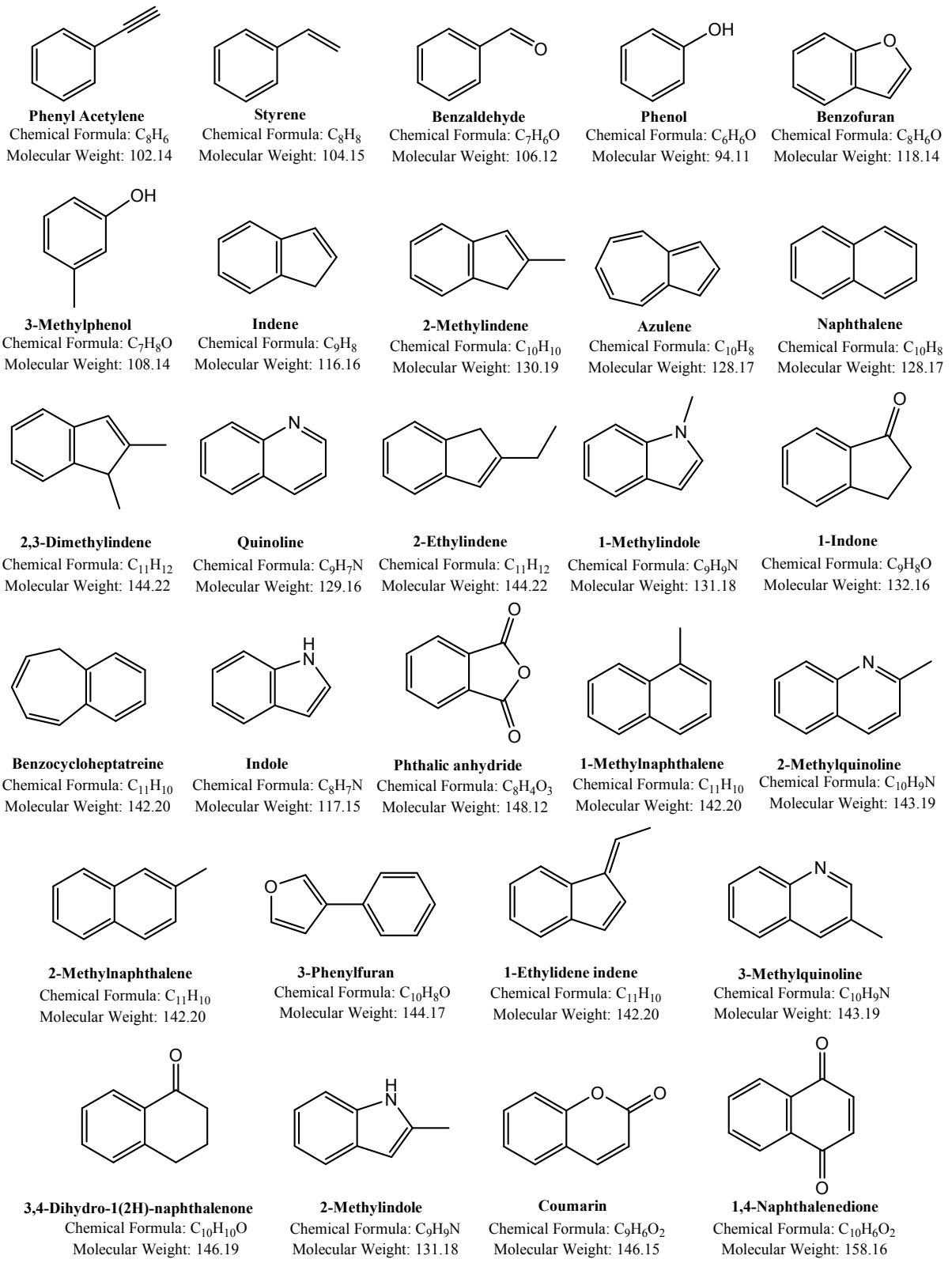
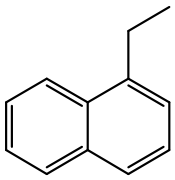
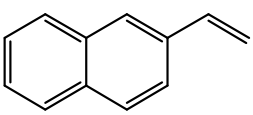


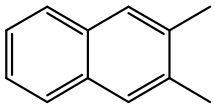
Figure 38: Pyrolytic products identified from naphthalene-containing analytes



1-Ethylnaphthalene
Chemical Formula: $C_{12}H_{12}$
Molecular Weight: 156.23



2-Ethenylnaphthalene
Chemical Formula: $C_{12}H_{10}$
Molecular Weight: 154.21



2,3-Dimethylnaphthalene
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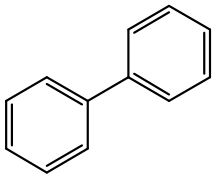
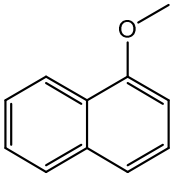
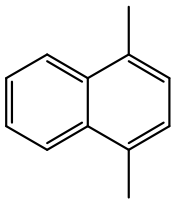
Molecular Weight: 156.23



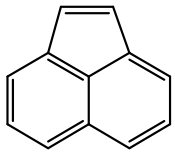
Biphenyl
Chemical Formula: $C_{12}H_{10}$
Molecular Weight: 154.21



1-Methoxynaphthalene
Chemical Formula: $C_{11}H_{10}O$
Molecular Weight: 158.20



1,4-Dimethylnaphthalene
Chemical Formula: $C_{12}H_{12}$
Molecular Weight: 156.23



Acenaphthylene
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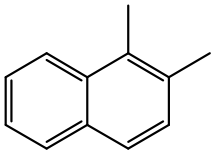
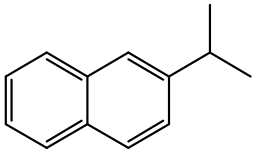
Molecular Weight: 152.20



1,2-Dimethylnaphthalene
Chemical Formula: $C_{12}H_{12}$
Molecular Weight: 156.23



2-(1-Methylethyl)naphthalene
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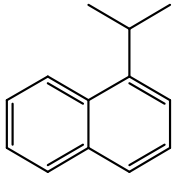
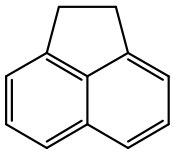
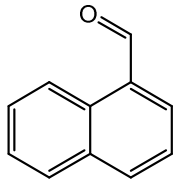
Molecular Weight: 170.26



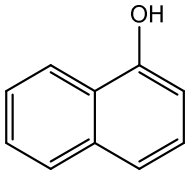
1-(1-Methylethyl)naphthalene
Chemical Formula: $C_{13}H_{14}$
Molecular Weight: 170.26



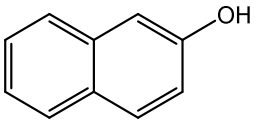
Acenaphthene
Chemical Formula: $C_{12}H_{10}$
Molecular Weight: 154.21



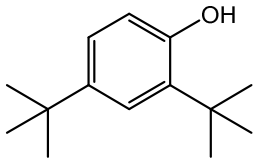
1-Naphthalenecarboxaldehyde
Chemical Formula: $C_{11}H_8O$
Molecular Weight: 156.18



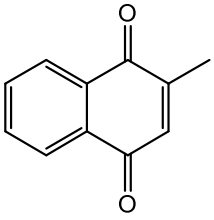
1-Naphthalenol
Chemical Formula: $C_{10}H_8O$
Molecular Weight: 144.17



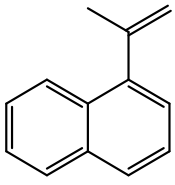
2-Naphthalenol
Chemical Formula: $C_{10}H_8O$
Molecular Weight: 144.17



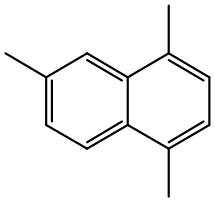
2,4-Di-t-butylphenol
Chemical Formula: $C_{14}H_{22}O$
Molecular Weight: 206.33



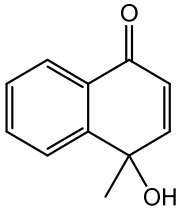
Menadione
Chemical Formula: $C_{11}H_8O_2$
Molecular Weight: 172.18



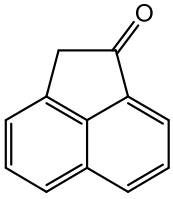
1-Isopropenylnaphthalene
Chemical Formula: $C_{13}H_{12}$
Molecular Weight: 168.24



1,4,6-Trimethylnaphthalene
Chemical Formula: $C_{13}H_{14}$
Molecular Weight: 170.26

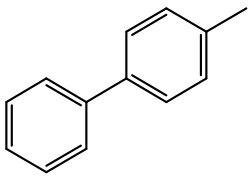


4-Hydroxy-4-methyl-naphthalen-1-one
Chemical Formula: $C_{11}H_{10}O_2$
Molecular Weight: 174.20

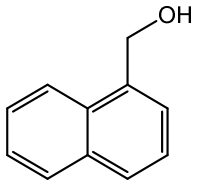


1(2H)-Acenaphthylenone
Chemical Formula: $C_{12}H_8O$
Molecular Weight: 168.20

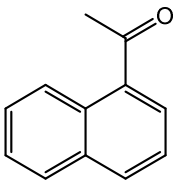
Figure 38 continued



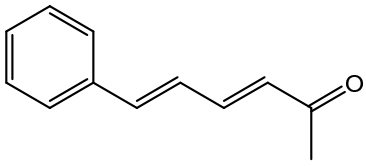
4-Methyl-1,1'-biphenyl
Chemical Formula: $C_{13}H_{12}$
Molecular Weight: 168.24



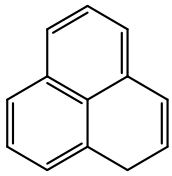
1-Naphthalenemethanol
Chemical Formula: $C_{11}H_{10}O$
Molecular Weight: 158.20



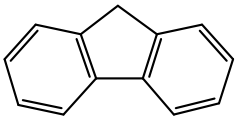
1-(1-Naphthalenyl)ethanone
Chemical Formula: $C_{12}H_{10}O$
Molecular Weight: 170.21



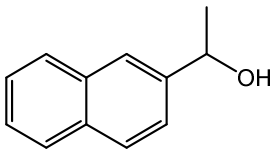
6-Phenyl-3,5-hexadien-2-one
Chemical Formula: $C_{12}H_{12}O$
Molecular Weight: 172.23



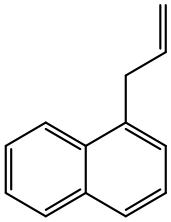
1H-Phenalene
Chemical Formula: $C_{13}H_{10}$
Molecular Weight: 166.22



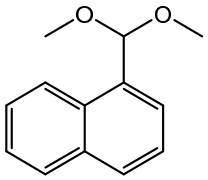
Fluorene
Chemical Formula: $C_{13}H_{10}$
Molecular Weight: 166.22



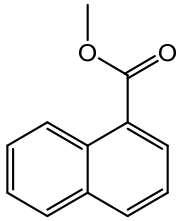
alpha-Methyl-2-naphthalenemethanol
Chemical Formula: $C_{12}H_{12}O$
Molecular Weight: 172.23



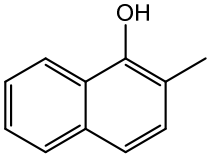
1-(2-Propenyl)naphthalene
Chemical Formula: $C_{13}H_{12}$
Molecular Weight: 168.24



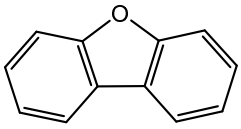
1-Dimethoxymethylnaphthalene
Chemical Formula: $C_{13}H_{14}O_2$
Molecular Weight: 202.25



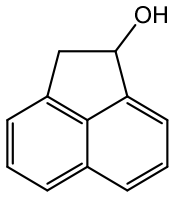
2-Naphthalenecarboxylic acid methyl ester
Chemical Formula: $C_{12}H_{10}O_2$
Molecular Weight: 186.21



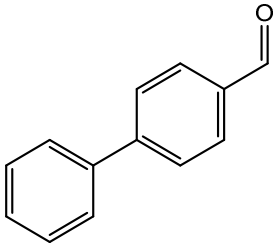
2-Methyl-1-naphthalenol
Chemical Formula: $C_{11}H_{10}O$
Molecular Weight: 158.20



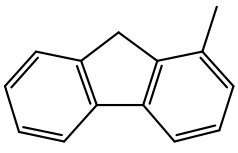
Dibenzofuran
Chemical Formula: $C_{12}H_8O$
Molecular Weight: 168.20



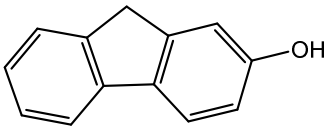
1-Acenaphthenol
Chemical Formula: $C_{12}H_{10}O$
Molecular Weight: 170.21



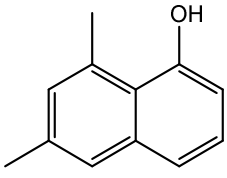
1,1-Biphenyl-4-carboxaldehyde
Chemical Formula: $C_{13}H_{10}O$
Molecular Weight: 182.22



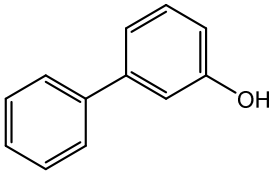
1-Methyl-9H-fluorene
Chemical Formula: $C_{14}H_{12}$
Molecular Weight: 180.25



2-Hydroxyfluorene
Chemical Formula: $C_{13}H_{10}O$
Molecular Weight: 182.22

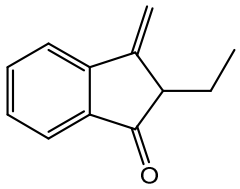


5,7-Dimethyl-1-naphthol
Chemical Formula: $C_{12}H_{12}O$
Molecular Weight: 172.23

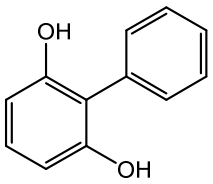


3-Hydroxybiphenyl
Chemical Formula: $C_{12}H_{10}O$
Molecular Weight: 170.21

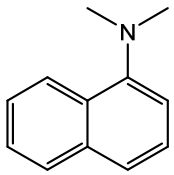
Figure 38 continued



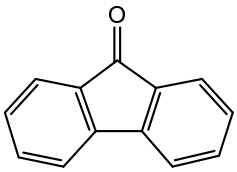
2-Ethyl-3-methylene-indan-1-one
Chemical Formula: $C_{12}H_{12}O$
Molecular Weight: 172.23



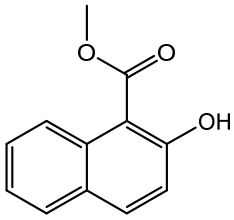
[1,1'-Biphenyl]-2,5-diol
Chemical Formula: $C_{12}H_{10}O_2$
Molecular Weight: 186.21



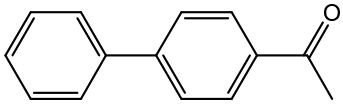
N,N-Dimethyl-1-naphthaleneamine
Chemical Formula: $C_{12}H_{13}N$
Molecular Weight: 171.24



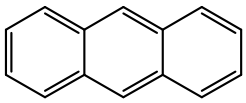
9H-Fluoren-9-one
Chemical Formula: $C_{13}H_8O$
Molecular Weight: 180.21



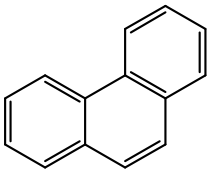
2-Hydroxy-1-naphthoic acid methyl ester
Chemical Formula: $C_{12}H_{10}O_3$
Molecular Weight: 202.21



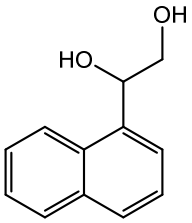
1-[1,1'-Biphenyl]-4-yl-ethanone
Chemical Formula: $C_{14}H_{12}O$
Molecular Weight: 196.25



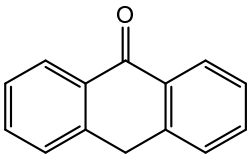
Anthracene
Chemical Formula: $C_{14}H_{10}$
Molecular Weight: 178.23



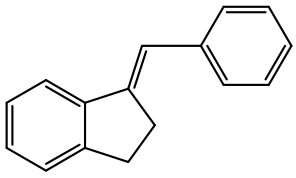
Phenanthrene
Chemical Formula: $C_{14}H_{10}$
Molecular Weight: 178.23



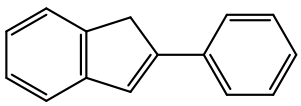
1-(1-Naphthyl)-1,2-ethanediol
Chemical Formula: $C_{12}H_{12}O_2$
Molecular Weight: 188.23



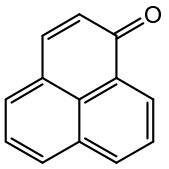
Anthrone
Chemical Formula: $C_{14}H_{10}O$
Molecular Weight: 194.23



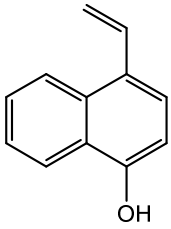
1-(phenylmethylene)indene
Chemical Formula: $C_{16}H_{14}$
Molecular Weight: 206.29



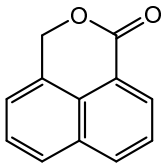
2-Phenyl-1H-indene
Chemical Formula: $C_{15}H_{12}$
Molecular Weight: 192.26



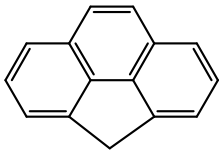
1H-Phenalen-1-one
Chemical Formula: $C_{13}H_8O$
Molecular Weight: 180.21



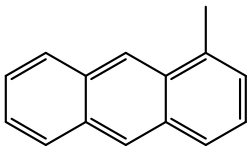
4-Hydroxy-1-naphthaldehyde
Chemical Formula: $C_{12}H_{10}O$
Molecular Weight: 170.21



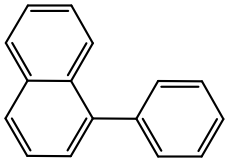
1H,3H-Naphtho[1,8-cd]pyran-1-one
Chemical Formula: $C_{12}H_8O_2$
Molecular Weight: 184.19



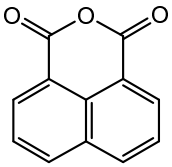
4H-Cyclopenta[def]phenanthrene
Chemical Formula: $C_{15}H_{10}$
Molecular Weight: 190.25



1-Methylanthracene
Chemical Formula: $C_{15}H_{12}$
Molecular Weight: 192.26

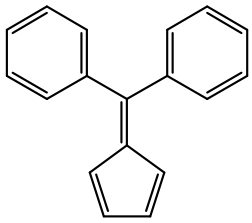


1-Phenyl-naphthalene
Chemical Formula: $C_{16}H_{12}$
Molecular Weight: 204.27

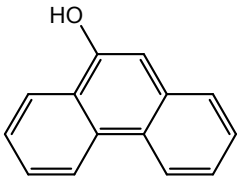


Naphthalic anhydride
Chemical Formula: $C_{12}H_6O_3$
Molecular Weight: 198.18

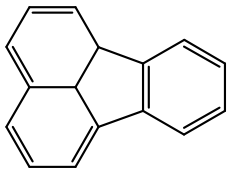
Figure 38 continued



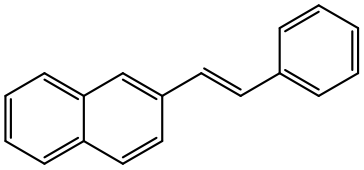
6,6-Diphenylfulvene
Chemical Formula: $C_{18}H_{14}$
Molecular Weight: 230.31



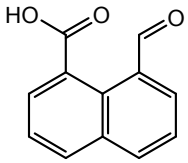
9-Phenanthrol
Chemical Formula: $C_{14}H_{10}O$
Molecular Weight: 194.23



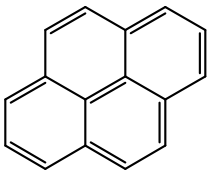
Fluoranthene
Chemical Formula: $C_{16}H_{12}$
Molecular Weight: 204.27



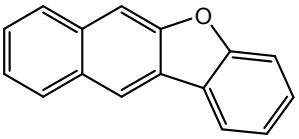
2-Styrylnaphthalene
Chemical Formula: $C_{18}H_{14}$
Molecular Weight: 230.31



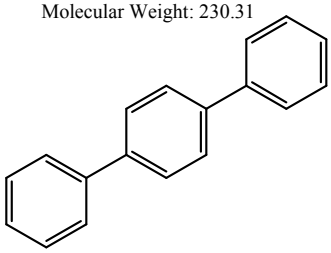
8-Formyl-1-naphthalene carboxylic acid
Chemical Formula: $C_{12}H_8O_3$
Molecular Weight: 200.19



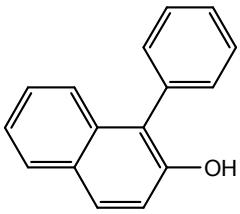
Pyrene
Chemical Formula: $C_{16}H_{10}$
Molecular Weight: 202.26



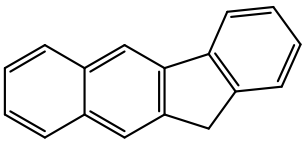
Benzo[b]naphtho[2,3-d]furan
Chemical Formula: $C_{16}H_{10}O$
Molecular Weight: 218.26



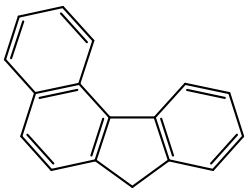
p-Terphenyl
Chemical Formula: $C_{18}H_{14}$
Molecular Weight: 230.31



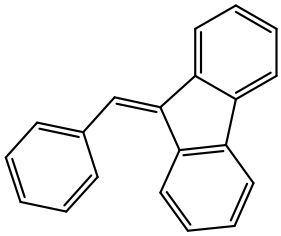
1-Phenyl-2-naphthalenol
Chemical Formula: $C_{16}H_{12}O$
Molecular Weight: 220.27



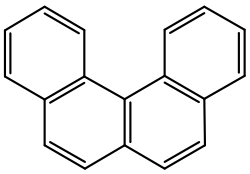
11H-Benzo[b]fluorene
Chemical Formula: $C_{17}H_{12}$
Molecular Weight: 216.28



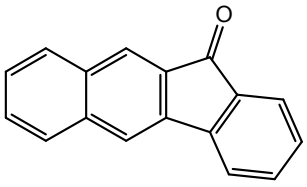
7H-Benzo[c]fluorene
Chemical Formula: $C_{17}H_{12}$
Molecular Weight: 216.28



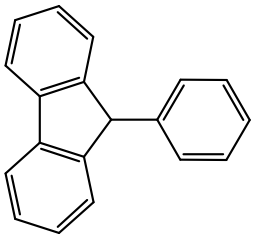
9-(Phenylmethylene) 9H-fluorene
Chemical Formula: $C_{20}H_{14}$
Molecular Weight: 254.33



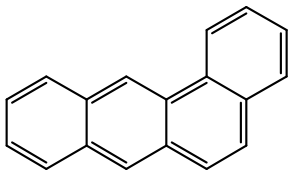
Benzo[c]phenanthrene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



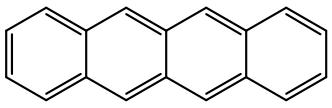
11H-Benzo[b]fluoren-11-one
Chemical Formula: $C_{17}H_{10}O$
Molecular Weight: 230.27



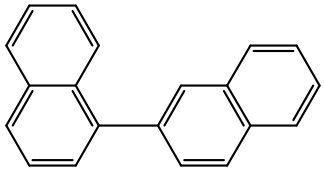
9-Phenyl-9H-fluorene
Chemical Formula: $C_{19}H_{14}$
Molecular Weight: 242.32



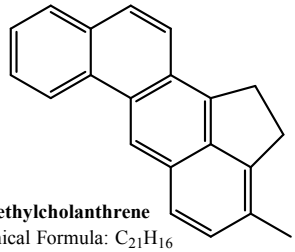
Benz[a]anthracene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



Naphthacene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29

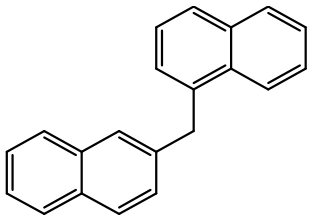


1,2'-Binaphthalene
Chemical Formula: $C_{20}H_{14}$
Molecular Weight: 254.33

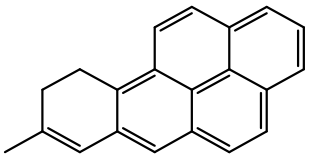


3-Methylcholanthrene
Chemical Formula: $C_{21}H_{16}$
Molecular Weight: 268.36

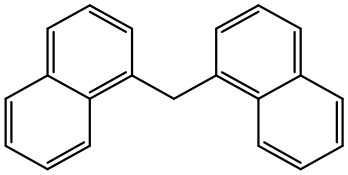
Figure 38 continued



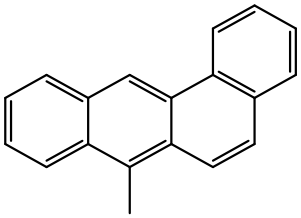
1-(2-naphthalenylmethyl)naphthalene
Chemical Formula: $C_{21}H_{16}$
Molecular Weight: 268.36



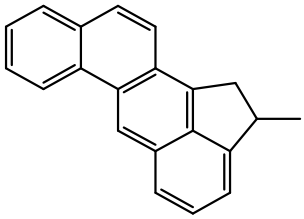
9,10-Dihydro-8-Methylbenzo[a]pyrene
Chemical Formula: $C_{21}H_{16}$
Molecular Weight: 268.36



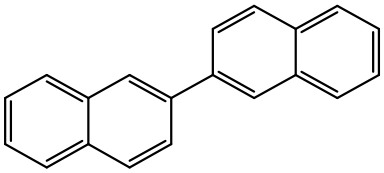
1,1'-methylenebis-Naphthalene
Chemical Formula: $C_{21}H_{16}$
Molecular Weight: 268.36



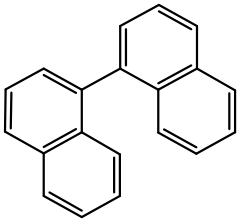
7-Methyl-benz[a]anthracene
Chemical Formula: $C_{19}H_{14}$
Molecular Weight: 242.32



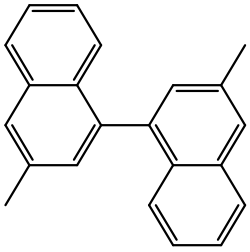
2-Methylcholanthrene
Chemical Formula: $C_{21}H_{16}$
Molecular Weight: 268.36



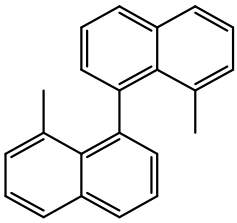
2,2'-Binaphthalene
Chemical Formula: $C_{20}H_{14}$
Molecular Weight: 254.33



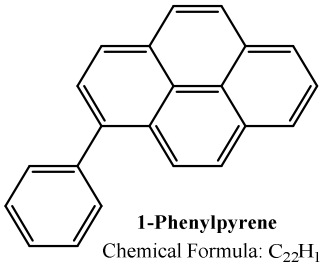
1,1'-Binaphthalene
Chemical Formula: $C_{20}H_{14}$
Molecular Weight: 254.33



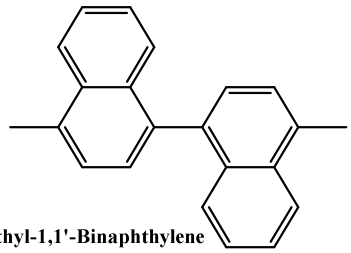
3,3'-Dimethyl-1,1'-Binaphthylene
Chemical Formula: $C_{22}H_{18}$
Molecular Weight: 282.39



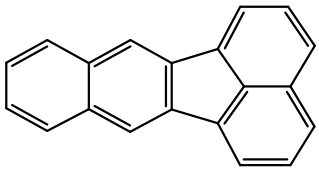
8,8'-Dimethyl-8,8'-Binaphthalene
Chemical Formula: $C_{22}H_{18}$
Molecular Weight: 282.39



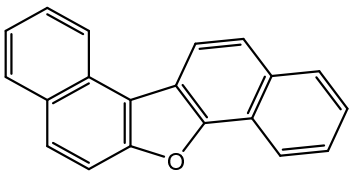
1-Phenylpyrene
Chemical Formula: $C_{22}H_{14}$
Molecular Weight: 278.35



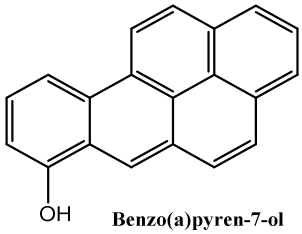
4,4'-Dimethyl-1,1'-Binaphthylene
Chemical Formula: $C_{22}H_{18}$
Molecular Weight: 282.39



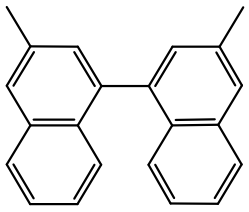
Benzo[k]fluoranthene
Chemical Formula: $C_{20}H_{12}$
Molecular Weight: 252.32



Dinaphtho[1,2-b:1',2'-d]furan
Chemical Formula: $C_{20}H_{12}O$
Molecular Weight: 268.32

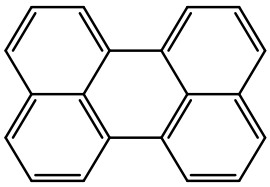


Benzo(a)pyren-7-ol
Chemical Formula: $C_{20}H_{12}O$
Molecular Weight: 268.32



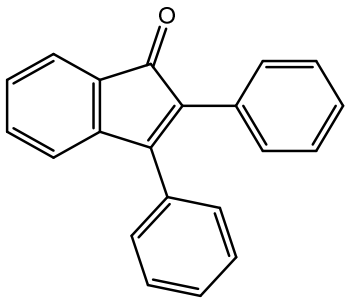
2,2'-Dimethyl-1,1'-Binaphthalene
Chemical Formula: $C_{22}H_{18}$
Molecular Weight: 282.39

Figure 38 continued



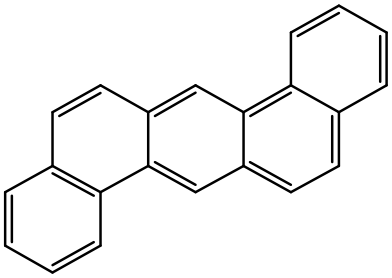
Perylene

Chemical Formula: $C_{20}H_{12}$
Molecular Weight: 252.32



2,3-Diphenyl-1H-Inden-1-one

Chemical Formula: $C_{21}H_{14}O$
Molecular Weight: 282.34



Dibenz[a,h]anthracene

Chemical Formula: $C_{22}H_{14}$
Molecular Weight: 278.35

Figure 38 continued

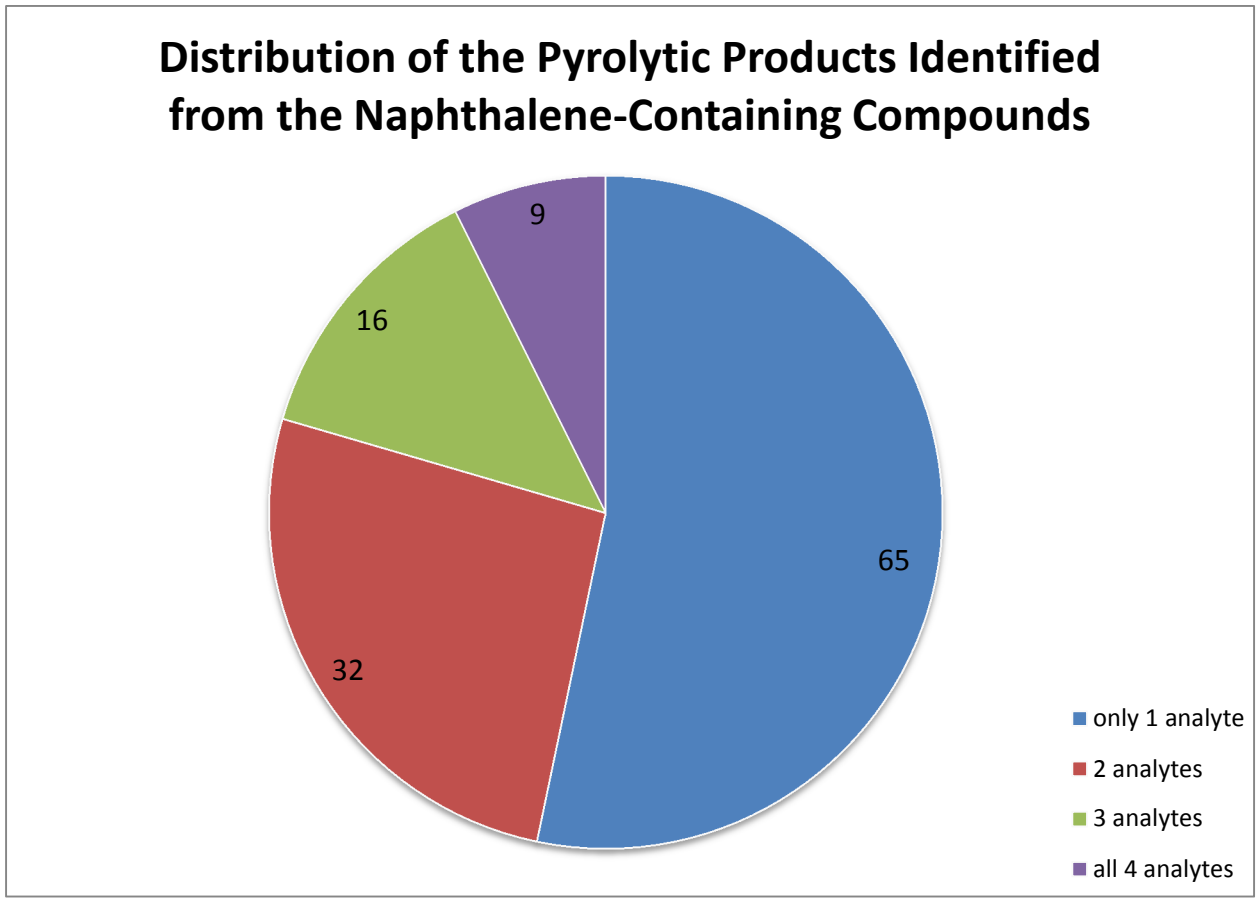


Figure 39: Inter-comparison of pyrolytic products between naphthalene-containing compounds

3.3.3 INDOLE-CONTAINING COMPOUNDS

A total of 111 pyrolytic products were identified from the four indole-containing compounds that were studied (***Table 9*** and ***Figure 40***). As would be expected, there were significantly more nitrogen-containing pyrolytic products; the majority of which were bridged or fused HCAs. Similar to the naphthalene containing compounds, an overwhelming number of products are of greater molecular weight than that of the indole-containing compounds themselves (117, 131, 145, and 187 amu). The greatest number of identifiable pyrolytic products was observed with 3-methylindole (59); followed by indole (44), indole-3-carboxaldehyde (36), and 1-pentylindole (26).

Figure 41 provides the distribution of the 111 pyrolytic products among the extracts of the four analytes and shows that two-thirds (66%) of the pyrolytic products were identified in only one of the indole-containing compounds. Four pyrolytic products were observed in extracts from each of the four analytes: indole, quinoline, benzo(h)quinoline, carbazole, and 9-anthracenececarbonitrile. An additional six products were observed in extracts from three of the four analytes: benzyl nitrile (phenyl-acetonitrile) naphthalene, oxindole, naphthalene-2-carbonitrile, and indole-5-carboxaldehye.

Seventy-three of the total compounds (66%) were only identifiable in an extract from one of the four indole-containing analytes. Four of the pyrolytic products observed are categorized as carcinogens. 2-Naphthylamine is classified by the IARC as known human carcinogen (Group 1). Naphthalene, identified in extracts of three of the four analytes, and carbazole, identified in extracts from all analytes are both Group 2B carcinogens. Fluorene, only identified in the indole extract, is listed in Group 3.

Table 9: Pyrolytic products identified from investigation of the indole-containing compounds

Retention Time (min)	Compound	ID	CG	Indole	3-CH_3 Indole	1-pentyl Indole	Indole-3-Carbox.
3.44	Benzonitrile	T	-	P	S		
4.35	4-Methylbenzonitrile	T	-	P	S		
4.41	o-Aminotoluene	**V**	-	P			
4.95	Benzyl nitrile	T	-	P	P		P
5.07	2-Hydroxybenzonitrile	T	-				P
5.37	Benzoic acid	T	-		P		
5.47	4-Aminostyrene	T	-		P		
5.54	3-Phenyl-2-propenenitrile	T	-		P, S		P
5.76	Naphthalene	**V**	2B	P	P		P
6.14	α-Methylene benzeneacetonitrile	T	-		P		
6.27	Quinoline	**V**	-	P	P	S	P, S
6.50	Isoquinoline	T	-		P	S	
6.58	1-Methylindole	**V**	-		P		
6.73	Indole	**V**	-	W, P, S	P, S	P*, S*	W, P, S
6.82	1-(2-Aminophenyl)ethanone	T	-		W, P, S		
7.01	2-Methylquinoline	**V**	-		P, S	S	
7.27	3-Acetylbenzonitrile	T	-		P		
7.28	Methyl anthranilate	T	-		S		P
7.48	1-Acetylisatin	T	-	S			
7.60	2(1H)-Quinolinone	T	-				W, P, S
7.62	1,3-Dimethylindole	T	-		P		
7.66	2-Methylindole	**V**	-		P, S	P, S	
7.68	3-Methylindole	**V**	-		W		P, S
7.88	2-Methyl-4H-3,1-benzoxazin-4-one	T	-		P, S		
7.98	1-Ethylnaphthalene	**V**	-		S		
8.16	1,2-Dimethylindole	T	-		P		
8.22	(Phenylmethylene)propanedinitrile	T	-	P	P		
8.34	2,4-Dimethylquinoline	T	-			S	
8.37	Oxindole	**V**	-	P	P, S		P, S
8.39	1-Methoxynaphthalene	**V**	-			P, S	P
8.45	2,6-Dimethylindole	T	-		P		

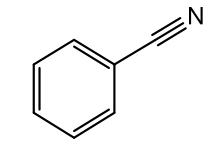
8.54	2,6-Dimethylphenyl isocyanate	T	-		W, S		
8.55	1-Ethylindole	T	-		P	P, S	
8.60	Indole-7-carboxaldehyde	T	-			S	S
8.61	Indole-3-carbonitrile	T	-	P			P
8.63	2,4,6-Trimethylbenzonitrile	T	-		P		
8.64	1,4-Dimethylindole	T	-		P		
8.71	2,3-Dimethylindole	T	-		P	P	
8.72	Naphthalene-1-carbonitrile	T	-			P	
8.75	Quinoline-3-carbonitrile	T	-	P	P		
8.79	Indole-3-acetaldehyde	T	-		P, S		
8.87	1-Naphthalenol	T	-			S	
8.88	(2-Acetylphenyl)formamide	T	-		W, P, S		
8.96	Naphthalene-2-carbonitrile	**V**	-	P	P	P	
8.98	2,4-Di-t-butylphenol	T	-		W, P		
9.01	2,5-Dimethylindole	T	-		P		
9.11	Quinoline-2-carbonitrile	T	-	P	P		
9.12	2-Ethyl-4-methyl-quinoline	T	-		P		
9.24	2-Naphthylamine	**V**	1		P	P, S	
9.27	N-(2-Acetylphenyl)acetamide	T	-		P, S		
9.31	4-Methyl-2(1H)-quinolinone	T	-			S	
9.33	4-Hydroxyindole	T	-	P			
9.37	3-Propylquinoline	T	-			S, P	
9.43	5-Hydroxyindole	T	-	P			
9.44	Indole-5-carbonitrile	T	-		P		P
9.45	1-Propylindole	T	-			P	
9.46	2,6-Dicyanotoluene	T	-	P			
9.69	Fluorene	**V**	3	P			
9.85	7-Methyl-8-quinolinol	T	-		S	W, S	
9.87	1-Pentylindole	**V**	-			W, P, S	
10.07	1-(2'-Methylaminophenyl)-1-pentanone	T	-			P, S	
10.17	Isatin	**V**	-		P		W, P, S
10.33	6-(1-Methylpropyl)quinoline	T	-			P	
10.57	1,3-Diacetylindole	T	-			S*	
10.63	4-(3-Methylbutyl)-1H-indole	T	-			P, S	

10.69	1-Pentyl-3-methylindole	T	-			P*, S*	
11.00	3,5-Dimethyl-2-phenylpiperidin-4-one	T	-			P*, S*	
11.03	Indole-3-acetonitrile	T	-		P		
11.06	Indole-3-carboxaldehyde	**V**	-	P, S			W, S
11.07	Indole-5-carboxaldehyde	T	-		S	S	P
11.29	Benzo(h)quinoline	T	-	P	P	S	P
11.48	Indole-3-carboxylic acid methyl ester	T	-				W, P, S
11.53	Carbazole	T	2B	P	P	P, S	P*
11.61	2-Methyl-indole-3-carboxaldehyde	T	-		P, S		
11.70	1-Methyl-3-acetylindole	T	-		P, S		
11.88	9H-Fluorene-2-carbonitrile	T	-		P		
12.04	Iminostilbene	T	-	P			
12.15	3-Methylcarbazole	T	-			S	
12.22	6-Phenylisoquinoline	T	-	P	P		
12.29	4H-Benzo[def]carbazole	T	-	P	P		
12.35	2-Methylcarbazole	T	-			P, S	
12.47	9-Methylcarbazole	T	-			P, S	
12.85	2-Phenylquinoline	T	-	P	P		
12.87	1-Anthracenamine	T	-	P			
12.95	4-Phenylisoquinoline	T	-	P	P		
13.10	2-Phenylindole	T	-	P	P		
13.24	3-Phenylindole	T	-	P		P	
13.27	Acenaphtho(1,2-b)pyridine	T	-				P
13.32	2-Methyl-benzo[f]quinoline	T	-	P			
13.38	Indeno(1,2,3-ij)isoquinoline	T	-	P			
13.43	9-Anthracenecarbonitrile	T	-	P	P		P
13.46	9-(Cyanomethylene)fluorene	T	-	P			
13.62	[1,1'-Biphenyl]-2,2'-dicarbonitrile	T	-	P			
14.59	Benzo(a)phenazine	T	-	P			
14.82	9-(Dicyanomethylene)fluorene	T	-	P			
14.83	7H-Benzo(c)carbazole	T	-	P			
15.17	4,4'-Dimethoxydiphenylamine	T	-			S	
15.55	2-(3-Isoquinolinyl)benzonitrile	T	-				P
15.62	4H-Benzo[e]pyrido[1,2-a]benzimidazole	T	-	P			P

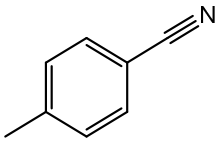
15.66	11H-Benzo[a]carbazole	T	-			S	
15.75	Benzo(b)phenazine	T	-	P, S			P
15.79	Dibenzo[c,h][2,6]naphthyridine	T	-				S
15.93	Benzo(b)carbazole	T	-			P	
16.11	Benzo[b]naphtho[2,3-d]furan	T	-		P		
16.13	1H-Phenanthro[9,10-c]pyrazole	T	-	P			
16.22	1H-Phenanthro[9,10-d]imidazole	T	-	P			
16.36	2-Methyl-1H-indeno[1,2-b]quinoxaline	T	-	P			
16.91	7-Methyl benz[a]anthracene	T	-			P	
17.23	1-(1H-Inden-2-yl)-1H-benzimidazole	T	-	P			
17.43	2-Phenyl-5H-imidazo(2,1-a)isoindole	T	-	P			
20.03	6,6'-Biquinoline	T	-		P		
20.75	2,2'-Biquinoline	T	-		P		

Identification (ID) refers to whether the pyrolytic product was tentatively identified, T, or verified with a reference material, **V**. The carcinogenic group (CG) indicates the IARC classification to which the analyte belongs [1, 2A, 2B, 3, or 4]. The extract in which the pyrolytic product is indicated with a single capital letter representative of each of the three extracts: W (wool), P (pipette), and S (solvent layer).

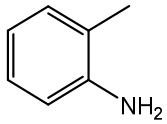
*****Observed as impurities in the analytical standard that was pyrolyzed and therefore may not be a true pyrolytic product



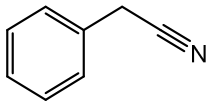
Benzonitrile
Chemical Formula: C_7H_5N
Molecular Weight: 103.12



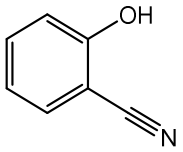
4-Methylbenzonitrile
Chemical Formula: C_8H_7N
Molecular Weight: 117.15



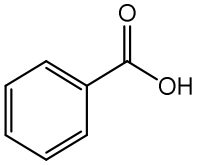
o-Aminotoluene
Chemical Formula: C_7H_9N
Molecular Weight: 107.16



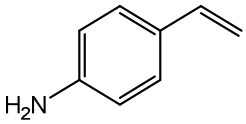
Benzyl Nitrile
Chemical Formula: C_8H_7N
Molecular Weight: 117.15



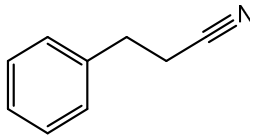
2-Hydroxybenzonitrile
Chemical Formula: C_7H_5NO
Molecular Weight: 119.12



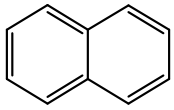
Benzoic acid
Chemical Formula: $C_7H_6O_2$
Molecular Weight: 122.12



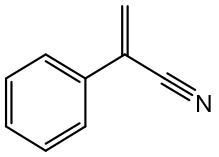
4-Aminostyrene
Chemical Formula: C_8H_9N
Molecular Weight: 119.17



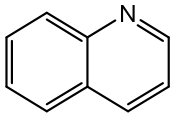
3-Phenyl-2-propenenitrile
Chemical Formula: C_9H_9N
Molecular Weight: 131.18



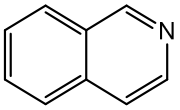
Naphthalene
Chemical Formula: $C_{10}H_8$
Molecular Weight: 128.17



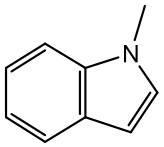
alpha-Methylene benzeneacetonitrile
Chemical Formula: C_9H_7N
Molecular Weight: 129.16



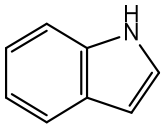
Quinoline
Chemical Formula: C_9H_7N
Molecular Weight: 129.16



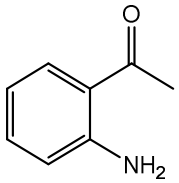
Isoquinoline
Chemical Formula: C_9H_7N
Molecular Weight: 129.16



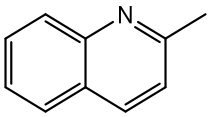
1-Methylindole
Chemical Formula: C_9H_9N
Molecular Weight: 131.18



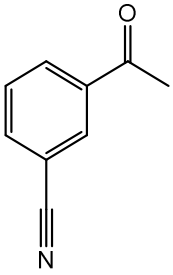
Indole
Chemical Formula: C_8H_7N
Molecular Weight: 117.15



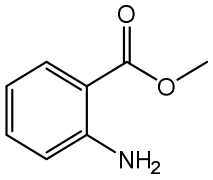
1-(2-aminophenyl)ethanone
Chemical Formula: C_8H_9NO
Molecular Weight: 135.17



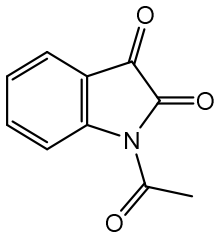
2-Methylquinoline
Chemical Formula: $C_{10}H_9N$
Molecular Weight: 143.19



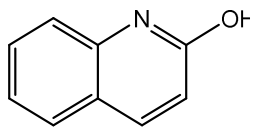
3-Acetylbenzonitrile
Chemical Formula: C_9H_7NO



Methyl anthranilate
Chemical Formula: $C_8H_9NO_2$

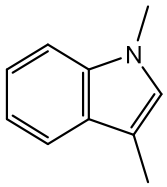


1-Acetylisatin
Chemical Formula: $C_{10}H_7NO_3$

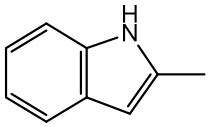


2(1H)-Quinolinone
Chemical Formula: C_9H_7NO

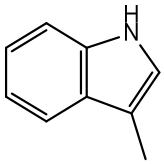
Figure 40: Pyrolytic products identified from indole-containing analytes



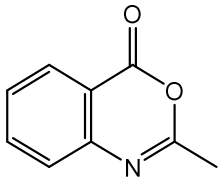
1,3-Dimethylindole
Chemical Formula: $C_{10}H_{11}N$
Molecular Weight: 145.21



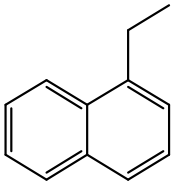
2-Methylindole
Chemical Formula: C_9H_9N
Molecular Weight: 131.18



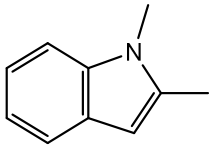
3-Methylindole
Chemical Formula: C_9H_9N
Molecular Weight: 131.18



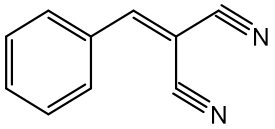
2-Methyl-4H-3,1-benzoxazin-4-one
Chemical Formula: $C_9H_7NO_2$
Molecular Weight: 161.16



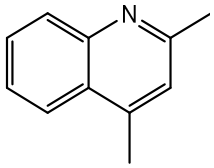
1-Ethylnaphthalene
Chemical Formula: $C_{12}H_{12}$
Molecular Weight: 156.23



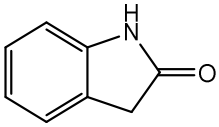
1,2-Dimethylindole
Chemical Formula: $C_{10}H_{11}N$
Molecular Weight: 145.21



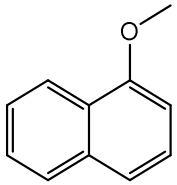
(Phenylmethylene)propanedinitrile
Chemical Formula: $C_{10}H_6N_2$
Molecular Weight: 154.17



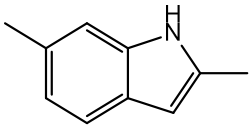
2,4-Dimethylquinoline
Chemical Formula: $C_{11}H_{11}N$
Molecular Weight: 157.22



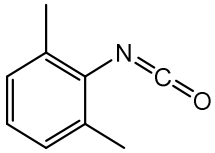
Oxindole
Chemical Formula: C_8H_7NO
Molecular Weight: 133.15



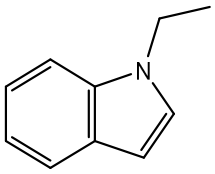
1-Methoxynaphthalene
Chemical Formula: $C_{11}H_{10}O$
Molecular Weight: 158.20



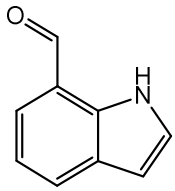
2,6-Dimethylindole
Chemical Formula: $C_{10}H_{11}N$
Molecular Weight: 145.21



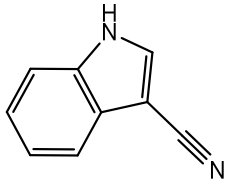
2,6-Dimethylphenyl isocyanate
Chemical Formula: C_9H_9NO
Molecular Weight: 147.18



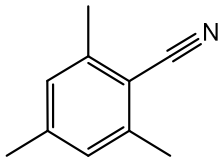
1-Ethylindole
Chemical Formula: $C_{10}H_{11}N$
Molecular Weight: 145.21



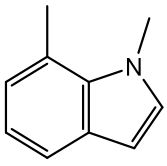
Indole-7-carboxaldehyde
Chemical Formula: C_9H_7NO
Molecular Weight: 145.16



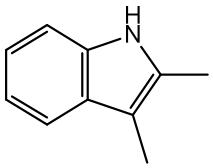
Indole-3-carbonitrile
Chemical Formula: $C_9H_6N_2$
Molecular Weight: 142.16



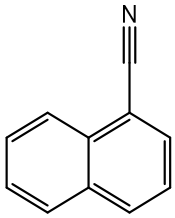
2,4,6-Trimethylbenzonitrile
Chemical Formula: $C_{10}H_{11}N$
Molecular Weight: 145.21



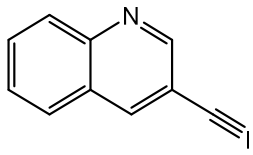
1,4-Dimethylindole
Chemical Formula: $C_{10}H_{11}N$
Molecular Weight: 145.21



2,3-Dimethylindole
Chemical Formula: $C_{10}H_{11}N$
Molecular Weight: 145.21

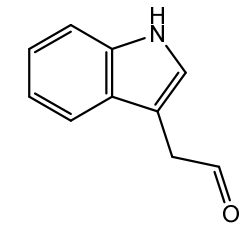


Naphthalene-1-carbonitrile
Chemical Formula: $C_{11}H_7N$
Molecular Weight: 153.18

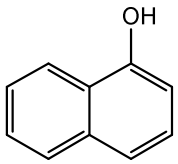


Quinoline-3-carbonitrile
Chemical Formula: $C_{10}H_6N_2$
Molecular Weight: 154.17

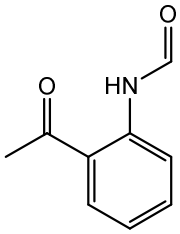
Figure 40 continued



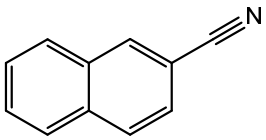
Indole-3-acetaldehyde
Chemical Formula: $C_{10}H_9NO$
Molecular Weight: 159.19



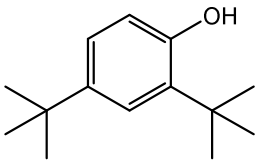
1-Naphthalenol
Chemical Formula: $C_{10}H_8O$
Molecular Weight: 144.17



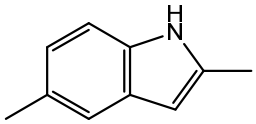
(2-Acetylphenyl)formamide
Chemical Formula: $C_9H_9NO_2$
Molecular Weight: 163.18



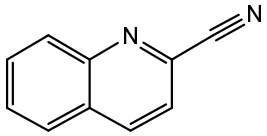
Naphthalene-2-carbonitrile
Chemical Formula: $C_{11}H_7N$
Molecular Weight: 153.18



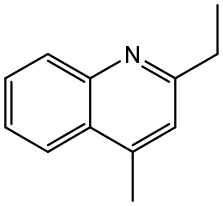
2,4-Di-t-butylphenol
Chemical Formula: $C_{14}H_{22}O$
Molecular Weight: 206.33



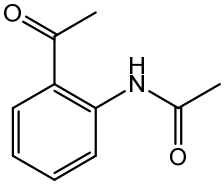
2,5-Dimethylindole
Chemical Formula: $C_{10}H_{11}N$
Molecular Weight: 145.21



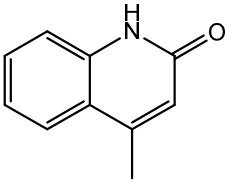
Quinoline-2-carbonitrile
Chemical Formula: $C_{10}H_6N_2$
Molecular Weight: 154.17



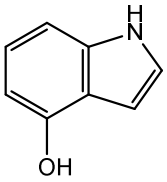
2-Ethyl-4-methyl-quinoline
Chemical Formula: $C_{12}H_{13}N$
Molecular Weight: 171.24



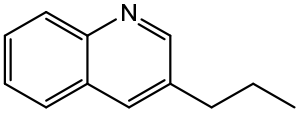
N-(2-Acetylphenyl)acetamide
Chemical Formula: $C_{10}H_{11}NO_2$
Molecular Weight: 177.20



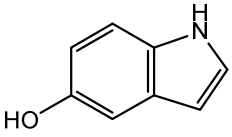
4-Methyl-2(1H)-quinolinone
Chemical Formula: $C_{10}H_9NO$
Molecular Weight: 159.19



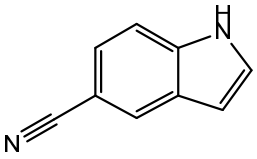
4-Hydroxyindole
Chemical Formula: C_8H_7NO
Molecular Weight: 133.15



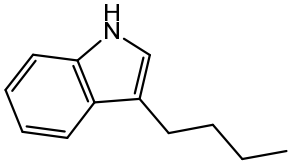
3-Propylquinoline
Chemical Formula: $C_{12}H_{13}N$
Molecular Weight: 171.24



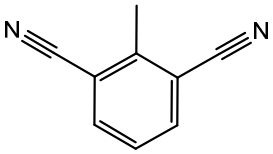
5-Hydroxyindole
Chemical Formula: C_8H_7NO
Molecular Weight: 133.15



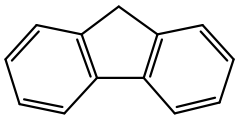
Indole-5-carbonitrile
Chemical Formula: $C_9H_6N_2$
Molecular Weight: 142.16



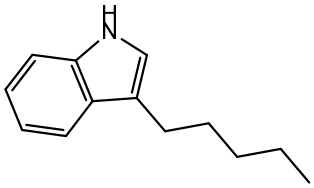
1-Propylindole
Chemical Formula: $C_{12}H_{15}N$
Molecular Weight: 173.26



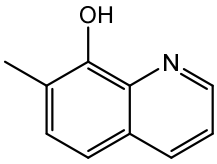
2,6-Dicyanotoluene
Chemical Formula: $C_9H_6N_2$
Molecular Weight: 142.16



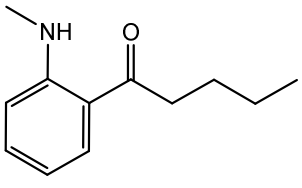
Fluorene
Chemical Formula: $C_{13}H_{10}$
Molecular Weight: 166.22



1-Pentylindole
Chemical Formula: $C_{13}H_{17}N$
Molecular Weight: 187.29

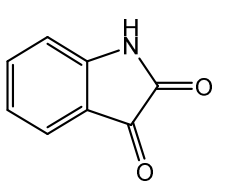


7-Methyl-8-quinolinol
Chemical Formula: $C_{10}H_9NO$
Molecular Weight: 159.19

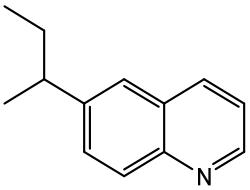


1-(2'-Methylaminophenyl)-1-pentano
Chemical Formula: $C_{12}H_{17}NO$
Molecular Weight: 191.27

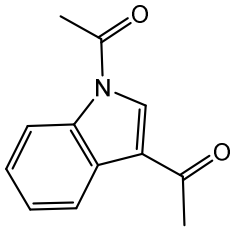
Figure 40 continued



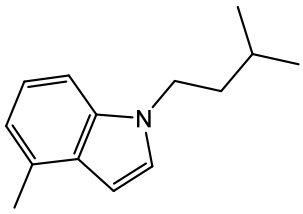
Isatin
Chemical Formula: $C_8H_5NO_2$
Molecular Weight: 147.13



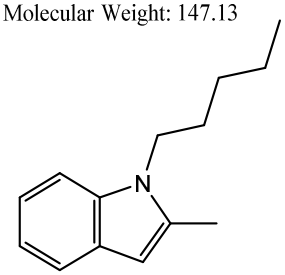
6-(1-Methylpropyl)quinoline
Chemical Formula: $C_{13}H_{15}N$
Molecular Weight: 185.27



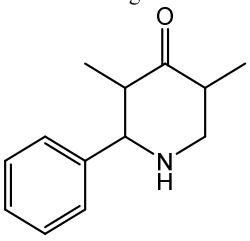
1,3-Diacetylindole
Chemical Formula: $C_{12}H_{11}NO_2$
Molecular Weight: 201.23



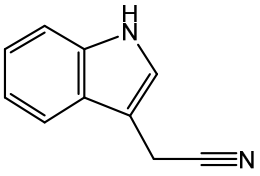
4-(3-Methylbutyl)-1H-indole
Chemical Formula: $C_{14}H_{19}N$
Molecular Weight: 201.31



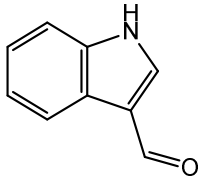
1-Pentyl-2-methylindole
Chemical Formula: $C_{14}H_{19}N$
Molecular Weight: 201.31



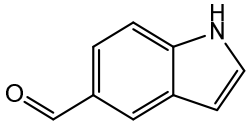
3,5-Dimethyl-2-phenylpiperidin-4-one
Chemical Formula: $C_{13}H_{17}NO$
Molecular Weight: 203.29



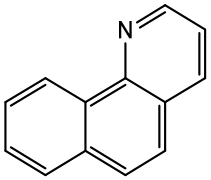
Indole-3-acetonitrile
Chemical Formula: $C_{10}H_8N_2$
Molecular Weight: 156.19



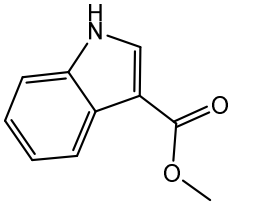
Indole-3-carboxaldehyde
Chemical Formula: C_9H_7NO
Molecular Weight: 145.16



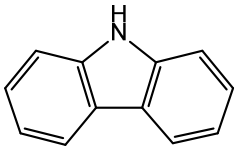
Indole-5-carboxaldehyde
Chemical Formula: C_9H_7NO
Molecular Weight: 145.16



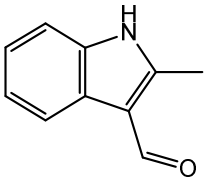
Benzo(h)quinoline
Chemical Formula: $C_{13}H_9N$
Molecular Weight: 179.22



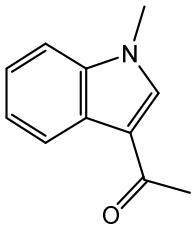
Indole-3-carboxylic acid methyl ester
Chemical Formula: $C_{10}H_9NO_2$
Molecular Weight: 175.19



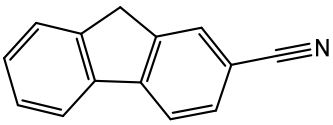
Carbazole
Chemical Formula: $C_{12}H_9N$
Molecular Weight: 167.21



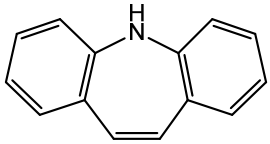
2-Methyl-indole-3-carboxaldehyde
Chemical Formula: $C_{10}H_9NO$
Molecular Weight: 159.19



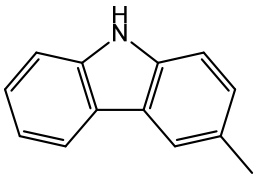
1-Methyl-3-acetylindole
Chemical Formula: $C_{11}H_{11}NO$
Molecular Weight: 173.22



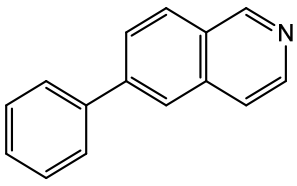
9H-Fluorene-2-carbonitrile
Chemical Formula: $C_{14}H_9N$
Molecular Weight: 191.23



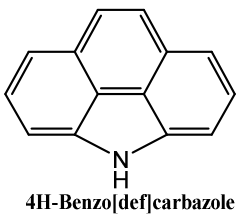
Iminostilbene
Chemical Formula: $C_{14}H_{11}N$
Molecular Weight: 193.25



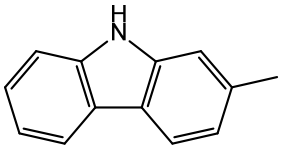
3-Methylcarbazole
Chemical Formula: $C_{13}H_{11}N$
Molecular Weight: 181.24



6-Phenylisoquinoline
Chemical Formula: $C_{15}H_{11}N$
Molecular Weight: 205.26

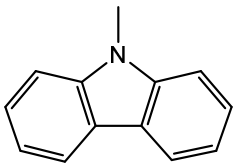


4H-Benzo[def]carbazole
Chemical Formula: $C_{14}H_9N$
Molecular Weight: 191.23

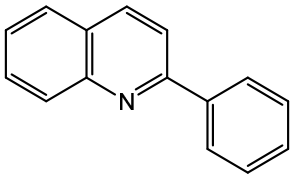


2-Methylcarbazole
Chemical Formula: $C_{13}H_{11}N$
Molecular Weight: 181.24

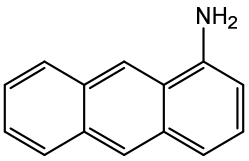
Figure 40 continued



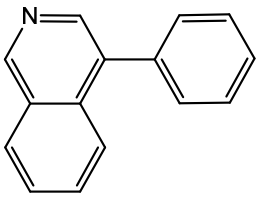
9-Methylcarbazole
Chemical Formula: $C_{13}H_{11}N$
Molecular Weight: 181.24



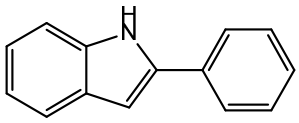
2-Phenylquinoline
Chemical Formula: $C_{15}H_{11}N$
Molecular Weight: 205.26



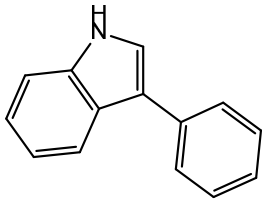
1-Anthracenamine
Chemical Formula: $C_{14}H_{11}N$
Molecular Weight: 193.25



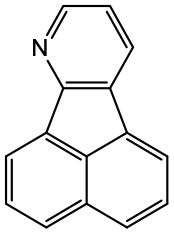
4-Phenylisoquinoline
Chemical Formula: $C_{15}H_{11}N$
Molecular Weight: 205.26



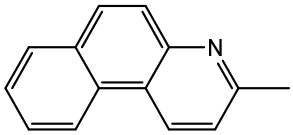
2-Phenylindole
Chemical Formula: $C_{14}H_{11}N$
Molecular Weight: 193.25



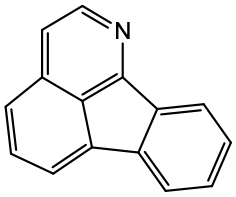
3-Phenylindole
Chemical Formula: $C_{14}H_{11}N$
Molecular Weight: 193.25



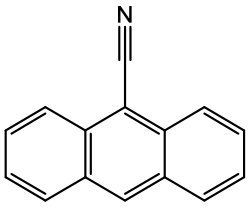
Acenaphtho(1,2-b)pyridine
Chemical Formula: $C_{15}H_9N$
Molecular Weight: 203.24



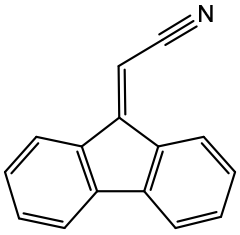
2-Methyl-benzo[f]quinoline
Chemical Formula: $C_{14}H_{11}N$
Molecular Weight: 193.25



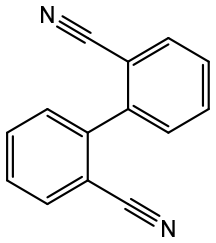
Indeno(1,2,3-ij)isoquinoline
Chemical Formula: $C_{15}H_9N$
Molecular Weight: 203.24



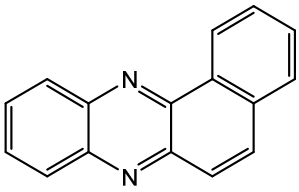
9-Anthracenecarbonitrile
Chemical Formula: $C_{15}H_9N$
Molecular Weight: 203.24



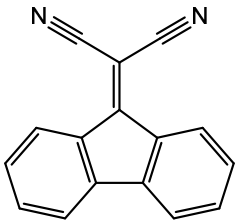
9-(Cyanomethylene)fluorene
Chemical Formula: $C_{15}H_9N$
Molecular Weight: 203.24



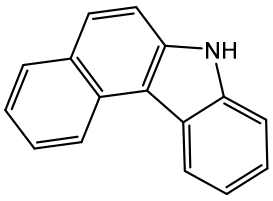
[1,1'-Biphenyl]-2,2'-dicarbonitrile
Chemical Formula: $C_{14}H_8N_2$
Molecular Weight: 204.23



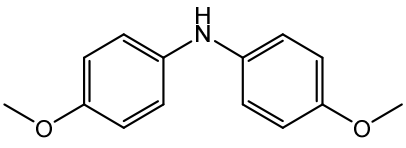
Benzo(a)phenazine
Chemical Formula: $C_{16}H_{10}N_2$
Molecular Weight: 230.27



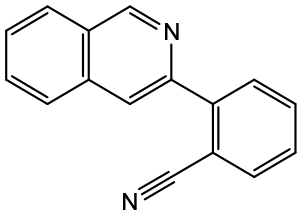
9-(Dicyanomethylene)fluorene
Chemical Formula: $C_{16}H_8N_2$
Molecular Weight: 228.25



7H-Benzo(c)carbazole
Chemical Formula: $C_{16}H_{11}N$
Molecular Weight: 217.27

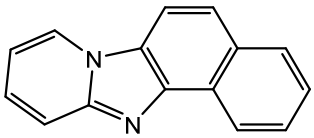


4,4'-Dimethoxydiphenylamine
Chemical Formula: $C_{14}H_{15}NO_2$
Molecular Weight: 229.28

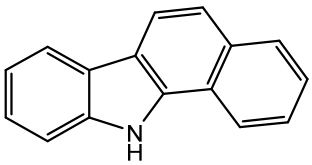


2-(3-Isoquinolinyl)-benzonitrile
Chemical Formula: $C_{16}H_{10}N_2$
Molecular Weight: 230.27

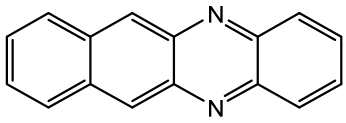
Figure 40 continued



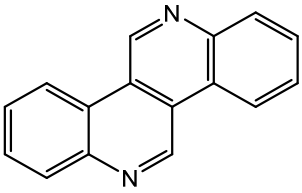
4H-Benzo[e]pyrido[1,2-a]benzimidazole
Chemical Formula: $C_{15}H_{10}N_2$
Molecular Weight: 218.26



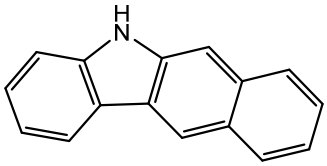
Benzo[a]carbazole
Chemical Formula: $C_{16}H_{11}N$
Molecular Weight: 217.27



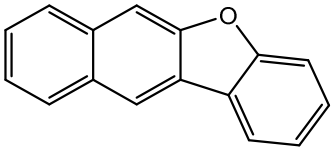
Benzo[b]phenazine
Chemical Formula: $C_{16}H_{10}N_2$
Molecular Weight: 230.27



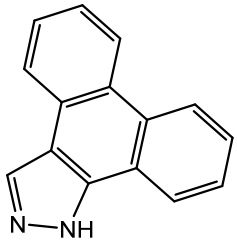
Dibenzo[c,h][2,6]naphthyridine
Chemical Formula: $C_{16}H_{10}N_2$
Molecular Weight: 230.27



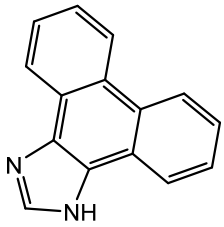
Benzo(b)carbazole
Chemical Formula: $C_{16}H_{11}N$
Molecular Weight: 217.27



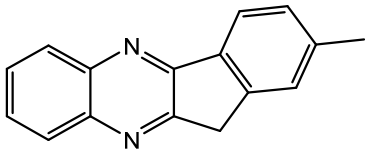
Benzo[b]naphtho[2,3-d]furan
Chemical Formula: $C_{16}H_{10}O$
Molecular Weight: 218.26



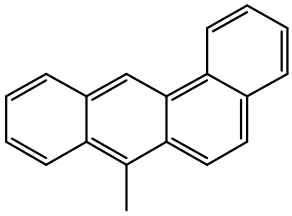
1H-Phenanthro[9,10-c]pyrazole
Chemical Formula: $C_{15}H_{10}N_2$
Molecular Weight: 218.26



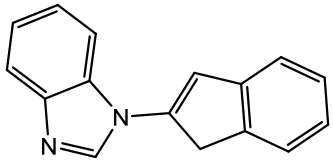
1H-Phenanthro[9,10-d]imidazole
Chemical Formula: $C_{15}H_{10}N_2$
Molecular Weight: 218.26



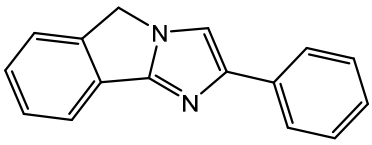
2-Methyl-1H-Indeno[1,2-b]quinoxaline
Chemical Formula: $C_{16}H_{12}N_2$
Molecular Weight: 232.29



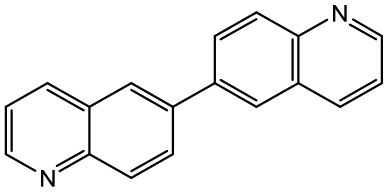
7-Methylbenz[a]anthracene
Chemical Formula: $C_{19}H_{14}$
Molecular Weight: 242.32



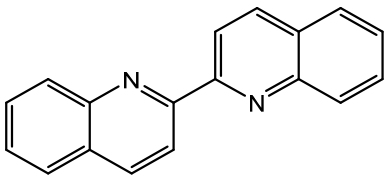
1-(1H-Inden-2-yl)-1H-benzimidazole
Chemical Formula: $C_{16}H_{12}N_2$
Molecular Weight: 232.29



2-Phenyl-5H-Imidazo(2,1-a)isoindole
Chemical Formula: $C_{16}H_{12}N_2$
Molecular Weight: 232.29



6,6'-Biquinoline
Chemical Formula: $C_{18}H_{12}N_2$
Molecular Weight: 256.31



2,2'-Biquinoline
Chemical Formula: $C_{18}H_{12}N_2$
Molecular Weight: 256.31

Figure 40 continued

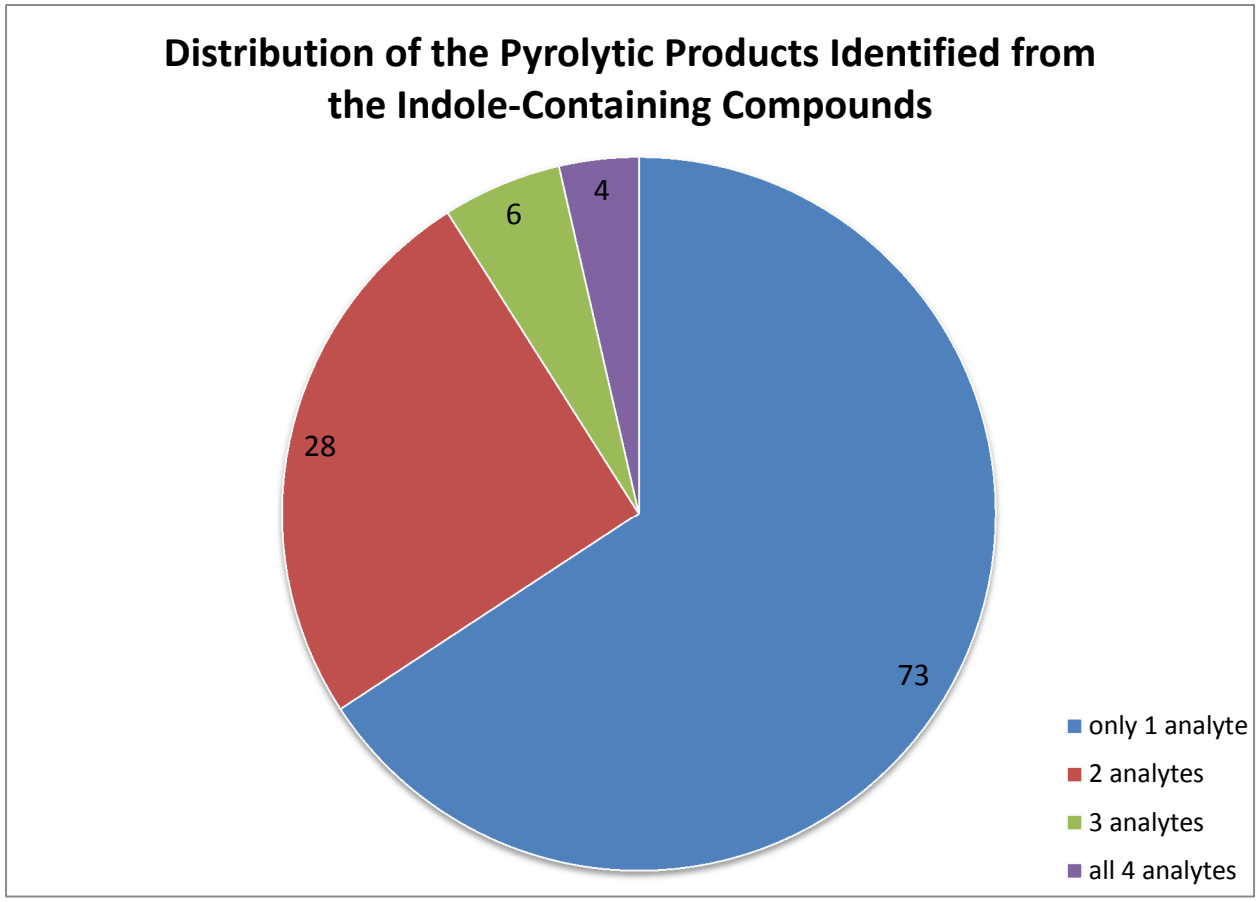


Figure 41: Inter-comparison of pyrolytic products between indole-containing compounds

3.3.4 3-NAPHTHOYLINDOLE

Fifty-nine total products were identified from the pyrolysis of 3-naphthoylindole (***Table 10*** and ***Figure 42***). Numerous pyrolytic products that were identified with 3-naphthoylindole were also observed with the naphthalene and indole-containing analytes; including naphthalene and indole themselves. No pyrolytic products were identified in the wool extract and none of the products were solely identified in solvent layer extract.

Thirty-six products were identified in only the pipette layer, with the remaining twenty-three compounds being identified in both the pipette and solvent layer. Twenty pyrolytic products identified from 3-naphthoylindole were not observed with the pyrolysis of the other analytes. Among these compounds were 4-membered ring systems, such as chrysene, two naphtho-quinoline species, and amino-substituted pyrene, chrysene, and benzo(c)phenanthrene.

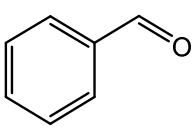
Ten products identified are specifically classified as carcinogens according to IARC. The Group 2B carcinogens 11H-Benzo[b]fluorene and carbazole and the Group 3 carcinogens phenol, acenaphthene, fluorene, and 7H-benzo[c]fluorene were only identified in the pipette layer. The Group 2B carcinogens naphthalene and chrysene, and the Group 3 carcinogens anthracene and fluoranthene, were identified in both the solvent layer and pipette extracts.

Table 10: Pyrolytic products identified from investigation of 3-naphthoylindole

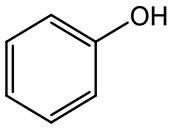
Retention Time (min)	Compound	ID	CG	Naphthoylindole
3.30	Benzaldehyde	T	-	P
3.41	Phenol	T	3	P
4.20	3-Methylphenol	T	-	P
4.26	Indene	T	-	P
4.34	4-Methylbenzonitrile	T	-	P
4.41	o-Aminotoluene	T	-	P
4.94	Benzyl nitrile	T	-	P
5.67	1,2-Naphthalenedione	T	-	P, S
5.80	Naphthalene	**V**	2B	P, S
6.25	Quinoline	**V**	-	P, S
6.73	Indole	**V**	-	P, S
7.13	2-Methylnaphthalene	T	-	P
7.69	2-Methylindole	**V**	-	P
7.87	1,4-Naphthalendione	T	-	P, S
8.10	2-Ethenylnaphthalene	T	-	P
8.34	Oxindole	**V**	-	P, S
8.46	Acenaphthylene	T	-	P, S
8.72	Naphthalene-1-carbonitrile	T	-	P, S
8.80	Acenaphthene	**V**	3	P
8.88	1-Naphthalenol	T	-	P, S
8.93	2-Naphthalenol	T	-	P, S
8.99	2,4-Di-t-butylphenol	T	-	P
9.67	Fluorene	**V**	3	P
9.85	2H-Naphtho[1,8-bc]furan-2-one	T	-	P, S
10.18	Dibenzofuran	T	-	P
10.28	N-Methyl-2-naphthamide	T	-	P
10.33	1-Naphthalenecarboxylic acid	T	-	P
10.86	9H-Fluoren-9-one	T	-	P
11.05	Indole-3-carbonitrile	T	-	P
11.26	Anthracene	**V**	3	P, S
11.54	Carbazole	T	2B	P

11.83	1-(Phenylmethylene)indene	T	-	P, S
12.02	Iminostilbene	T	-	P
12.64	1-Phenylnaphthalene	T	-	P
12.85	1-Anthraceneamine	T	-	P
13.09	2-Phenylindole	T	-	P
13.30	Fluoranthene	**V**	3	P, S
13.71	Benzo[b]naptho[2,3-d]furan	T	-	P
14.27	11H-Benzo[b]fluorene	T	2B	P
14.39	7H-Benzo[c]fluorene	T	3	P
14.57	Chrysene	T	3	P, S
14.63	5,6-Dihydrochrysene	T	-	P, S
15.04	9-(Phenylmethylene)-9H-fluorene	T	-	P, S
15.40	Benz[c]acridine	T	-	P
15.64	Naphtho(2,3-h)quinoline	T	-	P
15.67	11H-Benzo[a]carbazole	T	-	P, S
15.75	Naphthacene	T	-	P
15.81	1,2'-Binaphthalene	T	-	P, S
15.90	7H-Benz[de]anthracen-7-one	T	-	P
15.95	Naphtho(2,1-f)quinoline	T	-	P
16.02	1-Aminopyrene	T	-	P
16.11	N-(1-Naphthoyl)-sarcosine ethyl ester	T	-	P, S
16.21	Benzo(c)phenanthren-2-amine	T	-	P
16.54	Dibenzo(b,def)carbazole	T	-	P, S
16.73	6-Chrysenamine	T	-	P
17.03	Benzo(c)phenanthren-3-amine	T	-	P
17.13	Benzo(c)phenanthren-5-amine	T	-	P, S
17.97	12,13-Dihydro-7H-dibenzo(a,g)carbazole	T	-	P
18.90	3-Naphthoylindole	**V**	-	P, S

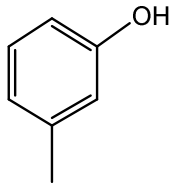
Identification (ID) refers to whether the pyrolytic product was tentatively identified, T, or verified with a reference material, **V**. The carcinogenic group (CG) indicates the IARC classification to which the analyte belongs [1, 2A, 2B, 3, or 4]. The extract in which the pyrolytic product is indicated with a single capital letter representative of each of the three extracts: W (wool), P (pipette), and S (solvent layer).



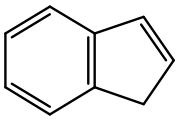
Benzaldehyde
Chemical Formula: C_7H_6O
Molecular Weight: 106.12



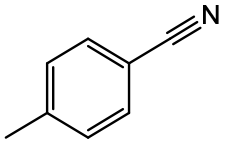
Phenol
Chemical Formula: C_6H_6O
Molecular Weight: 94.11



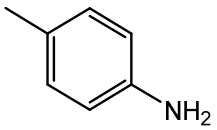
3-Methylphenol
Chemical Formula: C_7H_8O
Molecular Weight: 108.14



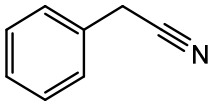
Indene
Chemical Formula: C_9H_8
Molecular Weight: 116.16



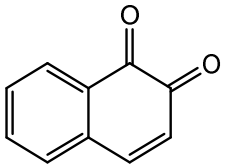
4-Methylbenzonitrile
Chemical Formula: C_8H_7N
Molecular Weight: 117.15



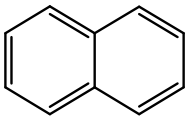
o-Aminotoluene
Chemical Formula: C_7H_9N
Molecular Weight: 107.16



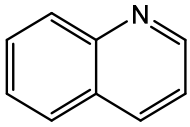
Benzyl Nitrile
Chemical Formula: C_8H_7N
Molecular Weight: 117.15



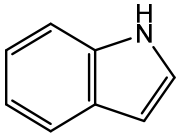
1,2-Naphthalenedione
Chemical Formula: $C_{10}H_6O_2$
Molecular Weight: 158.16



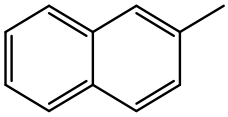
Naphthalene
Chemical Formula: $C_{10}H_8$
Molecular Weight: 128.17



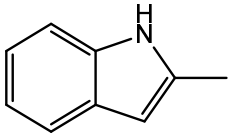
Quinoline
Chemical Formula: C_9H_7N
Molecular Weight: 129.16



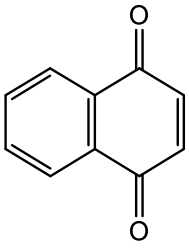
Indole
Chemical Formula: C_8H_7N
Molecular Weight: 117.15



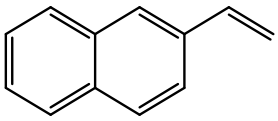
2-Methylnaphthalene
Chemical Formula: $C_{11}H_{10}$
Molecular Weight: 142.20



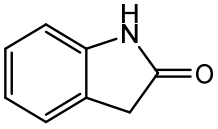
2-Methylindole
Chemical Formula: C_9H_9N
Molecular Weight: 131.18



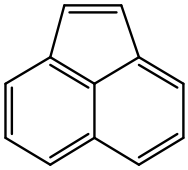
1,4-Naphthalenedione
Chemical Formula: $C_{10}H_6O_2$
Molecular Weight: 158.16



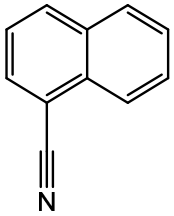
2-Ethenylnaphthalene
Chemical Formula: $C_{12}H_{10}$
Molecular Weight: 154.21



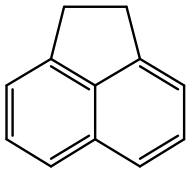
Oxindole
Chemical Formula: C_8H_7NO
Molecular Weight: 133.15



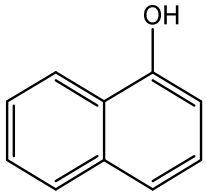
Acenaphthylene
Chemical Formula: $C_{12}H_8$
Molecular Weight: 152.20



Naphthalene-1-carbonitrile
Chemical Formula: $C_{11}H_7N$
Molecular Weight: 153.18

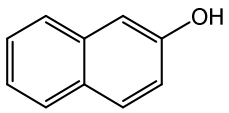


Acenaphthene
Chemical Formula: $C_{12}H_{10}$
Molecular Weight: 154.21

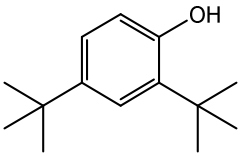


1-Naphthalenol
Chemical Formula: $C_{10}H_8O$
Molecular Weight: 144.17

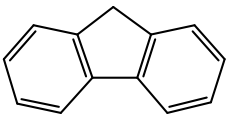
Figure 42: Pyrolytic products identified with 3-naphthoylindole



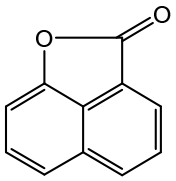
2-Naphthalenol
Chemical Formula: $C_{10}H_8O$
Molecular Weight: 144.17



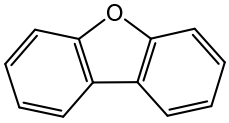
2,4-Di-t-butylphenol
Chemical Formula: $C_{14}H_{22}O$
Molecular Weight: 206.33



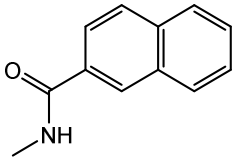
Fluorene
Chemical Formula: $C_{13}H_{10}$
Molecular Weight: 166.22



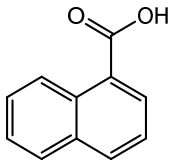
2H-Naphtho[1,8-bc]furan-2-one
Chemical Formula: $C_{11}H_6O_2$
Molecular Weight: 170.17



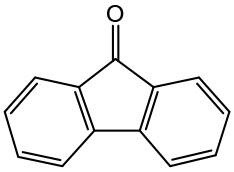
Dibenzofuran
Chemical Formula: $C_{12}H_8O$
Molecular Weight: 168.20



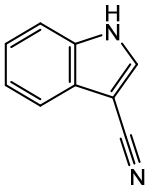
N-Methyl-2-naphthamide
Chemical Formula: $C_{12}H_{11}NO$
Molecular Weight: 185.23



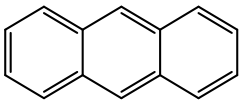
1-Naphthalenecarboxylic acid
Chemical Formula: $C_{11}H_8O_2$
Molecular Weight: 172.18



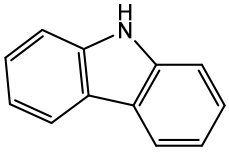
9H-Fluoren-9-one
Chemical Formula: $C_{13}H_8O$
Molecular Weight: 180.21



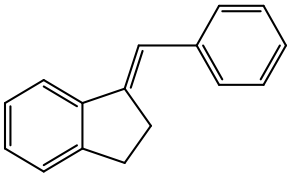
Indole-3-carbonitrile
Chemical Formula: $C_9H_6N_2$
Molecular Weight: 142.16



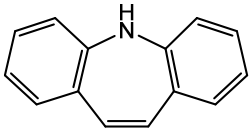
Anthracene
Chemical Formula: $C_{14}H_{10}$
Molecular Weight: 178.23



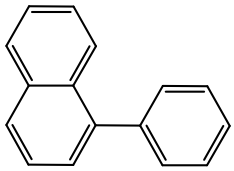
Carbazole
Chemical Formula: $C_{12}H_9N$
Molecular Weight: 167.21



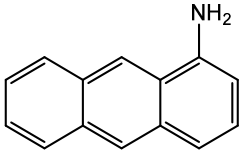
1-(Phenylmethylene)indene
Chemical Formula: $C_{16}H_{14}$
Molecular Weight: 206.29



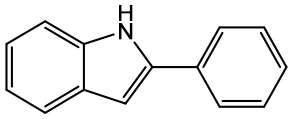
Iminostilbene
Chemical Formula: $C_{14}H_{11}N$
Molecular Weight: 193.25



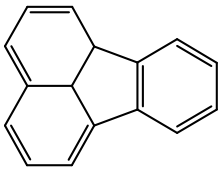
1-Phenylnaphthalene
Chemical Formula: $C_{16}H_{12}$
Molecular Weight: 204.27



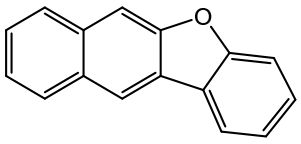
1-Anthracenamine
Chemical Formula: $C_{14}H_{11}N$
Molecular Weight: 193.25



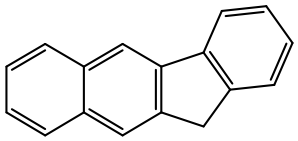
2-Phenylindole
Chemical Formula: $C_{14}H_{11}N$
Molecular Weight: 193.25



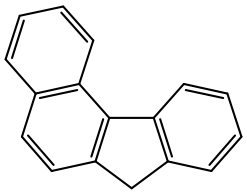
Fluoranthene
Chemical Formula: $C_{16}H_{12}$
Molecular Weight: 204.27



Benzo[b]naphtho[2,3-d]furan
Chemical Formula: $C_{16}H_{10}O$
Molecular Weight: 218.26

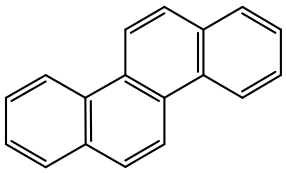


11H-Benzo[b]fluorene
Chemical Formula: $C_{17}H_{12}$
Molecular Weight: 216.28

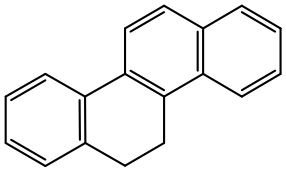


7H-Benzo[c]fluorene
Chemical Formula: $C_{17}H_{12}$
Molecular Weight: 216.28

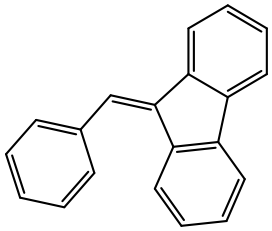
Figure 42 continued



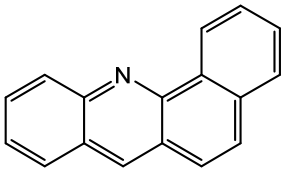
Chrysene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



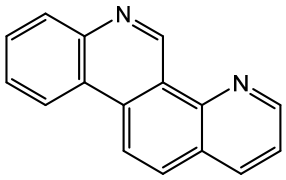
5,6-Dihydrochrysene
Chemical Formula: $C_{18}H_{14}$
Molecular Weight: 230.31



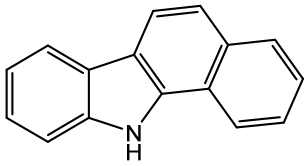
9-(phenylmethylene) 9H-Fluorene
Chemical Formula: $C_{20}H_{14}$
Molecular Weight: 254.33



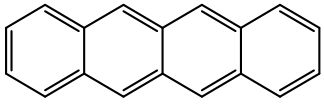
Benz[c]acridine
Chemical Formula: $C_{17}H_{11}N$
Molecular Weight: 229.28



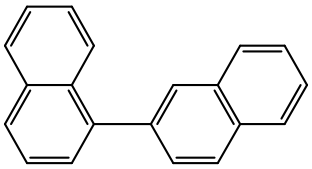
Naphtho(2,3-h)quinoline
Chemical Formula: $C_{16}H_{10}N_2$
Molecular Weight: 230.27



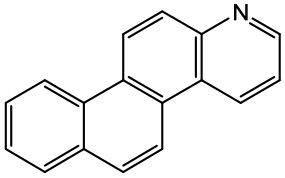
Benzo[a]carbazole
Chemical Formula: $C_{16}H_{11}N$
Molecular Weight: 217.27



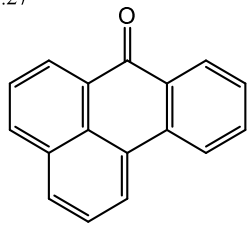
Naphthacene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



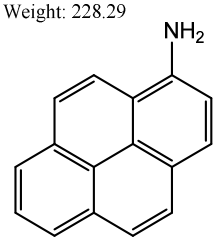
1,2'-Binaphthalene
Chemical Formula: $C_{20}H_{14}$
Molecular Weight: 254.33



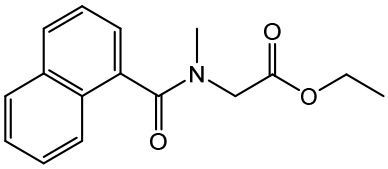
Naphtho(2,1-f)quinoline
Chemical Formula: $C_{17}H_{11}N$
Molecular Weight: 229.28



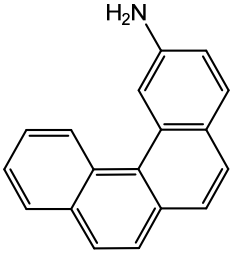
7H-Benz[de]anthracen-7-one
Chemical Formula: $C_{17}H_{10}O$
Molecular Weight: 230.27



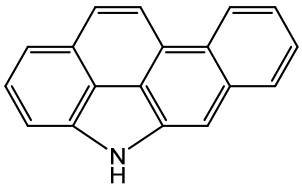
1-Aminopyrene
Chemical Formula: $C_{16}H_{11}N$
Molecular Weight: 217.27



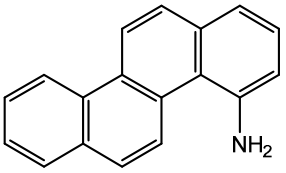
N-(1-Naphthoyl) sarcosine ethyl ester
Chemical Formula: $C_{16}H_{17}NO_3$
Molecular Weight: 271.32



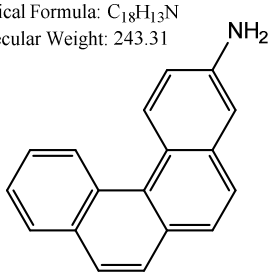
Benzo(c)phenanthren-2-amine
Chemical Formula: $C_{18}H_{13}N$
Molecular Weight: 243.31



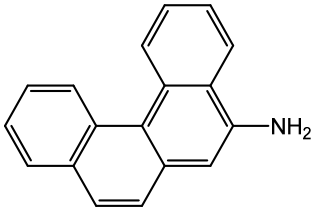
Dibenzo(b,def)carbazole
Chemical Formula: $C_{18}H_{11}N$
Molecular Weight: 241.29



6-Chrysenamine
Chemical Formula: $C_{18}H_{13}N$
Molecular Weight: 243.31

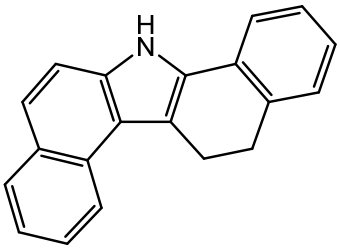


Benzo(c)phenanthren-3-amine
Chemical Formula: $C_{18}H_{13}N$
Molecular Weight: 243.31

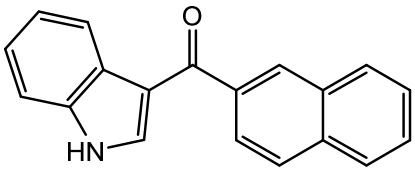


Benzo(c)phenanthren-5-amine
Chemical Formula: $C_{18}H_{13}N$
Molecular Weight: 243.31

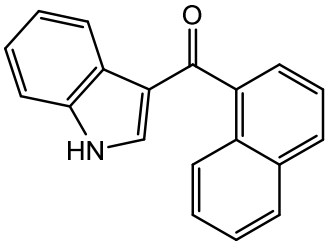
Figure 42 continued



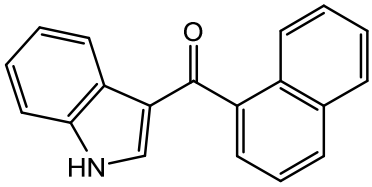
12,13-Dihydro-7H-dibenzo[a,g]carbazole
Chemical Formula: $C_{20}H_{15}N$
Molecular Weight: 269.35



3-(2-Naphthoyl)indole
Chemical Formula: $C_{19}H_{13}NO$
Molecular Weight: 271.32



1'-Naphthoylindole
Chemical Formula: $C_{19}H_{13}NO$
Molecular Weight: 271.32



3-(1-Naphthoyl)indole
Chemical Formula: $C_{19}H_{13}NO$
Molecular Weight: 271.32

Figure 42 continued

3.4.0 CONCLUSION

Given that several classes of synthetic cannabinoids are based upon the same molecular core and similar moieties, it is important to understand the pyrolytic fate of such chemicals. The nine compounds included in this study were purposefully selected as they represent smaller components of each of the four synthetic cannabinoids of interest: JWH-018, JWH-030, JWH-018, and UR-144. By experimentally identifying the products that are formed during the pyrolysis of these smaller components, insight regarding the possibility of generating certain chemicals during the process of smoking synthetic cannabinoids has been provided.

Twenty-one products that were identified are specifically classified as carcinogenic species by the International Agency on Research for Cancer. With synthetic cannabinoids consisting of these very same chemical components that generate carcinogenic or potentially carcinogenic pyrolytic products, there is reason for concern regarding the chronic use of such designer drugs.

Not all the pyrolytic products that were identified have been assessed by the NTP or IARC. The health effects of chronic exposure to a multitude of the pyrolytic products identified should therefore not be overlooked. For instance, although not listed as carcinogens by IARC, the U.S. EPA Integrated Risk Information System Database states there is “suggestive evidence of carcinogenic potential” associated with exposure to biphenyl, with aniline, 3-methylphenol, and quinoline listed as a “probable carcinogens” [94]. Likewise, 3-methylcholanthrene, which was identified in two of the naphthalene-containing compounds, is listed as a Category 1B carcinogen according the Globally Harmonised System of Classification and Labelling of Chemicals (GHS).

The results of this study indicate that a number of concerning PAHs and HCAs can be generated as pyrolytic products of the molecular moieties that make up specific synthetic cannabinoids. This does not necessarily imply that these compounds will be created with the synthetic cannabinoids themselves, but rather serves to provide insight into compounds that may be formed. In the following chapter, the results of the pyrolysis of the synthetic cannabinoids will be discussed and compared to the smaller components investigated in this chapter.

CHAPTER 4: PYROLYSIS OF SYNTHETIC CANNABINOIDS

4.1.0 INTRODUCTION

Since initially being identified in the U.S. in December 2008, synthetic cannabinoids have become one of the largest and most challenging forensic-related drug problems. Domestically, NFLIS indicated that 19,838 drug reports were analyzed between January 2014 and June 2014 involving synthetic cannabinoids [95]. In the European Union, there were 101 previously unreported NPS that were identified in the year 2014 alone; thirty of which were synthetic cannabinoids [96]. With the rapid and continuous influx of new compounds, forensic laboratories have struggled to prevent backlogs from rising. As such, much of the current body of research regarding synthetic cannabinoids has been primarily focused on the development of new methodology to detect the parent drug and/or the primary metabolites.

The lack of scientific articles discussing the toxicity and pharmacology of synthetic cannabinoids is in part hindered by the unavailability of reports detailing what are the pyrolytic products of synthetic cannabinoids. The specific focus of this study was to thoroughly evaluate the products that are generated upon pyrolysis of the synthetic cannabinoids JWH-018, JHW-030, JWH-081, and UR-144. These particular compounds were selected in part due to their prevalence in the beginning generations of synthetic cannabinoids, but more importantly because of the structural similarity to one another. Exhaustive efforts were taken to identify all pyrolytic products for these four compounds with the intent to provide a foundation for which future studies can build upon.

4.2.0 MATERIALS AND METHODS

JWH-018 (solid, purity ≥98.5%, CAS No: 209414-07-3), JWH-081 (solid, purity ≥99%, CAS No: 210179-46-7), and UR-144 (solid, purity ≥ 99%, CAS No: 1199943-44-6) were provided by the DEA Reference Materials Program. JWH-030 (20mg/mL dissolved in methanol, purity ≥98%, CAS No: 162934-73-8) was purchased from Cayman Chemicals (Ann Arbor, Michigan, USA). The molecular structures of these controlled substances can be found in **Figures 13** and **15**, respectively. The amount of synthetic cannabinoid that was pyrolyzed in each trial can be found in **Appendix D**.

4.2.1 EXPERIMENTAL

The pyrolyzed extracts obtained from each of the individual synthetic cannabinoids were analyzed using the same sample preparation, instrument conditions, and method parameters as previously discussed with the non-controlled substances. See **Section 3.2.0** for reference. The same data processing steps were also taken for all pyrolytic products listed in the data tables. Representative pyrograms of the wool, pipette, and solvent layer extracts for JWH-030, JWH-018, JWH-081, and UR-144 have been provided in **Appendix E**, **Appendix G**, **Appendix I**, and **Appendix K**, respectively.

4.3.0 RESULTS AND DISCUSSION

Given the unique structures of synthetic cannabinoids, it was anticipated that not all pyrolytic products would register with the commercially available NIST 2014 spectral database. To avoid elimination of potentially valuable compounds for pyrolytic products that were reproducibly observed and showed fragmentation patterns consistent with those known of synthetic cannabinoids, quality score was neglected. Any compound that was included as a result of the broadened criteria has been omitted from the data

tables and is instead discussed within the text of each respective section with an emphasis placed on proposing possible structures.

4.3.1 PYROLYSIS OF JWH-030

Nineteen pyrolytic products were identified with JWH-030. Reference standards were available to verify four of the reported products (see ***Table 11*** and ***Figure 43***), while the other species were tentatively identified. Possessing a naphthoyl-pyrrole core, it was expected that the pyrolytic products of JWH-030 would correlate with products identified from the naphthalene-containing compounds rather than those from the indole-containing compounds. Eleven of the nineteen JWH-030 products were observed with the naphthalene-containing compounds: naphthalene, 1,4-naphthalenedione, acenaphthylene, 1-naphthalenecarboxaldeyde, 1-naphthalenol, 2-naphthalenol, 1-(1-naphthalenyl)naphthalene, 1-dimethoxynaphthalene, 2-naphthalenecarboxlyic acid methyl ester, anthracene, and 1H-phenalen-1-one. Both naphthalene and anthracene are listed as Group 2B and Group 3 IARC carcinogens and as such, exposure to these compounds should be considered when evaluating the long-term health effects of smoking JWH-030.

Despite not possessing an indole or naphthoyl-indole core, there were some JWH-030 pyrolytic products that were observed with the 3-naphthoylindole and indole-compounds from the previous chapter. Both naphthalene and 1-naphthalenol, which were identified in many of the non-controlled substances, were identified as a result of the pyrolysis of JWH-030. 2H-naphtho[1,8-bc]furan-2-one and 1-naphthalenecarboxylic acid were also tentatively identified with JWH-030. Although these compounds may not necessarily be unique to 3-naphthoylindole, they were exclusive to 3-naphthoylindole in

the context of this study. The identification of the two products suggests that only the naphthoyl-pyrrole functionality, not the entire indole counterpart, is necessary to generate them during pyrolysis. A naphthoyl-pyrrole standard analogous to that of 3-naphthoylindole was unavailable for pyrolytic investigation to help resolve this issue.

Excluding the parent molecule, there were five pyrolytic products identified in the JWH-030 extracts that were not identified with the non-controlled substances: 2-naphthalenecarboxylic acid, 1-naphthamide, 2-phenylindole-3-carboxaldehyde, N-pentyl-2-naphthamide, and (4H)-thebenidinone. Reference materials were not available to verify whether these compounds could potentially be suitable pyromarkers. It is worth noting that the compound tentatively identified at 13.96 minutes as 2-phenylindole-3-carboxaldehyde has the same chemical formula ($C_{15}H_{11}NO$; MW =221.26) as that of 3-(1-naphthoyl)pyrrole but without a reference material this too was unable to be identified in this study.

There were five pyrolytic products consistently observed with appreciable abundance in the pyrograms that were unable to be identified. The m/z 127 and 155 ions, which correspond to the naphthalene and naphthoyl fragments, are routinely observed with the naphthoyl-indole and naphthoyl-pyrrole classes of synthetic cannabinoids. The mass spectrum of the unknown pyrolytic products at 13.15, 13.25, 13.90, 17.23, and 17.51 minutes are provided in **Appendix F**.

The unknowns at 13.15 and 13.25 minutes have nearly identical mass spectra, suggesting that they are isomeric variations of the same compound. In this particular case, the complexity of the spectra, or possible co-elution with another analyte, prevented a possible structure and/or explanation of the fragmentation pathway from

being reported; except the presumed likelihood that the compound contains a naphthoyl-entity. The two unknowns at 13.90 and 17.23 minutes had minimal fragmentation that could be used for structural elucidation, while the unidentified compound at 17.51 minutes was uninterpretable. No decision can be made at this time regarding the potential of these compounds as specific pyromarkers for JWH-030.

Table 11: Pyrolytic products identified from investigation of JWH-030

Retention Time (min)	Compound	ID	CG	JWH-030
5.80	Naphthalene	**V**	2B	P, S
7.88	1,4-Naphthalenedione	T	-	P, S
8.47	Acenaphthylene	T	-	S
8.85	1-Naphthalenecarboxaldehyde	T	-	P, S
8.89	1-Naphthalenol	T	-	P, S
8.95	2-Naphthalenol	T	-	P
9.53	1-(1-Naphthalenyl)ethanone	T	-	P, S
9.86	2H-Naphtho[1,8-bc]furan-2-one	T	-	P, S
9.90	1-Dimethoxylmethylnaphthalene	T	-	P
9.96	2-Naphthalenecarboxylic acid methyl ester	T	-	P, S
10.39	1-Naphthalenecarboxylic acid	T	-	P, S
10.55	2-Naphthalenecarboxylic acid	T	-	P
11.28	Anthracene	**V**	3	P
11.59	1-Naphthamide	T	-	P
12.03	1H-Phenalen-1-one	**V**	-	P, S
13.96	2-Phenylindole-3-carboxaldehyde	T	-	P
14.35	N-Pentyl-2-naphthamide	T	-	P, S
15.45	5(4H)-Thebenidinone	T	-	P
17.00	JWH-030	**V**	-	P, S

Identification (ID) refers to whether the pyrolytic product was tentatively identified, T, or verified with a reference material, **V**. The carcinogenic group (CG) indicates the IARC classification to which the analyte belongs [1, 2A, 2B, 3, or 4]. The extract in which the pyrolytic product is indicated with a single capital letter representative of each of the three extracts: W (wool), P (pipette), and S (solvent layer).

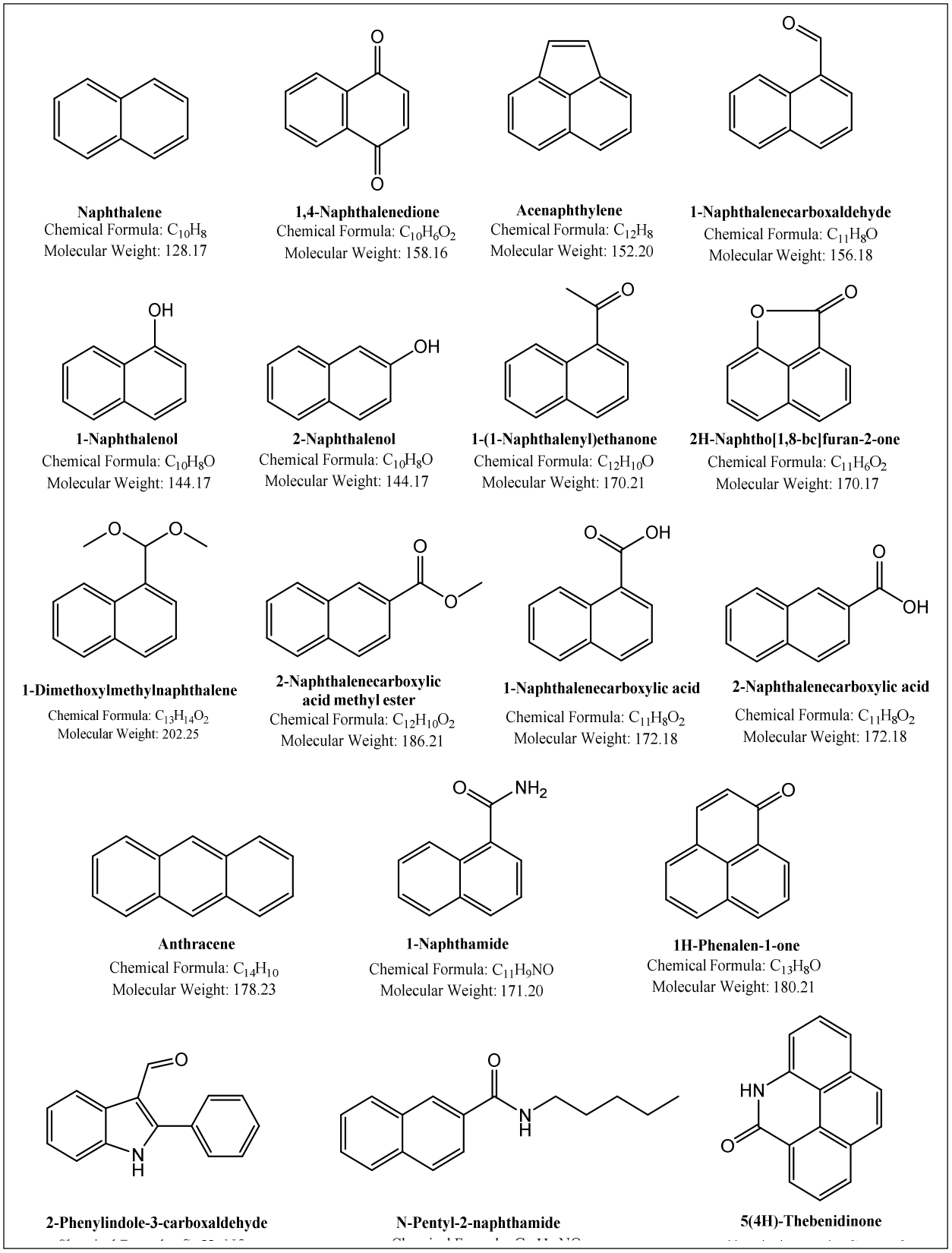
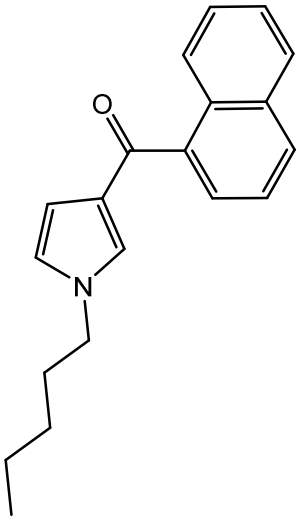


Figure 43: Proposed pyrolytic products of JWH-030



JWH-030
Chemical Formula: $C_{20}H_{21}NO$
Molecular Weight: 291.39

Figure 43 continued

4.3.2 PYROLYSIS OF JWH-018

The presence of an additional benzene ring is the only structural difference between JHW-018 (indole) and JWH-030 (pyrrole). This minor but significant structural appendage was expected to have a profound effect on the type of products observed with JWH-018 than were noticed with JWH-030. From a broad perspective, it was expected that a greater proportion of products identified with 3-naphthoylindole and indole compounds would be present because of the indole, and consequently naphthoyl-indole, moiety that JWH-018 does possess. Overall, there were fifty-two pyrolytic products identified with JWH-018 (see ***Table 12*** and ***Figure 44***).

Nineteen of the fifty-two products identified with JWH-018 were also observed in the previous chapter with the indole-compounds (not independent of other analytes pyrolyzed); representing a proportionally greater amount compared to that of JWH-030. Seven JWH-018 pyrolytic products (isoquinoline, 1-(2-aminophenyl)ethanone, 2(1H)-quinolinone, 1-pentylindole, indole-3-carboxaldehyde, and both 2- and 3-methylcarbazole) were found exclusively in the indole extracts. Another six compounds (1,2-naphthalenedione, naphthalene-1-carbonitrile, chrysene, N-(1-naphthoyl)-sarcosine ethyl ether and dibenzo(b,def)carbazole) were exclusively identified in the 3-naphthoyldinole extracts. There does not appear to be significant enough difference between the products that are observed with JWH-018 and JWH-030 and the different moieties that they contain.

There were five JWH-018 pyrolytic products (4-isocyanatoacetophenone, 5- and 7-hydroxy-1-naphthalenecarbonitrile, 1-naphthoic acid 3-methylbutyl ester, and benz[a]anthracene-7-carbonitrile) that were not observed with the non-controlled substances. The utility that the two hydroxy-1-naphthalenecarbonitrile species and 1-

naphthoic acid 3-methylbutyl ester may have as pyromarkers of the synthetic cannabinoid JWH-018 is uncertain. Although none of these were identified with the other three synthetic cannabinoids and vice versa, the hydroxylated naphthalene species may consequently be a metabolite and esters are rapidly hydrolyzed within the body and would likely not be detectable for a needed duration. Additionally, the synthetic cannabinoid JWH-071 (ethyl-analog of JWH-018) was identified as a product. Although not suitable as a pyromarker, the identification of another synthetic cannabinoid suggests that pyrolysis may consequently result in an individual being exposed to multiple receptor agonists.

In addition to JWH-071 being identified, there were three pyrolytic products from JHW-018 that were not able to be identified, but contain characteristic m/z ions of 155 and 127 which make them potentially valuable targets (see **Appendix H**). The unknown at 17.05 minutes is second in abundance to only JWH-018 itself, but due to minimal fragmentation it is difficult to predict the identity of this compound. The unknown has a molecular ion of m/z 283, but the intensity of the m/z 155 and 127 ions dominates the spectra. The existence of such fragmentation implies that the connectivity to the naphthoyl core is weak and could possibly suggest that the molecular structure contains a nitrogen atom in an adjacent position.

The unknown pyrolytic product eluting at 17.36 minutes was of considerably less abundance compared to the previous, but is an interesting notion. The spectral databases provided a match to that of 3-naphthoylindole but the retention time is inconsistent with that of the reference material. The unknown does have ions consistent with 3-naphthoylindole but the ratio of the m/z 144 is much different and a noticeable

difference is observed with the m/z 116 ion as well. It is possible that this compound is a 1'-naphthyl or 2-naphthoyl isomer for which a reference material was not available to compare a retention and spectrum with.

The final unknown pyrolytic product which showed ions characteristic of synthetic cannabinoids was observed at 20.78 minutes. The molecular ion of this unknown is believed to be m/z 339 and has reasonable consistency with that of another synthetic cannabinoid, JHW-022. However, a drastic difference in the abundance of the m/z 310 ion is noticed; much greater with the unknown than that of JWH-022. Additionally, there is an appreciably abundant ion at m/z 214 which is not found in JWH-022. This compound is likely to be very structurally similar to JWH-022 but was unable to be definitely characterized in this study.

Table 12: Pyrolytic products identified from investigation of JWH-018

Retention Time (min)	Compound	ID	CG	JWH-018
3.42	Phenol	T	3	P
4.19	3-Methylphenol	T	-	P
4.95	Benzyl nitrile	T	-	P
5.61	3-Phenyl-2-propenenitrile	T	-	P
5.68	1,2-Naphthalenedione	T	-	P
5.80	Naphthalene	**V**	2B	P
6.26	Quinoline	**V**	-	P, S
6.50	Isoquinoline	T	-	P
6.84	1-(2-Aminophenyl)ethanone	T	-	P
6.99	1-Methylnaphthalene	**V**	-	P
6.73	Indole	**V**	-	S
7.02	2-Methylquinoline	T	-	P
7.16	2-Methylnaphthalene	T	-	P
7.49	4-Isocyanatoacetophenon	T	-	W, S
7.53	3-Methylquinoline	**V**	-	P
7.63	2(1H)-Quinolinone	T	-	P
7.88	1,4-Naphthalenedione	T	-	P
8.01	1-Ethylnaphthalene	**V**	-	P
8.13	2-Ethenylnaphthalene	T	-	P
8.34	Oxindole	**V**	-	P
8.48	Acenaphthylene	T	-	P
8.75	Naphthalene-1-carbonitrile	T	-	P
8.80	Acenaphthene	**V**	-	P
8.86	1-Naphthalenecarboxaldehyde	T	-	P
8.89	1-Naphthalenol	T	-	P
9.28	1(2H)-Acenaphthylenone	T	-	P
9.87	1-Pentylindole	**V**	-	P, S
9.94	2-Naphthalenecarboxylic acid methyl ester	T	-	S
10.05	5-Hydroxy-1-naphthalenecarbonitrile	T	-	P
10.18	Dibenzofuran	T	-	P
10.44	7-Hydroxy-1-naphthalenecarbonitrile	T	-	P

11.05	Indole-3-carboxaldehyde	**V**	-	P
11.27	Anthracene	**V**	3	P
11.55	Carbazole	T	-	P
11.83	1-(Phenylmethylene)indene	T	-	P
12.05	1H-Phenalen-1-one	**V**	-	P
12.17	3-Methylcarbazole	T	-	P
12.37	2-Methylcarbazole	T	-	P
12.67	1-Phenylnaphthalene	T	-	P
12.92	3-Methylbutyl ester 1-naphthoic acid	T	-	P
13.32	Fluoranthene	**V**	3	P
14.29	11H-Benzo[b]fluorene	T	3	P
14.60	Chrysene	T	3	P
15.69	11H-Benzo[a]carbazole	T	-	P
15.75	Naphthacene	T	-	P
15.83	1,2'-Binaphthalene	T	-	P
16.11	N-(1-Naphthoyl)-sarcosine ethyl ester	T	-	P, S
16.57	Dibenzo(b,def)carbazole	T	-	P
17.71	Benz[a]anthrancene-7-carbonitrile	T	-	S
18.48	JWH-071	T	-	P
18.95	3-(1-Naphthoyl)indole	**V**	-	W, P
20.31	JWH-018	**V**	-	W, P, S

Identification (ID) refers to whether the pyrolytic product was tentatively identified, T, or verified with a reference material, **V**. The carcinogenic group (CG) indicates the IARC classification to which the analyte belongs [1, 2A, 2B, 3, or 4]. The extract in which the pyrolytic product is indicated with a single capital letter representative of each of the three extracts: W (wool), P (pipette), and S (solvent layer).

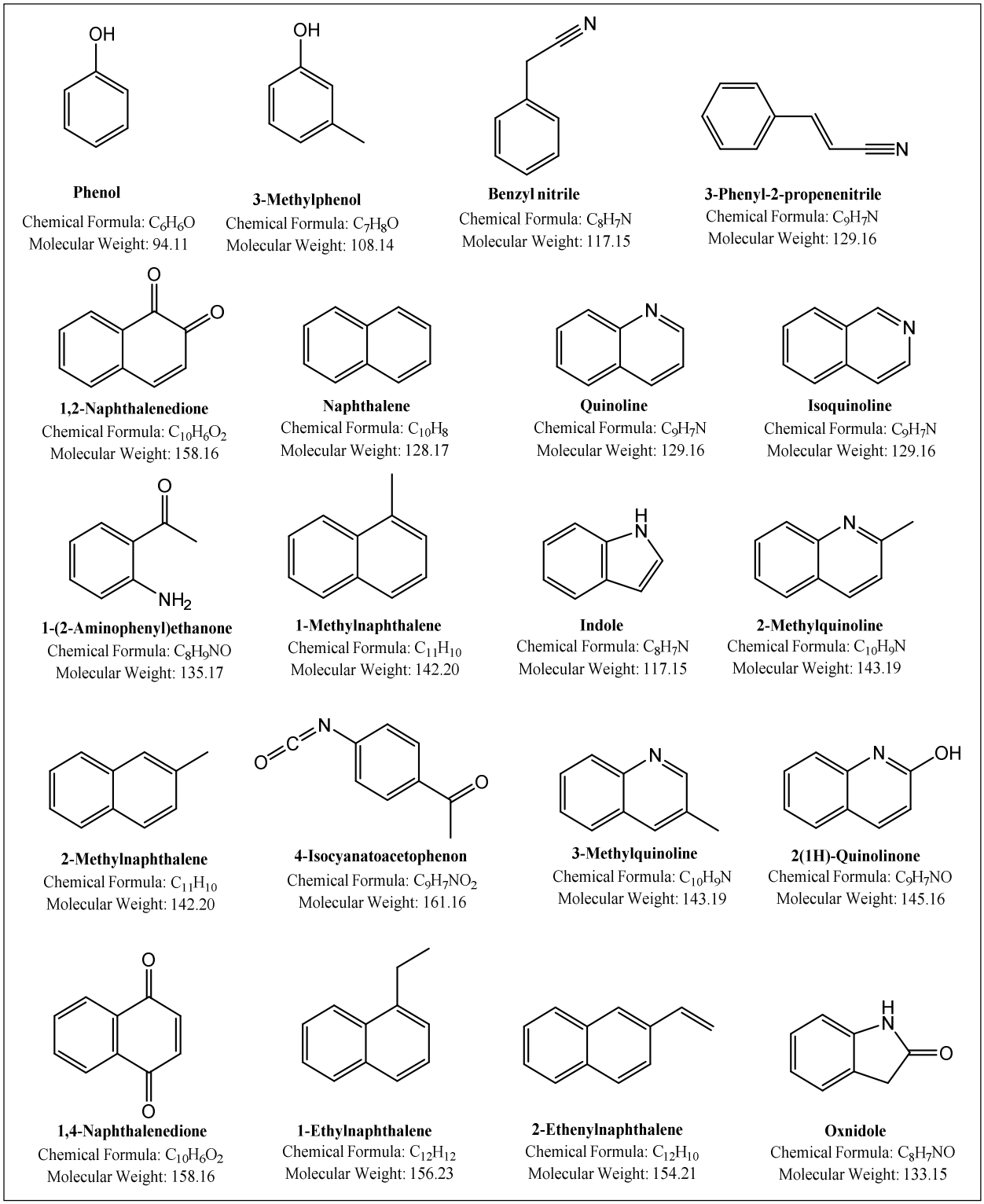


Figure 44: Proposed pyrolytic products of JWH-018

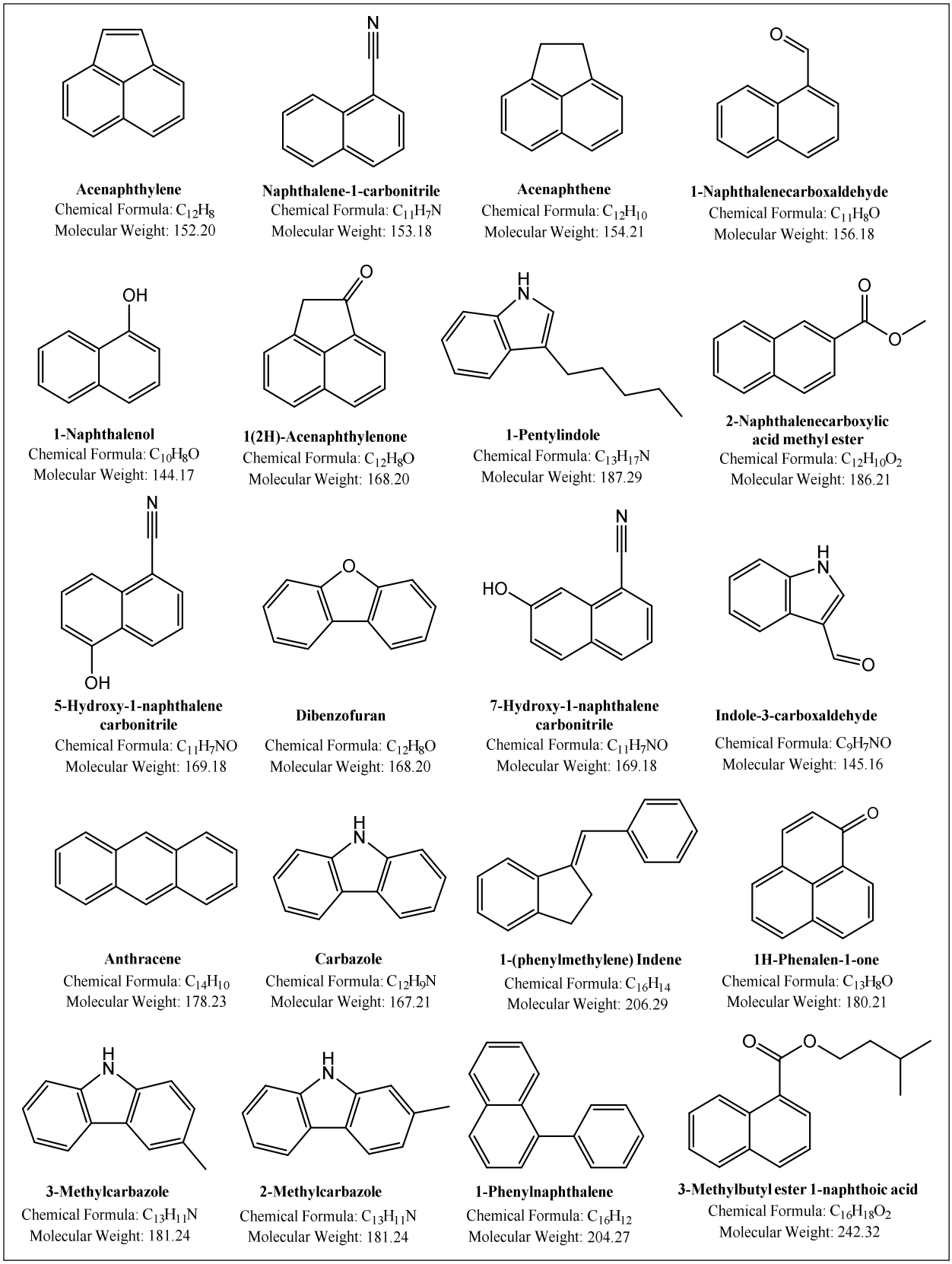
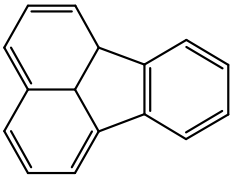
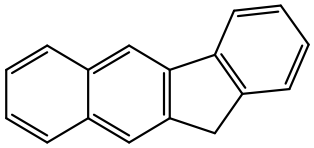


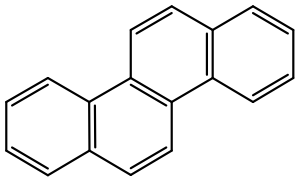
Figure 44 continued



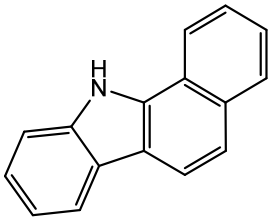
Fluoranthene
Chemical Formula: $C_{16}H_{12}$
Molecular Weight: 204.27



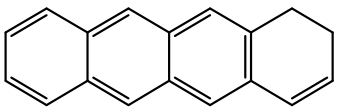
11H-Benzo[b]fluorene
Chemical Formula: $C_{17}H_{12}$
Molecular Weight: 216.28



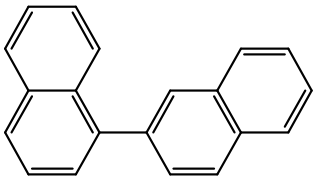
Chrysene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



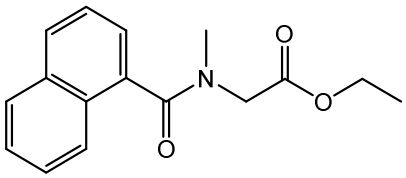
11H-Benzo[a]carbazole
Chemical Formula: $C_{16}H_{11}N$
Molecular Weight: 217.27



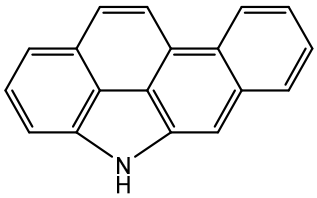
Naphthacene
Chemical Formula: $C_{18}H_{14}$
Molecular Weight: 230.31



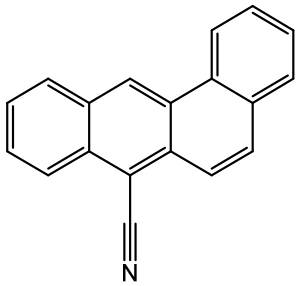
1,2'-Binaphthalene
Chemical Formula: $C_{20}H_{14}$
Molecular Weight: 254.33



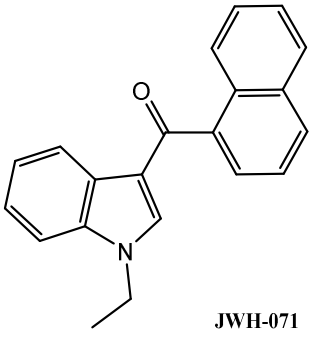
N-(1-Naphthoyl) sarcosine ethyl ester
Chemical Formula: $C_{16}H_{17}NO_3$
Molecular Weight: 271.32



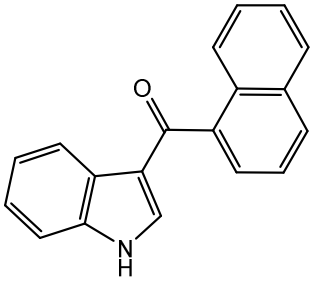
Dibenzo(b,def)carbazole
Chemical Formula: $C_{18}H_{11}N$
Molecular Weight: 241.29



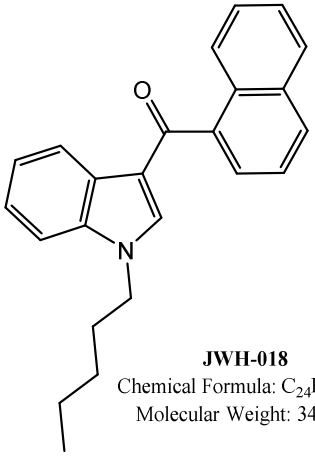
Benz[a]anthracene-7-carbonitrile
Chemical Formula: $C_{19}H_{11}N$
Molecular Weight: 253.30



JWH-071
Chemical Formula: $C_{21}H_{17}NO$
Molecular Weight: 299.37



3-(1-Naphthoyl)indole
Chemical Formula: $C_{19}H_{13}NO$
Molecular Weight: 271.32



JWH-018
Chemical Formula: $C_{24}H_{23}NO$
Molecular Weight: 341.45

Figure 44 continued

4.3.3 PYROLYSIS OF JWH-081

Of the four synthetic cannabinoids that were studied, JWH-081 was the only one with an additional oxygen-atom outside of the carbonyl group. JWH-081 also happens to be the highest molecular weight compound of the group being investigated; structurally differing from JWH-018 only in that it possesses a 4-methoxy-naphthyl substituent where JWH-018 has a hydrogen atom. In the previous chapter, it was determined that the greatest number of identifiable pyrolytic products stemmed from the pyrolysis of 1-methoxynaphthalene (73) and the same trend was observed among the synthetic cannabinoids with sixty-three products being identified with JWH-081 (see ***Table 13*** and ***Figure 45***).

With the methoxy-naphthyl moiety, it was suspected that an increased number of the pyrolytic products from 1-methoxynaphthalene would also be identified in the JWH-081 extracts. However, only five of the thirty-three pyrolytic products that were exclusive to 1-methoxynaphthalene (in ***Table 8***) were identified with JWH-018. This was slightly more than the two (3-methylquinoline and 11H-benzo[b]fluorene) identified in the JWH-018 extracts and there were no such products identified in the JWH-030 extracts. Of these particular compounds, the three products observed with JWH-081 (1,1'-biphenyl-4-carboxaldehyde, 2-hydroxyfluorene, and dinaphtho[1,2-b:1',2'-d]furan) were oxygenated species; unlike the two previously mentioned products.

There were seventeen pyrolytic products identified with JWH-081 that were not observed with any of the non-controlled substances (including 1-methoxynaphthalene). Additionally, the isocyanatoacetophenone species at 7.49 minutes was the only pyrolytic product that was identified in either JWH-018 or JWH-030. After eliminating the compounds that were identified with UR-144, there were twelve compounds exclusively

identified as pyrolytic products of JWH-081: 1- and 3-methylisoquinoline, isoquinoline-1-carbonitrile, 2-ethenylquinoline, 2,4-dimethylquinoline, 3-phenylpyridine, 4-hydroxymethyl-naphthalen-1-one, 1-naphthalenol acetate, 7-methyl-1-naphthalenol, phenanthridine, 9-phenylacridine, and benz(a)acridine.

Similar to the type of products observed with JWH-018, there are a number of chemicals that may potentially cause detrimental health effects. Four particular compounds are classified as Group 2B carcinogens and another six are listed in Group 3. A number of substituted quinoline and isoquinoline species were identified that may be cause for concern given that quinoline is listed as a probable human carcinogen by the NTP. Additionally, JWH-018 was identified in the extracts of JWH-081, indicating that the methoxy-group is susceptible to bond cleavage.

A current review of scientific literature indicates that one article and one abstract have examined the possibility that burning one synthetic cannabinoid can consequently generate additional synthetic cannabinoids [97, 98]. A 2013 study by Donohue *et. al.* showed that the synthetic cannabinoids JWH-018 and JWH-022 (4-pentenyl analog of JWH-018) were identifiable in the charred remains of the synthetic cannabinoid AM-2201 (N-pentyl fluorine analog of JWH-018). This idea was followed up with in a 2014 abstract by RTI International which reported identifying JWH-022 as a pyrolytic product of JWH-018, five products (including JWH-018 and JWH-022) as a result of AM-2201, and JWH-167 as a result of JWH-250. JWH-167 and JWH-250 which belong to the phenylacetyl-indole class of cannabimimetics are analogous to naphthoyl-indoles JWH-018 and JWH-081 and correlate with the observations of this study.

There were four pyrolytic products at 17.06 minutes, 19.05 minutes, 21.54 minutes and 21.74 minutes that could not be identified (see **Appendix H**). The first of these unknowns was previously discussed in the section with JWH-018. A small peak at 19.05 minutes was unable to be structurally elucidated based upon limited fragments. The molecular ion of this unknown is presumed to be the m/z ion at 313, which could suggest a molecular structure consistent with that of JWH-072 (the propyl analog of JWH-018). However, the relative intensity of the molecular ion peak in the unknown is significantly lower than that of JWH-072. The base peak of m/z 185 did not afford much assistance in structural elucidation of this compound and this compound has therefore been included as an unidentified pyrolytic product of interest.

The third and fourth unknown products at 21.54 and 21.74 minutes partially co-eluted with each other. The compound at 21.54 minutes has a prominent molecular ion of m/z 301. The second base peak was m/z 144, which would lead one to believe that an indolyl-moiety is present. A possible molecular structure for this unknown could be a 3-naphthoylindole core with a carboxaldehyde substituent replacing the methoxy-group, but further consideration would need to be taken in structurally elucidating this unknown. The last unknown compound was identified because of a large base peak of m/z 214 and the second most intense ion being m/z 144. The presence of each ion implies that the molecule contains an indolyl-moiety with an attached pentyl-group. However, there was difficulty identifying the substituents of this core without abundant higher m/z ions in the spectra.

Table 13: Pyrolytic products identified during investigation of JHW-081

Retention Time (min)	Compound	ID	CG	JWH-081
6.26	Quinoline	**V**	-	P, S
6.49	Isoquinoline	T	-	P
6.73	Indole	**V**	-	P, S
7.02	2-Methylquinoline	T	-	P
7.36	1-Methylisoquinoline	T	-	P
7.49	3-Isocyanatoacetophenone	T	-	P
7.53	3-Methylquinoline	**V**	-	P
7.68	2-Methylindole	T	-	P
7.71	4-Methylquinoline	T	-	P, S
7.90	1,4-Naphthalenedione	T	-	P, S
8.19	Isoquinoline-1-carbonitrile	T	-	P
8.26	2-Ethenylquinoline	T	-	P
8.36	2,4-Dimethylquinoline	T	-	P
8.63	3-Phenylpyridine	T	-	P
8.66	3-Methylisoquinoline	T	-	P
8.73	Naphthalene-1-carbonitrile	T	-	P
8.87	1-Naphthalenol	T	-	P, S
8.95	Naphthalene-2-carbonitrile	**V**	-	P
9.15	4-Hydroxy-methyl-naphthalen-1-one	T	-	P
9.28	1(2H)-Acenaphthylenone	T	-	P, S
9.46	Indole-5-carbonitrile	T	-	P
9.49	1-Naphthalenol acetate	T	-	P
9.83	7-Methyl-1-naphthalenol	T	-	P
9.87	1-Pentylindole	**V**	-	P, S
10.19	Dibenzofuran	T	-	P
10.30	1,1'-Biphenyl-4-carboxaldehyde	T	-	P
10.42	2-Hydroxyfluorene	T	-	P
10.59	1-Pentyl-2-methylindole	T	-	P
10.87	9H-Fluoren-9-one	T	-	P
11.02	3,5-Dimethyl-2-phenylpiperidin-4-one	T	-	P
11.06	Indole-3-carboxaldehyde	**V**	-	P

11.28	Anthracene	**V**	3	P, S
11.37	Phenanthrene	T	3	P
11.39	Benzo[f]quinoline	T	-	P
11.55	Carbazole	T	2B	P, S
11.74	Phenanthridine	T	-	P
11.83	1-(Phenylmethylene)indene	T	-	P
12.04	1H-Phenalen-1-one	**V**	-	P
12.16	3-Methylcarbazole	T	-	P
12.36	2-Methylcarbazole	T	-	P
12.49	9-Methylcarbazole	T	-	P
12.66	1-Phenylnaphthalene	T	-	P
13.32	Fluoranthene	**V**	3	P
13.46	9-(Cyanomethylene)fluorene	T	-	P
13.67	Pyrene	T	3	P
13.72	Benzo[b]naphtho[2,3-d]furan	T	-	P
14.29	11H-Benzo[b]fluorene	T	3	P
14.41	7H-Benzo[c]fluorene	T	-	P
14.59	Chrysene	T	3	P
15.36	Benzo[c]phenanthrene	T	2B	P
15.44	9-Phenylacridine	T	-	P
15.65	Benz(a)acridine	T	-	P
15.68	11H-Benzo[a]carbazole	T	-	P
15.72	Benz[a]anthracene	**V**	2B	P
15.77	Naphthacene	T	-	P, S
15.83	1,2'-Binaphthalene	T	-	P
15.96	Naphtho(2,1-f)quinoline	T	-	P
16.02	1-Aminopyrene	T	-	P
16.56	Dibenzo(b,def)carbazole	T	-	P
17.47	Benzo[k]fluoranthene	T	2B	P
17.53	Dinaphtho[1,2-b:1',2'-d]furan	T	-	P, S
20.32	JWH-018	**V**	-	P
23.48	JWH-081	**V**	-	P, S

Identification (ID) refers to whether the pyrolytic product was tentatively identified, T, or verified with a reference material, **V**. The carcinogenic group (CG) indicates the IARC classification to which the analyte belongs [1, 2A, 2B, 3, or 4]. The extract in which the pyrolytic product is indicated with a single capital letter representative of each of the three extracts: W (wool), P (pipette), and S (solvent layer).

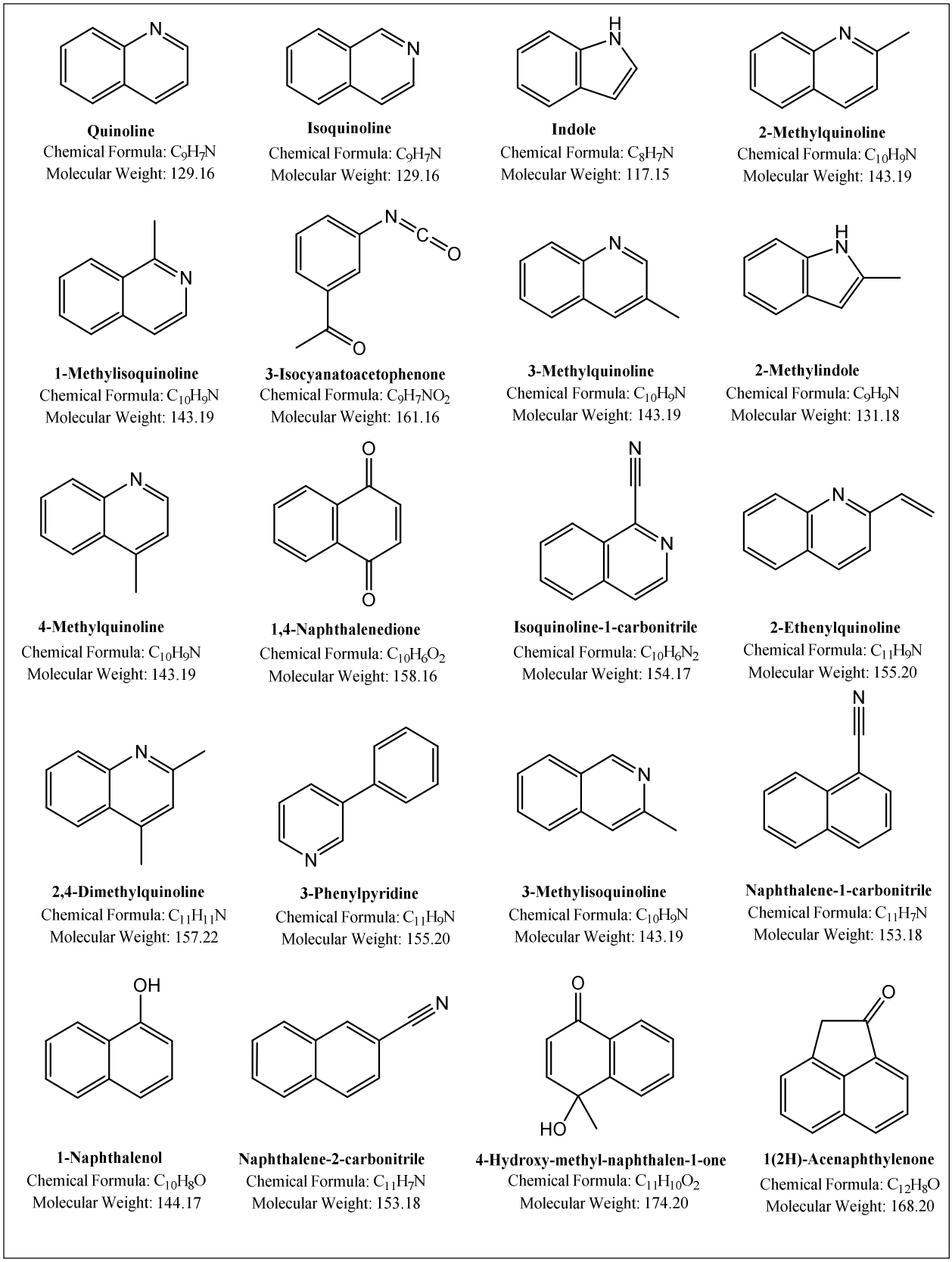


Figure 45: Proposed pyrolytic products of JWH-081

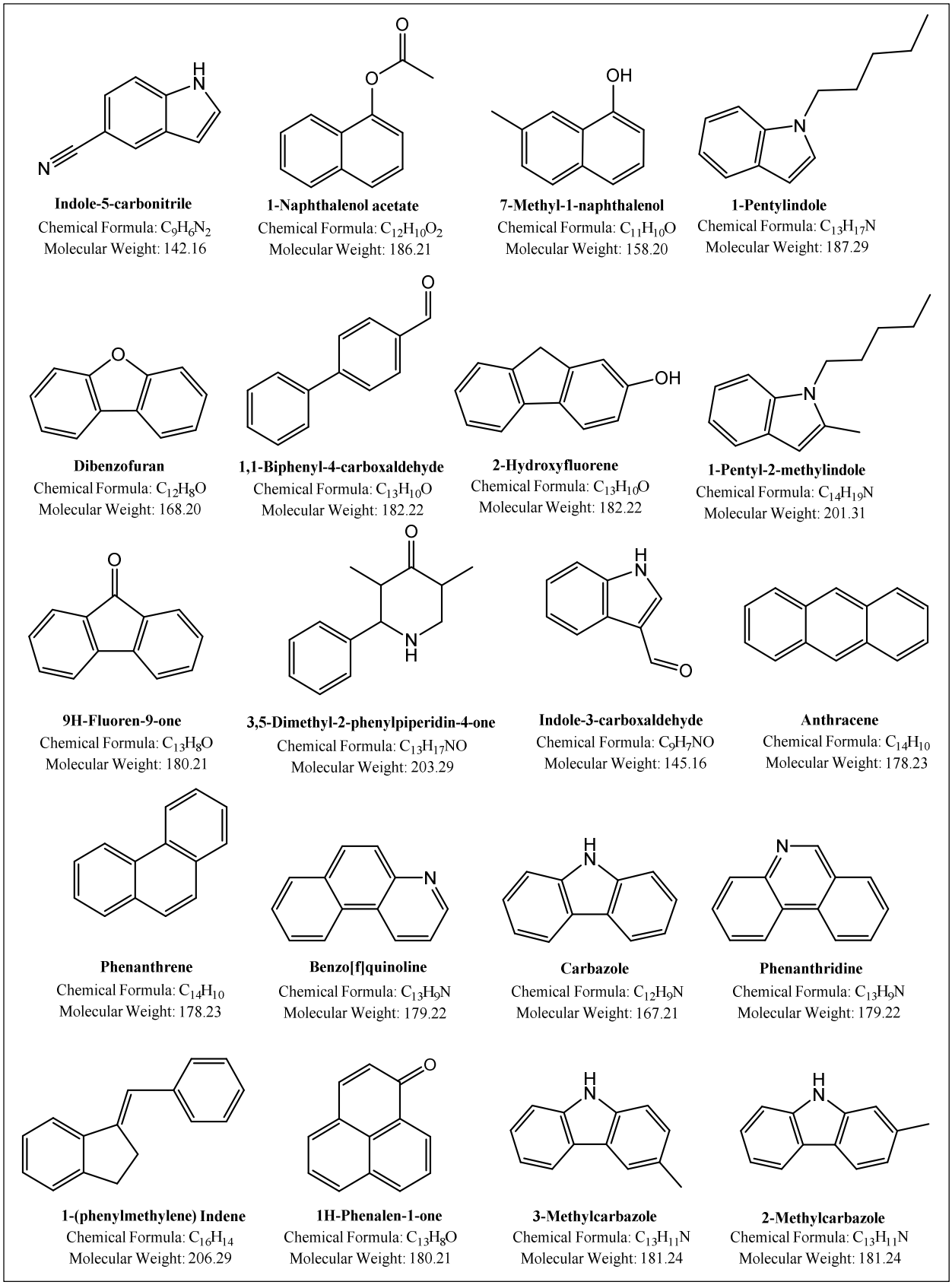
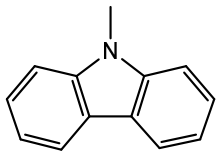
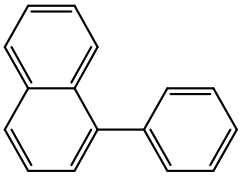


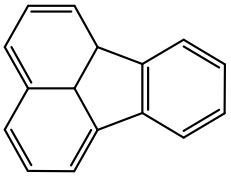
Figure 45 continued



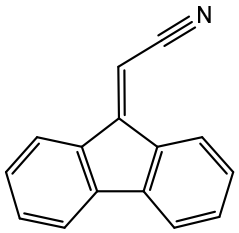
9-Methylcarbazole
Chemical Formula: $C_{13}H_{11}N$
Molecular Weight: 181.24



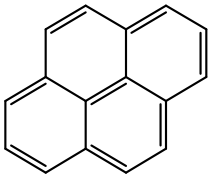
1-Phenyl-naphthalene
Chemical Formula: $C_{16}H_{12}$
Molecular Weight: 204.27



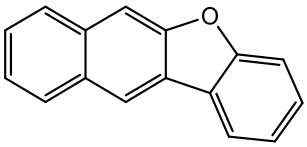
Fluoranthene
Chemical Formula: $C_{16}H_{12}$
Molecular Weight: 204.27



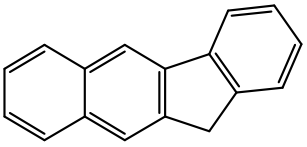
9-(Cyanomethylene)fluorene
Chemical Formula: $C_{15}H_9N$
Molecular Weight: 203.24



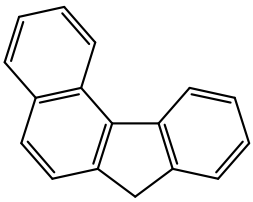
Pyrene
Chemical Formula: $C_{16}H_{10}$
Molecular Weight: 202.26



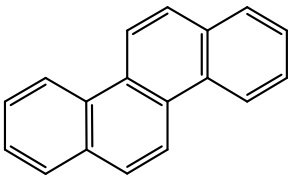
Benzo[b]naphtho[2,3-d]furan
Chemical Formula: $C_{16}H_{10}O$
Molecular Weight: 218.26



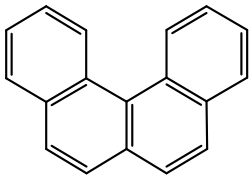
11H-Benzo[b]fluorene
Chemical Formula: $C_{17}H_{12}$
Molecular Weight: 216.28



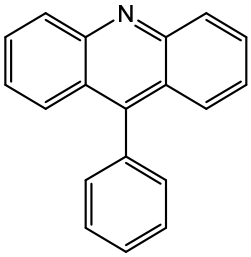
7H-Benzo[c]fluorene
Chemical Formula: $C_{17}H_{12}$
Molecular Weight: 216.28



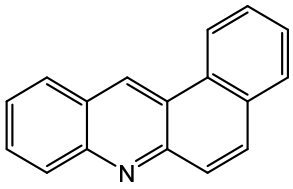
Chrysene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



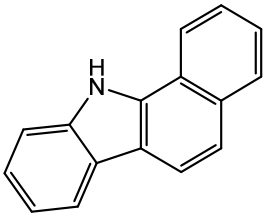
Benzo[c]phenanthrene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



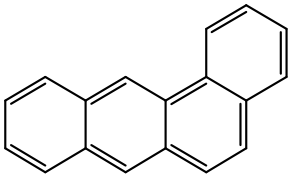
9-Phenylacridine
Chemical Formula: $C_{19}H_{13}N$
Molecular Weight: 255.32



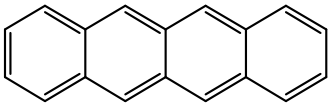
Benz(a)acridine
Chemical Formula: $C_{17}H_{11}N$
Molecular Weight: 229.28



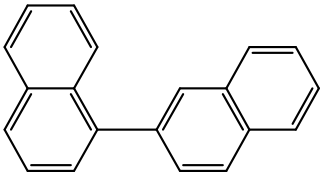
11H-Benzo[a]carbazole
Chemical Formula: $C_{16}H_{11}N$
Molecular Weight: 217.27



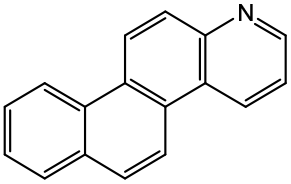
Benz[a]anthracene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



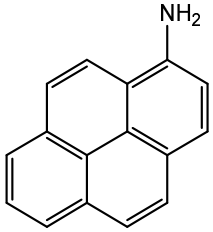
Naphthacene
Chemical Formula: $C_{18}H_{12}$
Molecular Weight: 228.29



1,2'-Binaphthalene
Chemical Formula: $C_{20}H_{14}$
Molecular Weight: 254.33

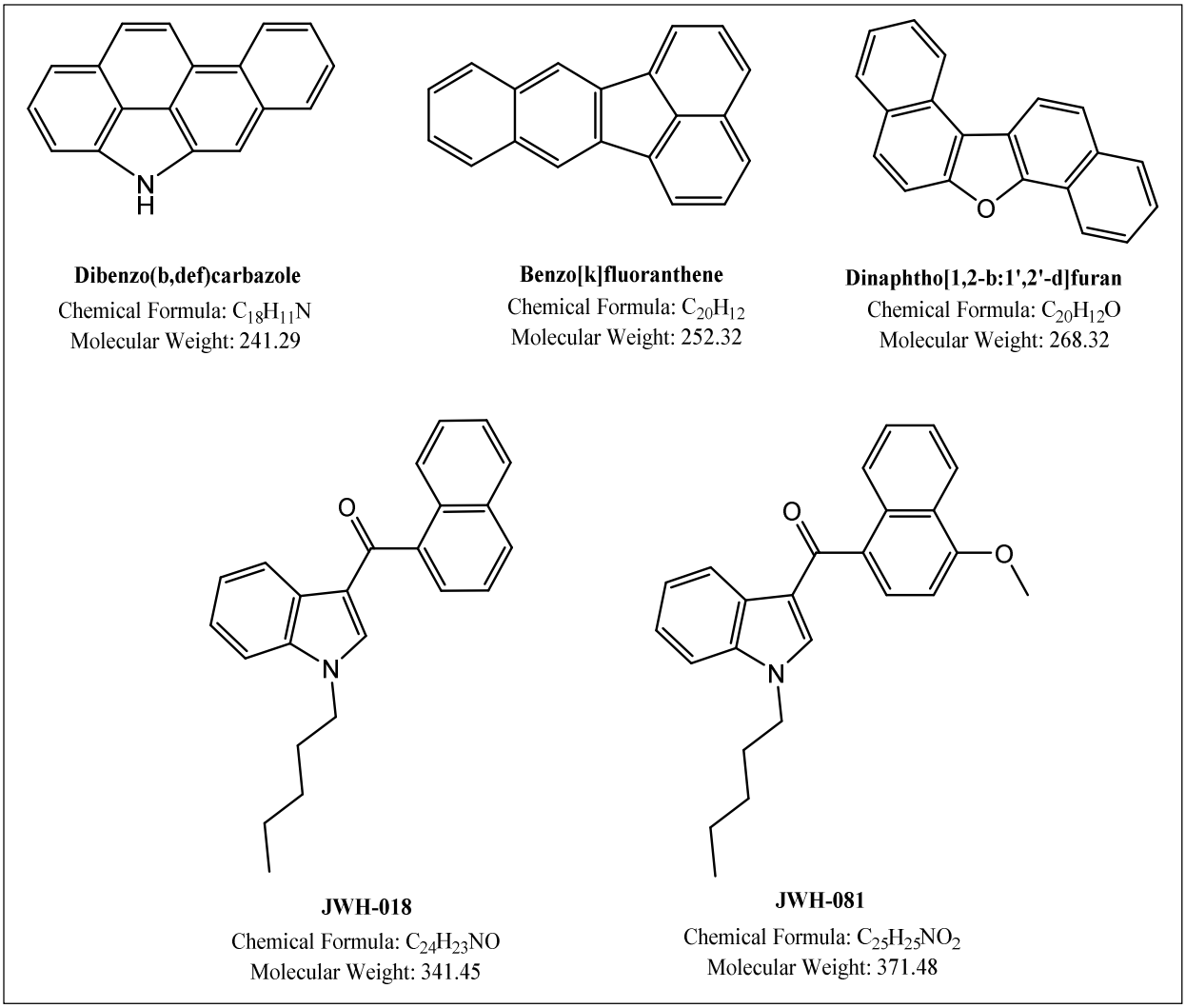


Naphtho(2,1-f)quinoline
Chemical Formula: $C_{17}H_{11}N$
Molecular Weight: 229.28



1-Aminopyrene
Chemical Formula: $C_{16}H_{11}N$
Molecular Weight: 217.27

Figure 45 continued



Dibenzo(b,def)carbazole
Chemical Formula: $C_{18}H_{11}N$
Molecular Weight: 241.29

Benzo[k]fluoranthene
Chemical Formula: $C_{20}H_{12}$
Molecular Weight: 252.32

Dinaphtho[1,2-b:1',2'-d]furan
Chemical Formula: $C_{20}H_{12}O$
Molecular Weight: 268.32

JWH-018
Chemical Formula: $C_{24}H_{23}NO$
Molecular Weight: 341.45

JWH-081
Chemical Formula: $C_{25}H_{25}NO_2$
Molecular Weight: 371.48

Figure 45 continued

4.3.4 PYROLYSIS OF UR-144

Forty-eight compounds were identified as pyrolytic products of the synthetic cannabinoid UR-144. The overall dissimilarity these products had from those identified in the other synthetic cannabinoid enables a number of compounds to be suggested as possible pyromarkers of UR-144. Five of the total UR-144 products were identified in the naphthalene-containing compounds, but only one (3-methylquinoline) was solely identified in the extracts from the naphthalene-compounds. This correlates with the absence of a naphthalene group in UR-144.

The absence of naphthalene appears to have a direct effect on the number of products which pose a carcinogenic threat to users. Carbazole (Group 2B) and fluoranthene (Group 3) were detected, but with fluoranthene being the only compound identified which had more than three fused rings, no other health hazards can be explicitly provided without future research first being conducted on the health hazards of the number of substituted PAHs that were observed.

UR-144 had the largest percentage of compounds (57%) that were only identified in its extracts when compared to the others. Of the twenty-eight products identified only with UR-144, twenty-two were not identified with the non-controlled compounds studied in the previous chapter. ***Table 14*** shows the total list of pyrolytic products that were identified and the corresponding structures are provided in ***Figure 46***.

Table 14: Pyrolytic products identified during investigation of UR-144

Retention Time (min)	Compound	ID	CG	UR-144
3.37	Isocyanatobenzene	T	-	P
3.44	Aniline	T	-	P
4.92	Dihydro-4,4,5,5-tetramethyl-2(3H)furanone	T	-	P, S
6.51	Isoquinoline	T	-	P, S
6.76	Indole	**V**	-	P, S
6.80	N-Phenylformamide	T	-	P
7.02	2-Methylquinoline	**V**	-	S
7.29	2-Aminobenzoic acid methyl ester	T	-	P
7.47	N-Phenylacetamide	T	-	P
7.49	4-Isocyanatoacetophenone	T	-	P, S
7.53	3-Methylquinoline	T	-	P, S
7.63	2(1H)-Quinolinone	T	-	P
7.69	2-Methylindole	T	-	P
7.71	4-Methylquinoline	T	-	S
8.14	1-Methyl-2-indolinone	T	-	P
8.33	Oxindole	**V**	-	P, S
8.55	2,3-Dimethylphenylisocyanate	T	-	S
8.63	2,4,6-Trimethylbenzonitrile	T	-	P
8.76	N-Acetylindole	T	-	P, S
8.88	Naphthalene-1-carbonitrile	T	-	P
8.94	Naphthalene-2-carbonitrile	**V**	-	P, S
9.33	2-Acetylindole	T	-	P, S
9.39	3-Propylquinoline	T	-	P
9.46	N-Ethyl-1-naphthalenamine	T	-	P, S
9.81	Indole-3-ethanol	T	-	P
9.88	1-Pentylindole	**V**	-	P, S
10.01	1-Isocyanatonaphthalene	T	-	P, S
10.18	Isatin	**V**	-	P, S
10.27	1H-Cyclopenta[b]quinoxaline-1,2,3-trione	T	-	P
10.35	6-(1-Methylpropyl)quinoline	T	-	P, S
10.44	7-Hydroxy-2-naphthalenecarbonitrile	T	-	P

10.59	1-Pentyl-2-methylindole	T	-	P
11.00	3,5-Dimethyl-2-phenylpiperidin-4-one	T	-	P, S
11.05	Indole-3-carboxaldehyde	**V**	-	P, S
11.29	4'-(1-Pyrroyl)acetophenone	T	-	P, S
11.39	Acridine	T	-	P, S
11.55	Carbazole	T	2B	P, S
11.61	1-(1H-Indol-3-yl)ethanone	T	-	P
11.74	1-Methyl-3-acetylindole	T	-	P
12.17	3-Methylcarbazole	T	-	P
12.37	2-Methylcarbazole	T	-	P, S
12.49	9-Methylcarbazole	T	-	P
12.94	1,4-Dimethylcarbazole	T	-	P
13.32	Fluoranthene	**V**	3	P
13.44	3,4-Dimethylcarbazole	T	-	P, S
15.17	10-Pentyl-10H-acridin-9-one	T	-	P, S
16.02	UR-144	**V**	-	P, S
16.18	UR-144 Degradant	**V**	-	P, S

Identification (ID) refers to whether the pyrolytic product was tentatively identified, T, or verified with a reference material, **V**. The carcinogenic group (CG) indicates the IARC classification to which the analyte belongs [1, 2A, 2B, 3, or 4]. The extract in which the pyrolytic product is indicated with a single capital letter representative of each of the three extracts: W (wool), P (pipette), and S (solvent layer).

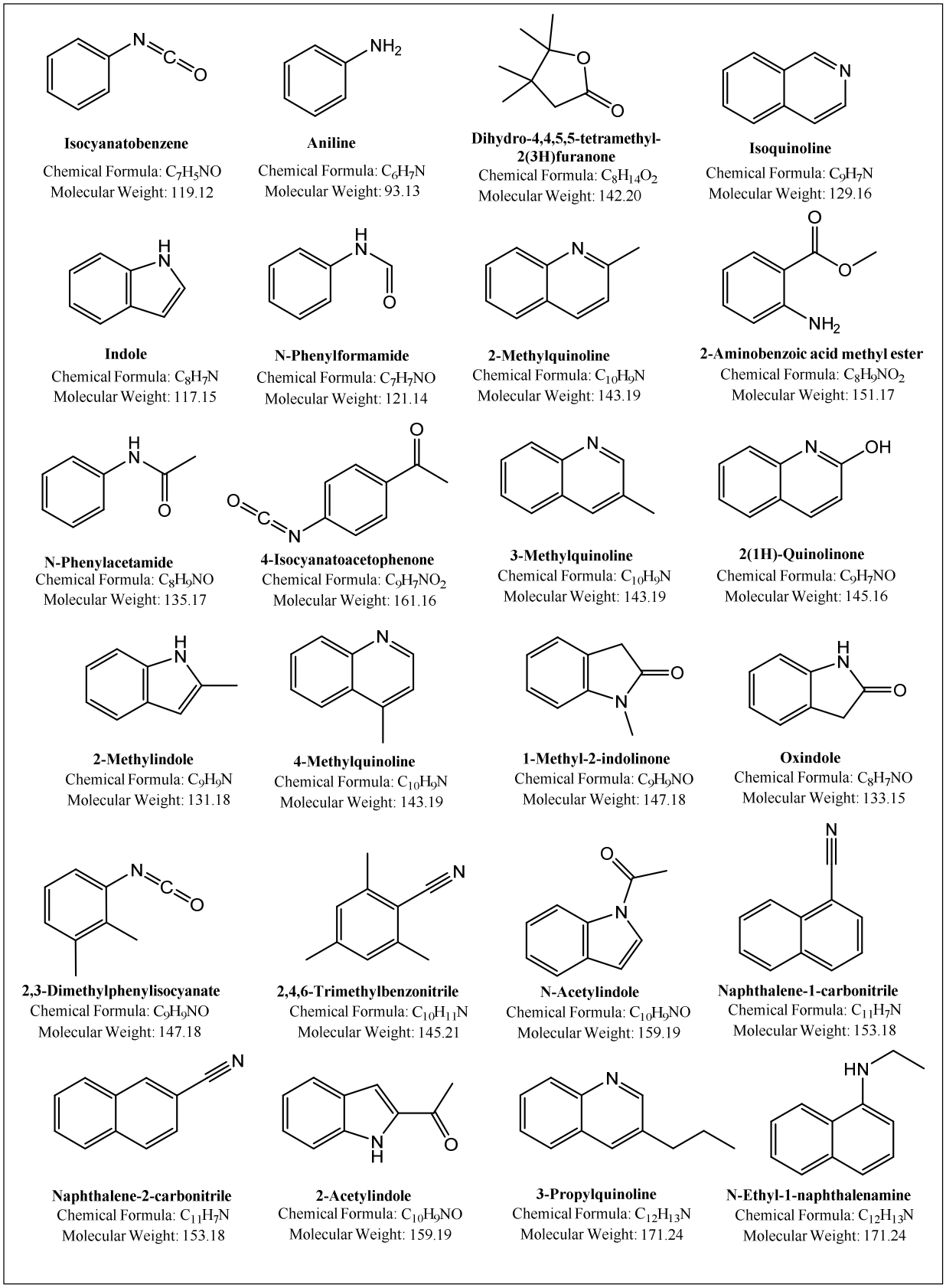


Figure 46: Proposed pyrolytic products for UR-144

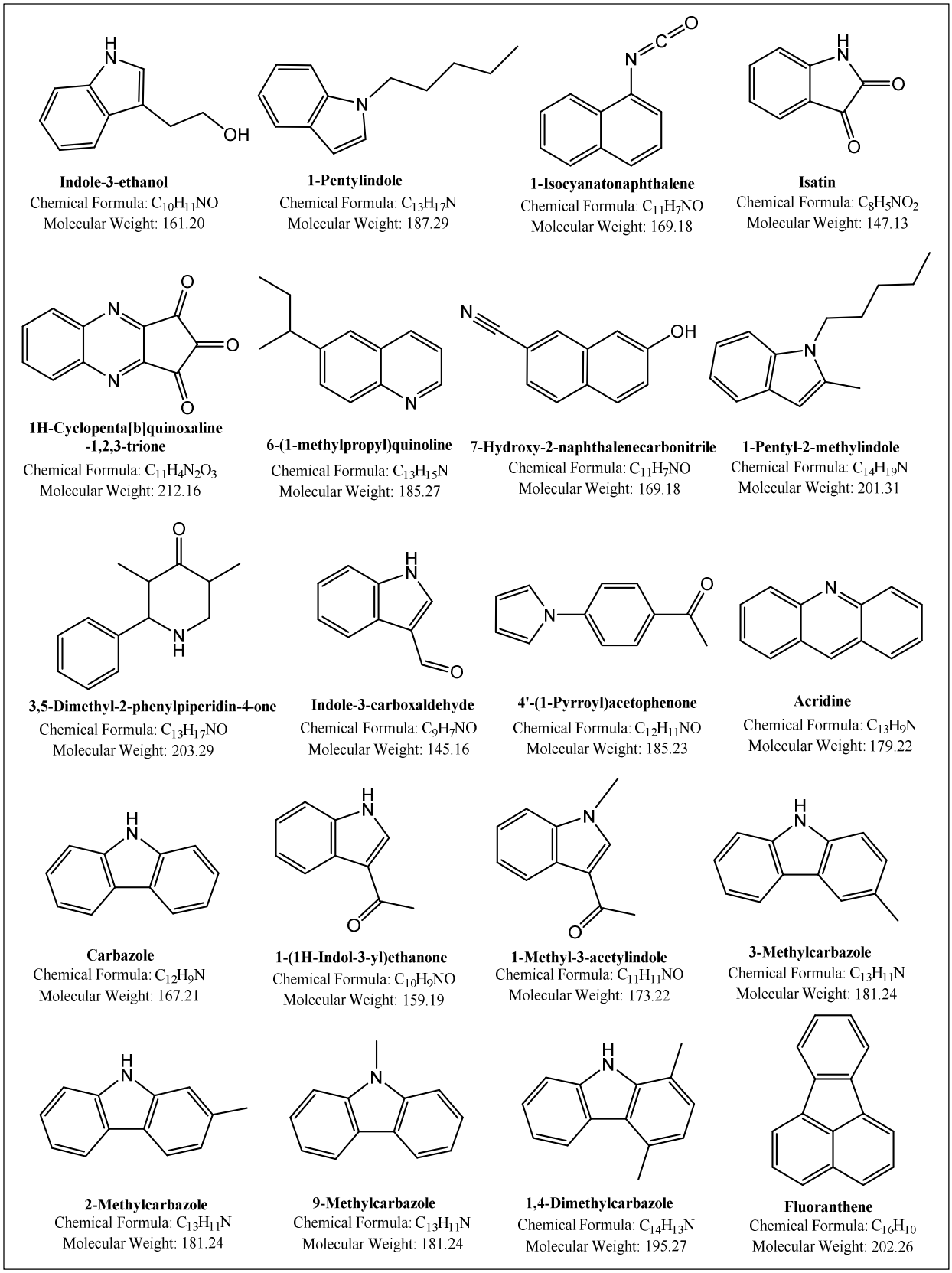


Figure 46 continued

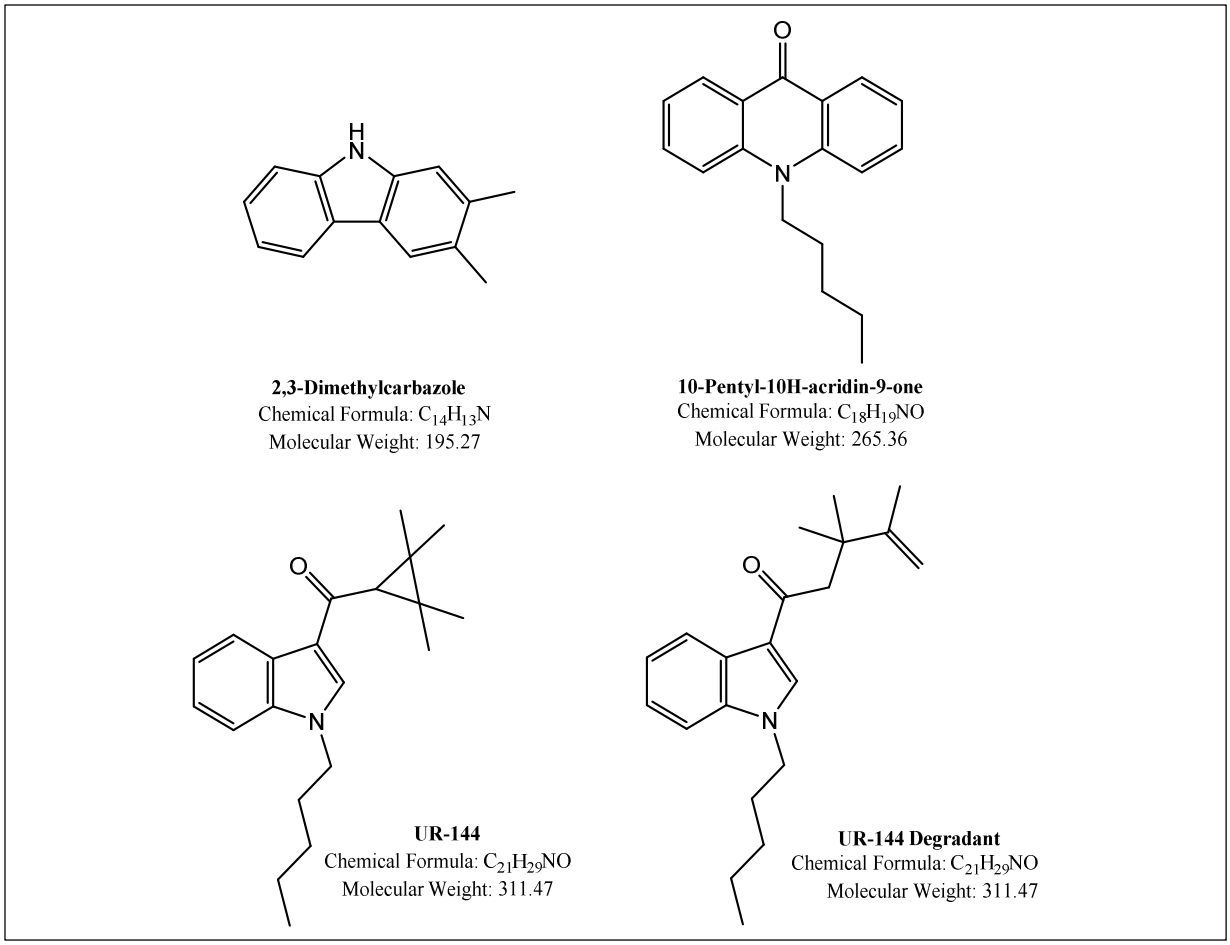


Figure 46 continued

There were four specific pyrolytic products that were unable to be tentatively identified or verified with reference materials and are thus not listed in the data table. Plausible structures and names for two of the unknown compounds have been proposed based upon the mass spectral fragmentation patterns observed. The first unknown pyrolytic product (**UPP1**) which eluted at 13.62 minutes was the second most abundant product observed in the pyrogram; the most abundant product being the UR-144 Degradant at 16.18 minutes. The combined pyrogram and mass spectra for **UPP1** is provided in ***Figure 47***. The molecular ion and base peak for **UPP1** were determined to be m/z 229 and m/z 214, respectively. The proposed fragmentation pathway for **UPP1** is provided in ***Figure 48*** and provides plausible explanations for the m/z 144, 172, and 130.

A structure has also been proposed for a second unknown pyrolytic product (**UPP2**) which was identified at 14.10 minutes. The combined pyrogram and mass spectra for **UPP2** is provided in ***Figure 49***, with the proposed fragmentation pathway illustrated in ***Figure 50***. The molecular ion, m/z 243, and base peak, m/z 214, of **UPP2** suggest similarity to that of **UPP1**. The m/z 144 fragment ion was also used when proposing a molecular structural for this product.

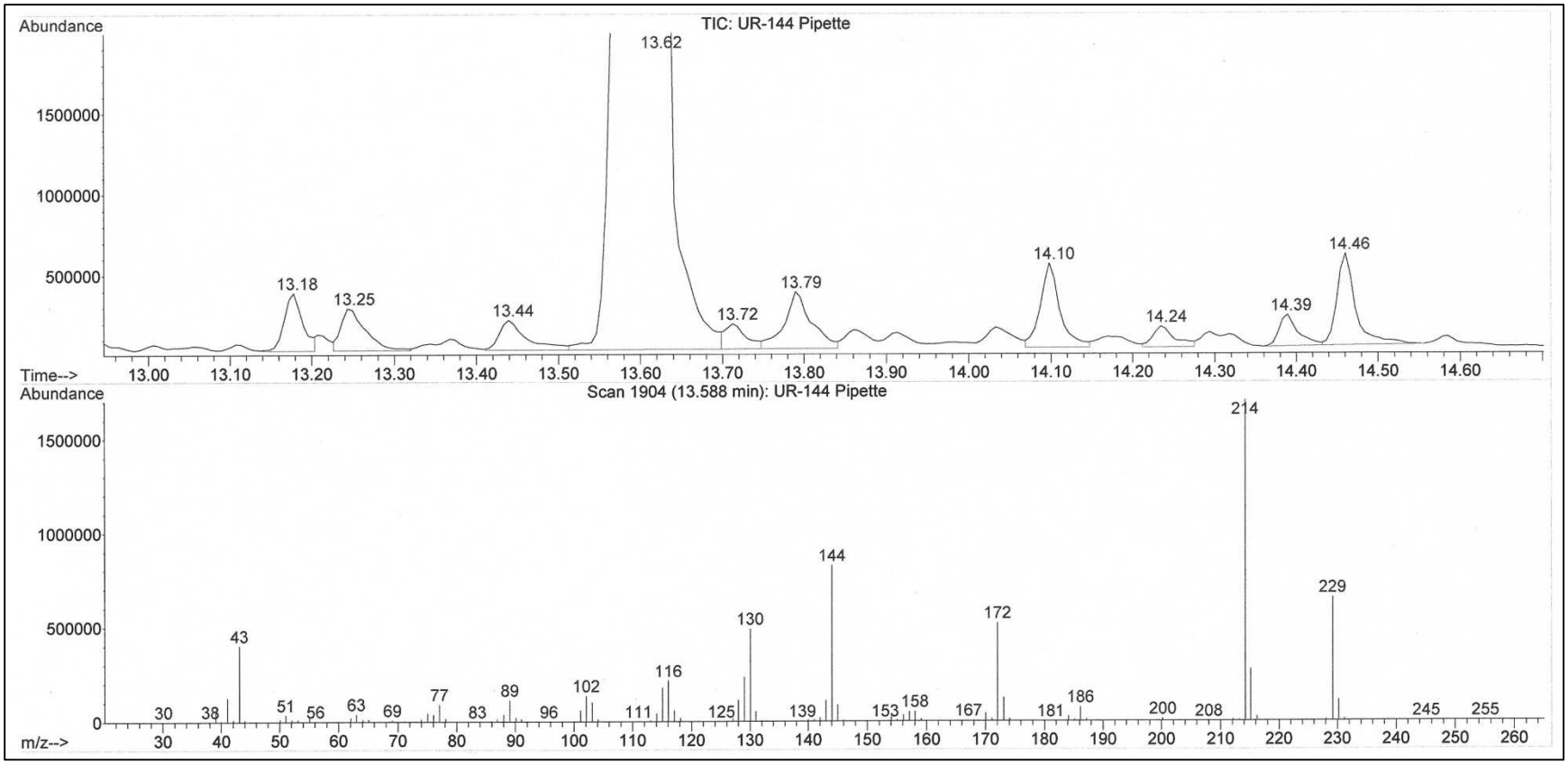


Figure 47: Pyrogram and mass spectrum for UPP1

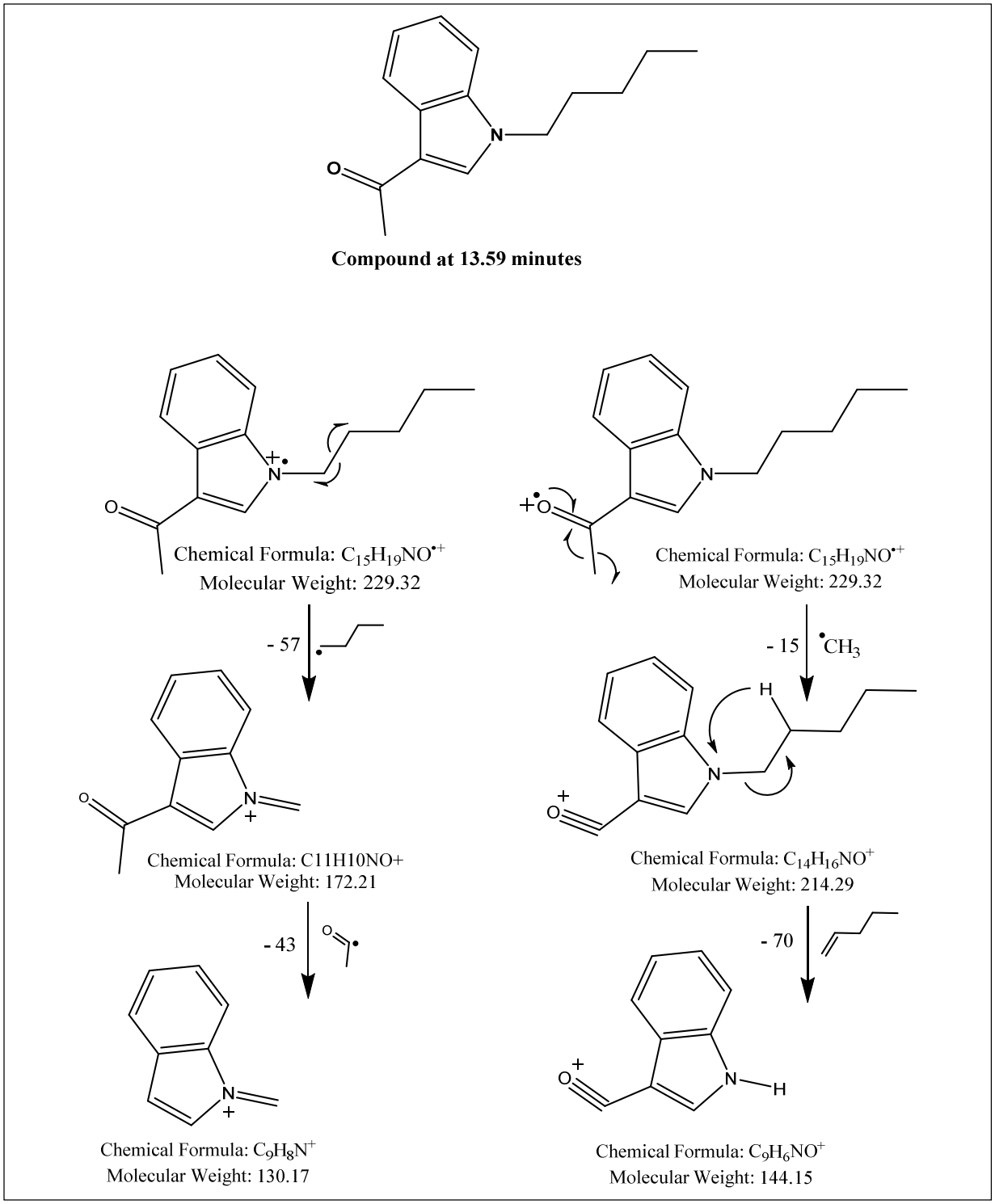


Figure 48: Proposed fragmentation pattern for UPP1

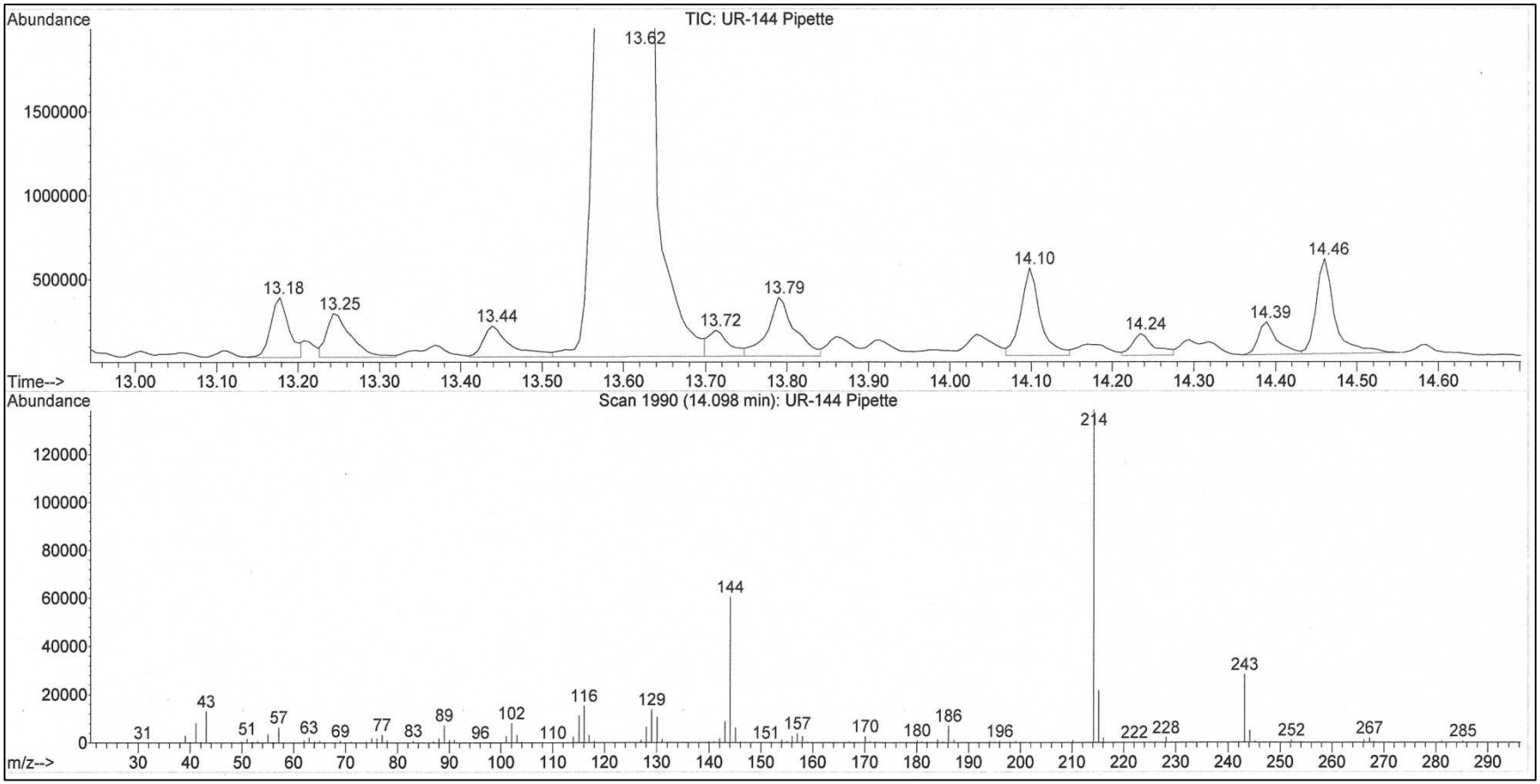


Figure 49: Pyrogram and mass spectrum for UPP2

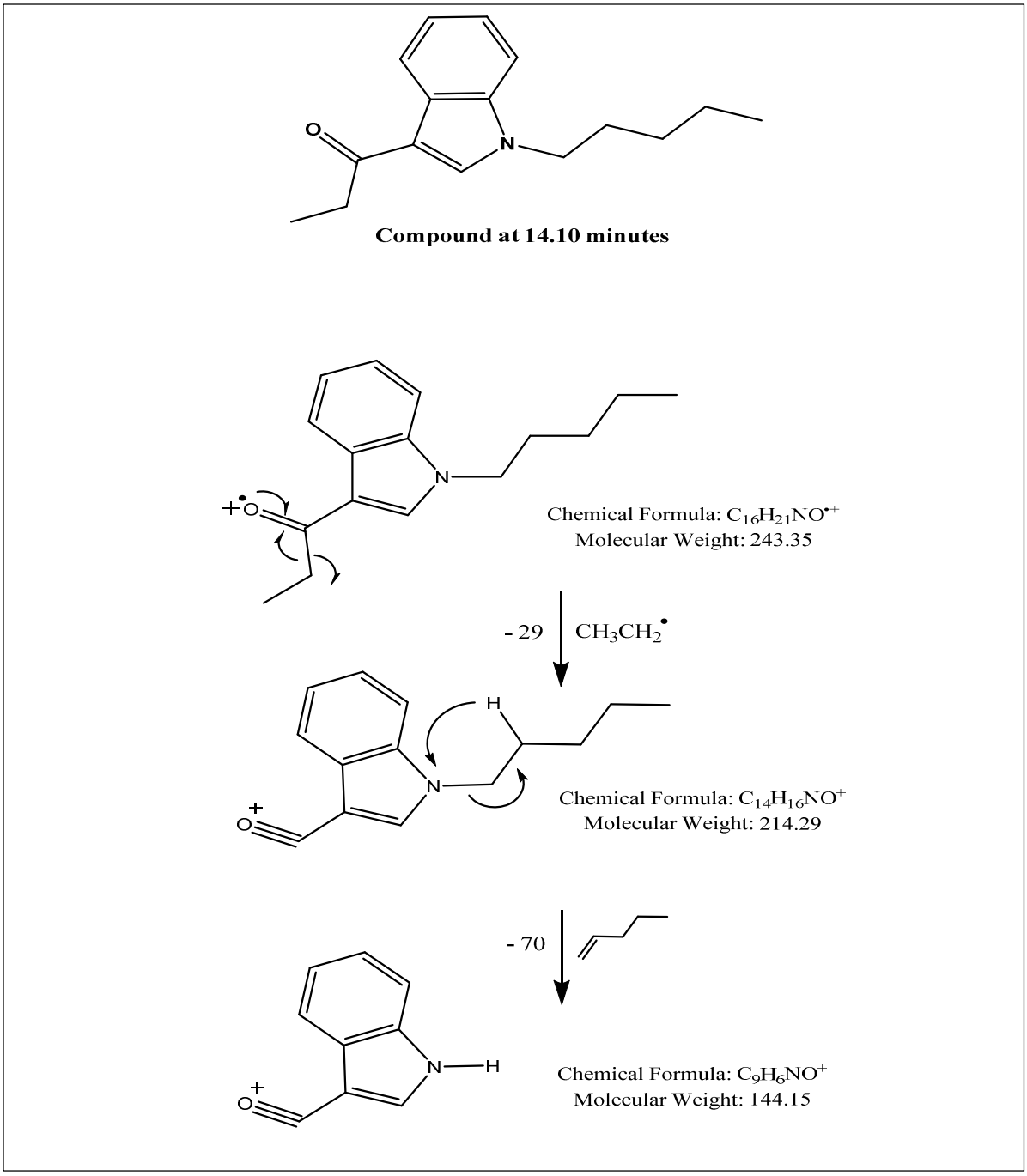


Figure 50: Proposed fragmentation pathway for UPP2

Structural interpretation for the two other unknown pyrolytic products identified at 15.23 minutes (**UPP3**) and 16.79 minutes (**UPP4**) was unsuccessful. **UPP4** was the third most abundant product observed in the UR-144 pyrogram and showed spectral similarity to that of UR-144 and the UR-144 Degradant. A low intensity molecular ion of m/z 311, with an even lower intensity ion with m/z 296, was observed in the mass spectrum for this unknown. The base peak ion with m/z 229 suggests that the 1-pentyl-3-acetylindole moiety is present in this particular pyrolytic product and that the carbon-carbon bond connecting the rest of the molecular structure is highly susceptible to bond cleavage.

UPP3 was found to contain the characteristic ions (m/z 311 molecular ion and fragment ion m/z 296 ion) for a compound related to UR-144 or the UR-144 Degradant (see **Appendix L**). Unlike the **UPP4**, the base peak of the spectra was the m/z 296 ion, which corresponds to the loss of a methyl radical. All additional ions (m/z 252, 210, 180, 167, 144, 130, and 109) in the spectra were of considerably low intensity and suggest that the pyrolytic product is relatively capable of stabilizing the excess energy imparted during the ionization process (i.e. extensive conjugation or aromaticity).

4.4.0 CONCLUSION

The pyrolysis of four synthetic cannabinoids has confirmed the presence of pyrolytic products observed with that of the smaller components investigated in the previous study. Compared to the results from the indole and naphthalene containing compounds, a similar number of pyrolytic products were observed with each synthetic cannabinoid. The greatest number of pyrolytic products was identified with 1-methoxynaphthalene and JHW-081, suggesting that a molecule containing a methoxy group will be more reactive than other similar compounds without the functional group. The fewest number of products were identified with 1-methylnaphthalene and JWH-030, indicating the relatively unreactive nature of a lone methyl-substituent connected to an aromatic system.

Ranging from phenol to chrysene, there were a number of compounds generated during the pyrolysis of the synthetic cannabinoids which are considered to be serious health hazards; excluding UR-144. Such compounds with a varying level of carcinogenic concern, as defined by IARC, may pose serious long-term health effects to chronic users of synthetic cannabinoids. The unknown health effects of the remaining pyrolytic products (heterocyclic amines, etc.) are also concerning.

While not every product was able to be identified, it was found that structurally similar synthetic cannabinoids could be generated during pyrolysis; as was evident with the result of JWH-018 and JWH-081. With the possibility that more than one synthetic cannabinoid already exists in herbal incense products, the generation of multiple biologically active compounds could be contributing to the severe effects observed with the use of these particular designer drugs.

FUTURE DIRECTIONS

Future work should begin with the structural elucidation of the specified unknown pyrolytic products of JWH-030, JWH-018, JHW-081, and UR-144 discussed in Chapter 4. The ability to characterize these specific compounds may lead to the identification of potential pyromarkers and/or biologically active compounds. Identifying unique pyromarkers of these drugs would provide forensic toxicologists with a valuable analyte that, in the absence of the parent drug, can indicate use of a given substance.

Investigating the acute and chronic effects of the most abundant pyrolytic products is another potential avenue for continued research. While this work is strictly qualitative, future work could include the verification and quantification of levels associated with the pyrolytic product(s) of interest using reference materials. Cell cultures could then be dosed with a corresponding concentration to assess toxicity or other desired cellular responses and outputs. The bind affinity of the prominent pyrolytic products for both the CB_1 and CB_2 receptors would also be beneficial information that has not yet been reported.

One additional area of interest would be to pyrolyze the plant material that synthetic cannabinoids are commonly sprayed onto. While the synthetic cannabinoids are typically viewed as the primary ingredient of herbal incense products and are certainly the main focus of forensic chemists, the pyrolysis of the plant material would provide insight regarding the generation of other potentially harmful compounds.

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97. Donohue, K. M.; Steiner, R. R. JWH-018 and JWH-022 as Combustion Products of AM2201. *Microgram* **2013***, 9*, 52-56.

98. Daw, R. C.; Grabenauer, Pande, P. G.; Cox, A. O.; Kovach, A. L.; Davis, K. H.; Wiley, J. J.; Stout, P. R.; Thomas, B. F. Pyrolysis of Synthetic Cannabinoids in Herbal Products. *Drug and Alcohol Dependence* **2014**, 140, e44.

APPENDIX A – AMOUNT OF EACH NON-CONTROLLED SUBSTANCE PYROLYZED

Listed below is the amount of chemical substance that was pyrolyzed in each trial. Also included is the amount of quartz wool that was used to 'sandwich' the chemical. For liquid analytes, the pipettes were allowed to air-dry overnight (~18 hours) before pyrolysis.

Naphthalene

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	64.61 mg	5.46 mg	16.32 mg
Trial #2	59.62 mg	5.67 mg	25.39 mg
Trial #3	46.52 mg	7.34 mg	22.69 mg

1-Methylnaphthalene (density = 1.001 g/mL)

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	62.48 mg	40 µL	13.50 mg
Trial #2	61.36 mg	40 µL	24.08 mg
Trial #3	69.71 mg	40 µL	20.96 mg

1-Methoxynaphthalene (density = 1.094 g/mL)

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	60.64 mg	40 µL	11.06 mg
Trial #2	59.01 mg	40 µL	15.88 mg
Trial #3	70.16 mg	40 µL	15.79 mg

1-Ethylnaphthalene (density = 1.008 g/mL)

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	55.81 mg	40 µL	21.16 mg
Trial #2	59.05 mg	40 µL	15.45 mg
Trial #3	60.26 mg	40 µL	19.69 mg

Indole

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	62.47 mg	8.48 mg	11.16 mg
Trial #2	57.35 mg	9.86 mg	15.90 mg
Trial #3	55.21 mg	5.06 mg	22.64 mg

3-Methylindole

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	51.05 mg	5.29 mg	15.75 mg
Trial #2	48.98 mg	6.18 mg	15.73 mg
Trial #3	49.51mg	5.80 mg	19.78 mg

1-Pentylindole

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	52.57 mg	40 µL	18.45 mg
Trial #2	51.21 mg	40 µL	15.44 mg
Trial #3	45.29 mg	40 µL	23.63 mg

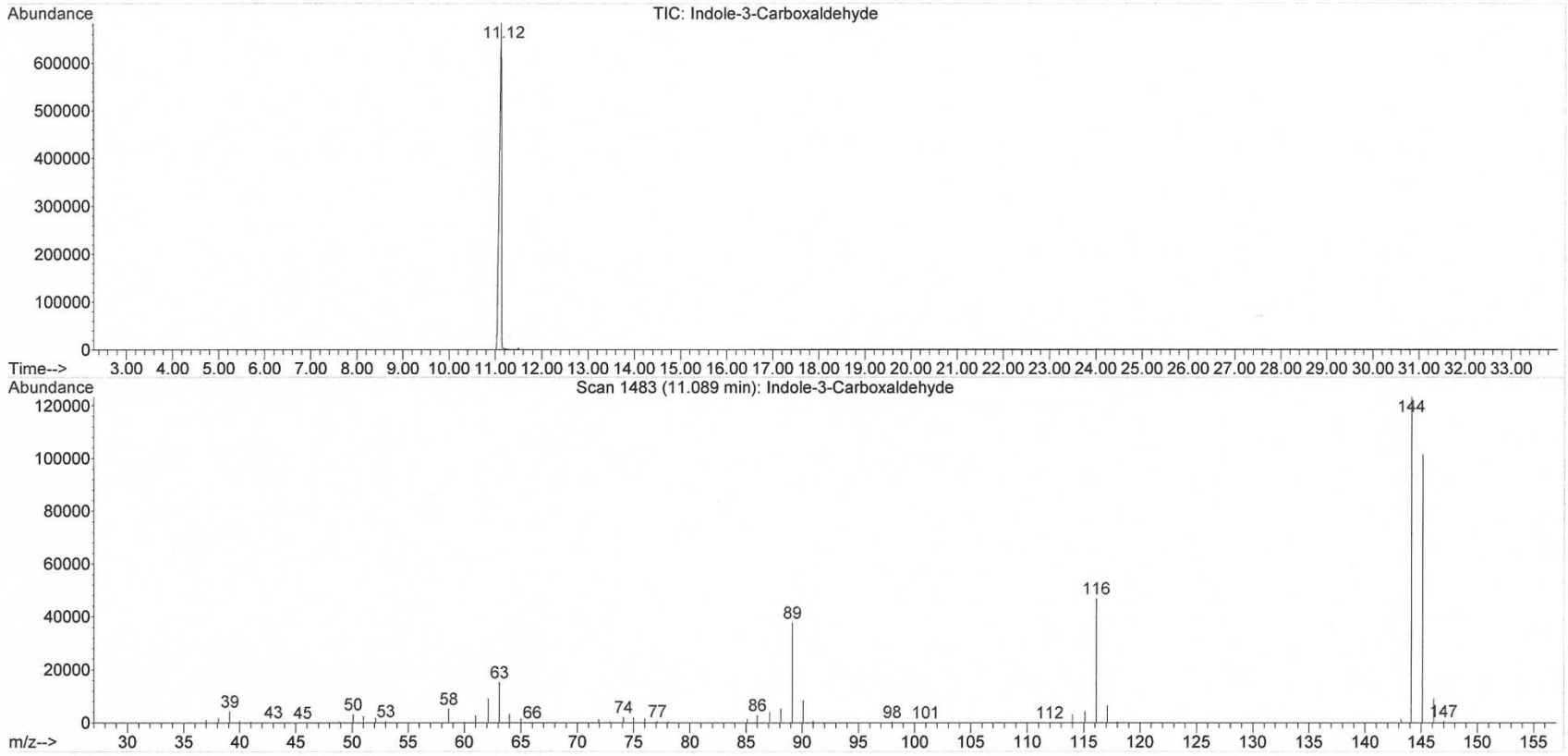
Indole-3-Carboxaldehyde

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	48.35 mg	4.99 mg	14.24 mg
Trial #2	50.70 mg	4.47 mg	14.71 mg
Trial #3	44.04 mg	4.87 mg	19.10 mg

3-Naphthoylindole

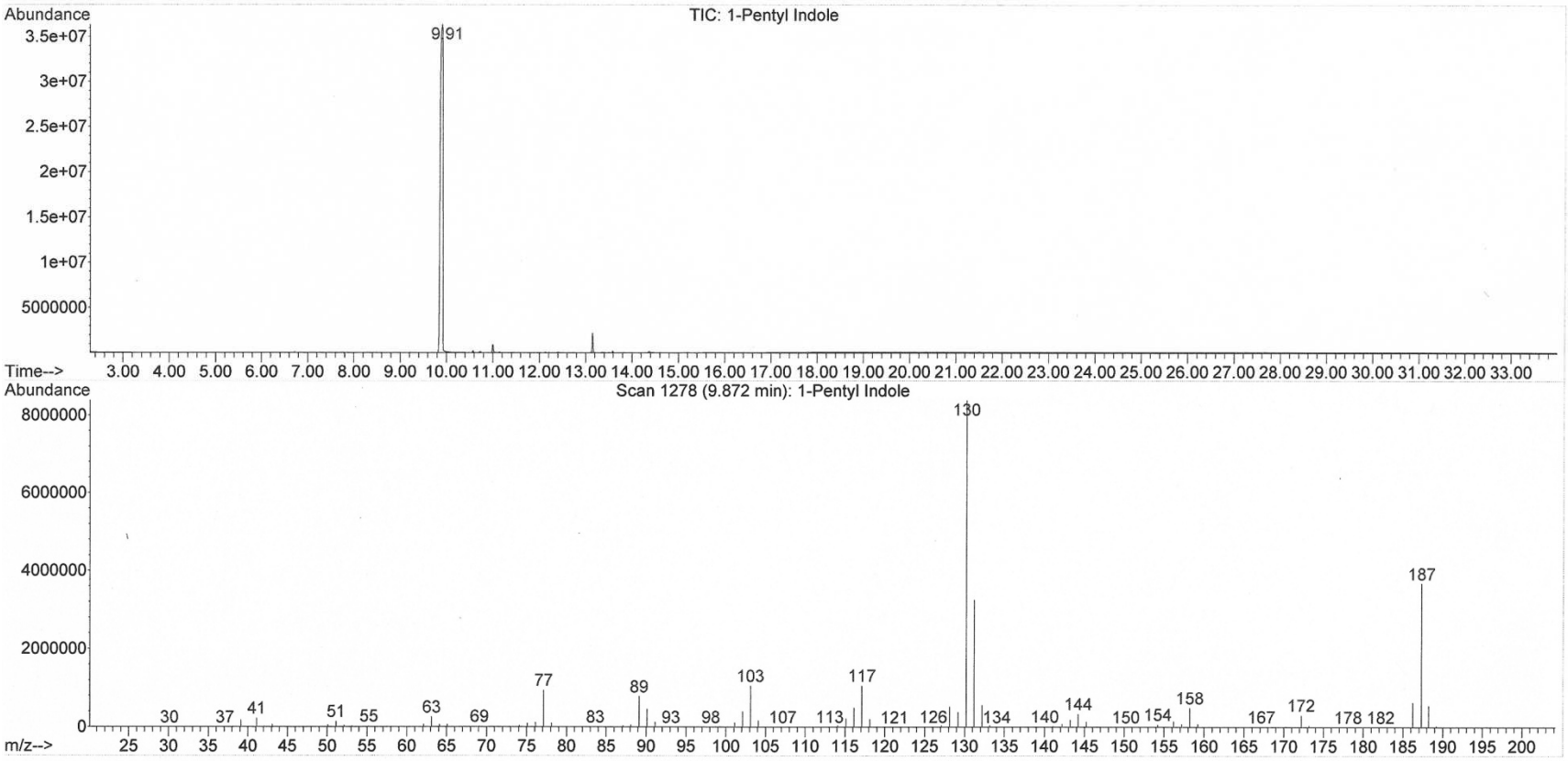
	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	56.65 mg	3.99 mg	17.77 mg
Trial #2	50.52 mg	3.12 mg	23.65 mg
Trial #3	50.08 mg	4.43 mg	23.01 mg

APPENDIX B – TIC OF INDOLE-3-CARBOXALDEHYDE, 1-PENTYLINDOLE, AND 3-NAPHTHOYLINDOLE STANDARDS



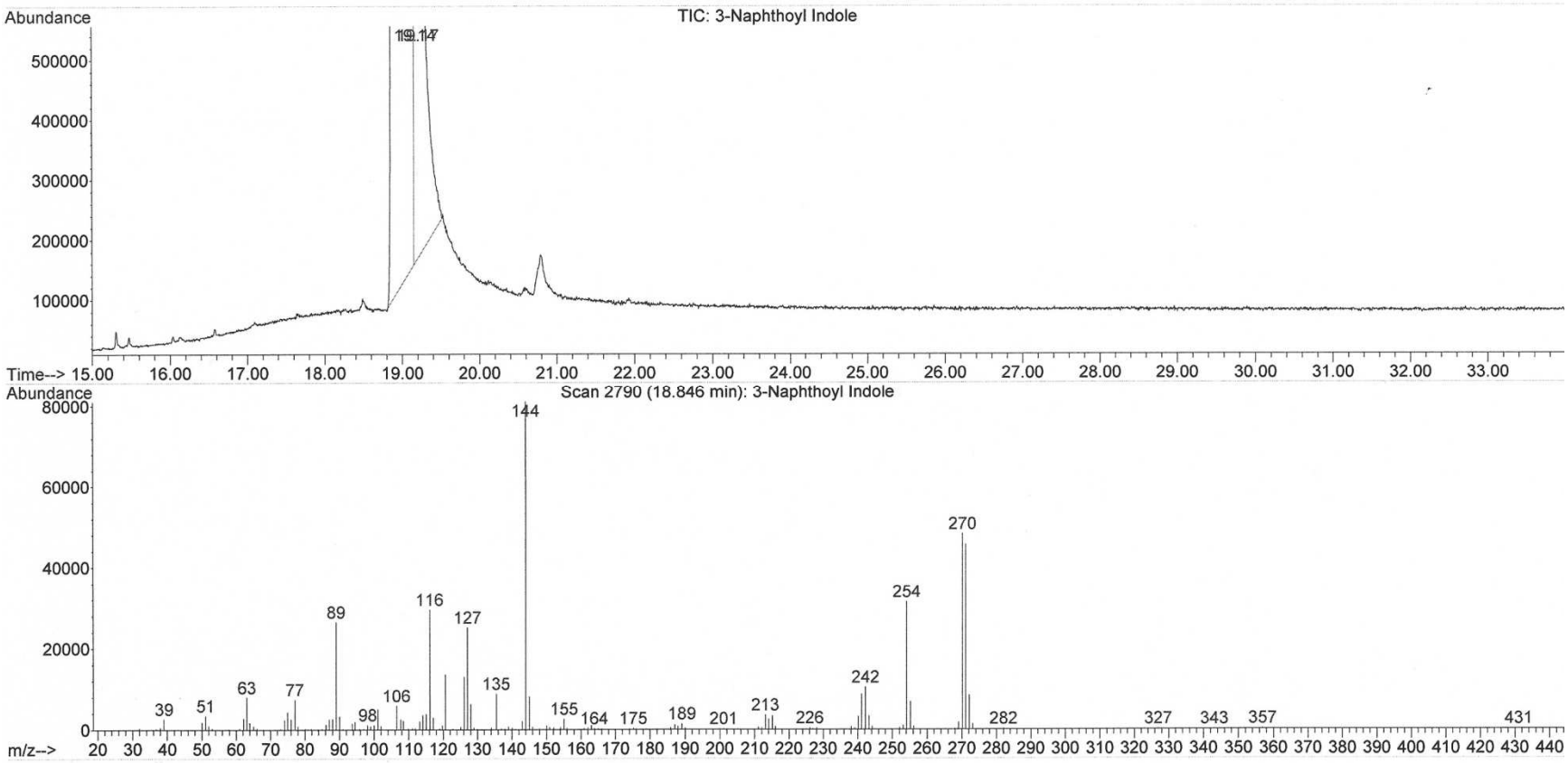
Qualitative EI GC-MS total ion chromatogram and mass spectrum for the indole-3-carboxaldehyde standard

Indole-3-carboxaldehyde was provided for this project via in-house synthesis (without an associated purity). The lone impurity detected at 11.53 minutes was identified as indole-3-carboxylic acid methyl ester using the NIST 2014 database (mass spectrum not shown).



Qualitative EI GC-MS total ion chromatogram and mass spectrum for the 1-pentlyindole standard

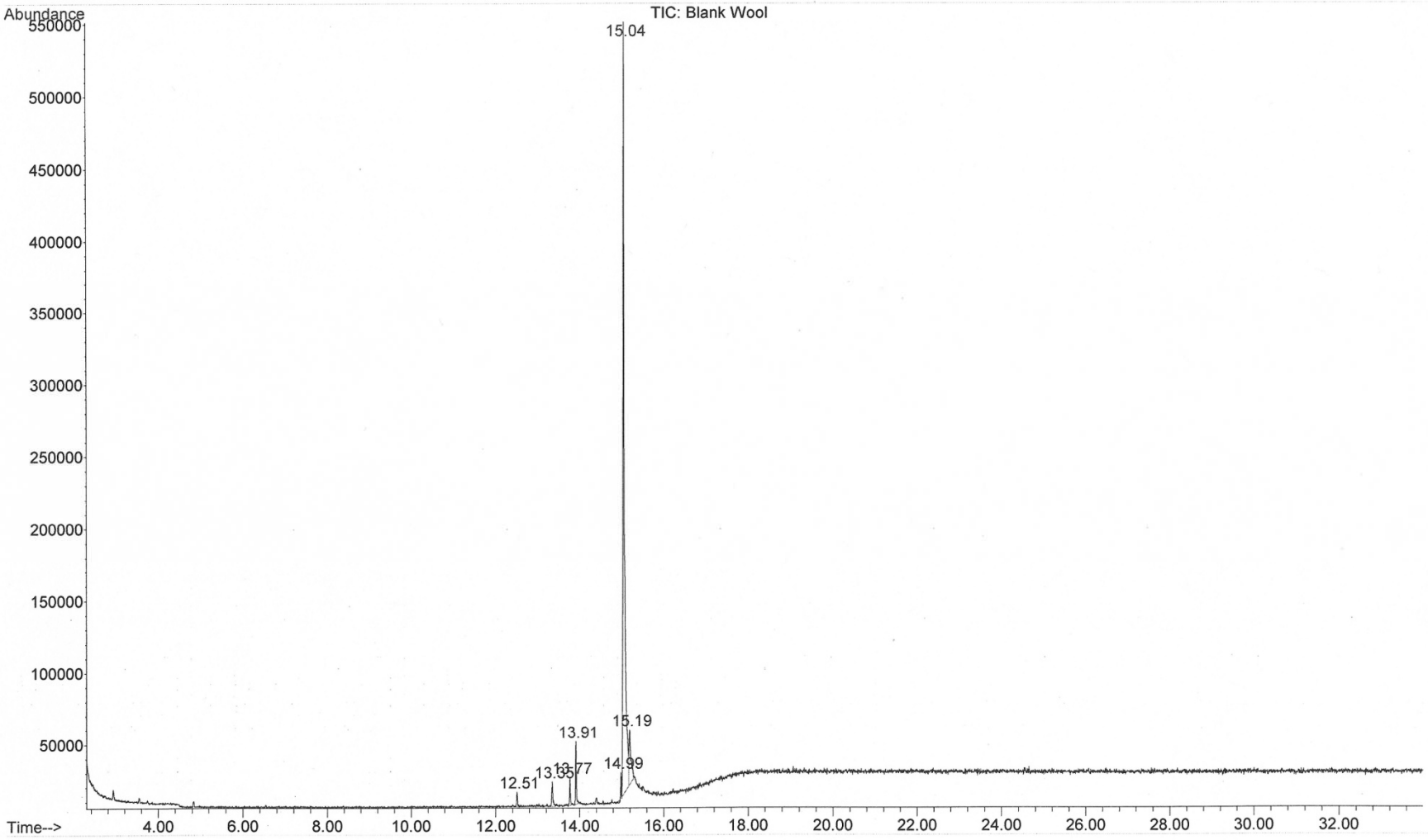
1-pentylindole was provided for this project via in-house synthesis (without an associated purity). The impurities present at 6.73, 10.69, and 11.00 minutes were identified as indole, 1-pentyl-3-methylindole, and 3,5-dimethyl-2-phenylpiperidin-4-one using the NIST 2014 MS database (individual mass spectra not shown).



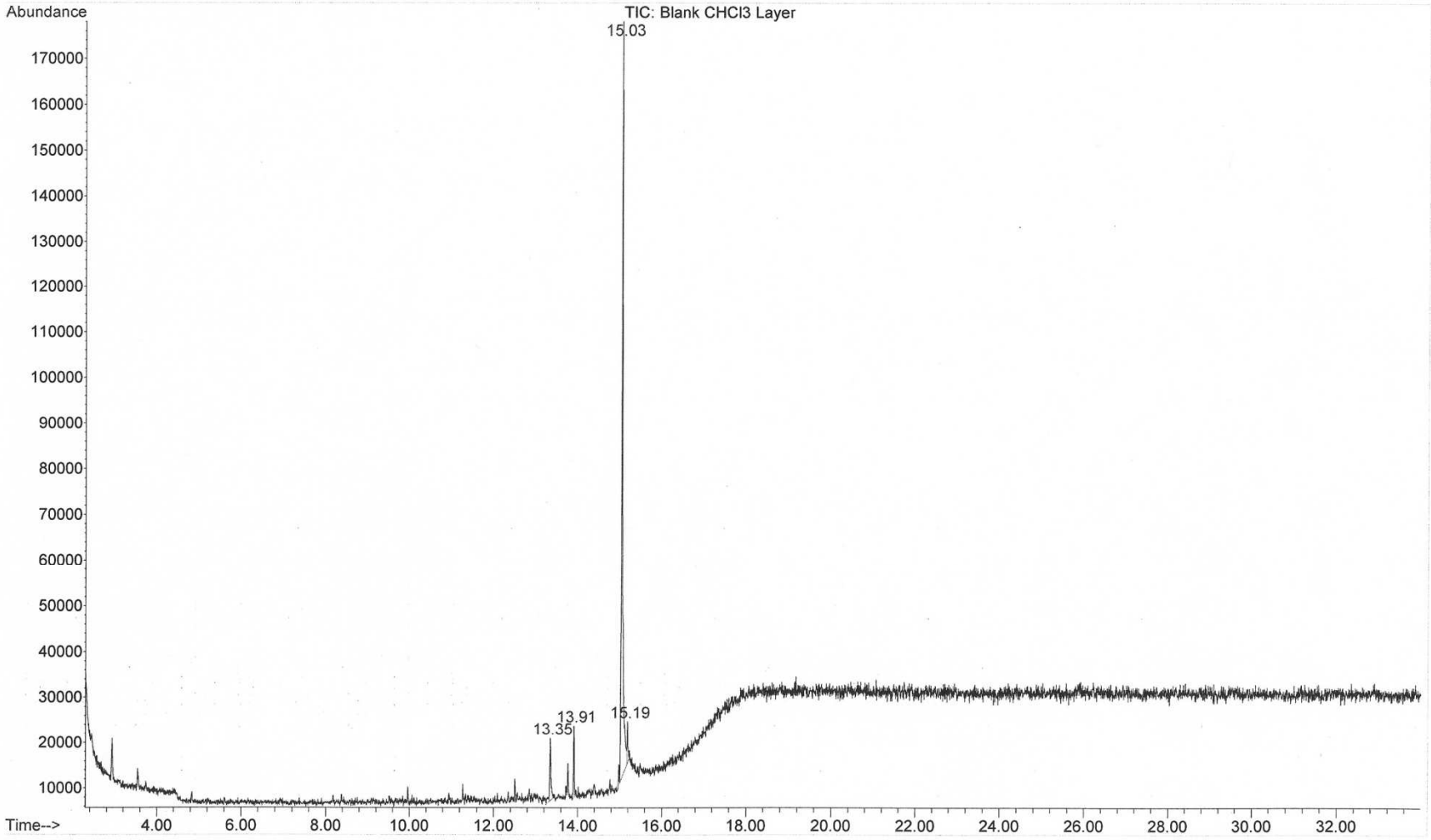
Qualitative EI GC-MS total ion chromatogram and mass spectrum for the 3-naphthoylindole standard

3-naphthyolindole was also provided for this project via in-house synthesis (without an associated purity). Trace amounts of indole at 6.74 min, N-Methyl-2-naphthamide at 10.28 min, and 1,4-dihydro-1,4-methanonaphthalene-6-carboxylic acid at 11.41 min were identified using the NIST 2014 MS library (not displayed in the given figure above). A small impurity, that was unable to be identified (base peak m/z 305; secondary base peak m/z 288), at 20.80 minutes did exist but was considered negligible.

APPENDIX C – PYROGRAMS REPRESENTATIVE OF THE MATRIX COMPONENTS IN BLANK WOOL AND BLANK SOLVENT LAYER EXTRACTS



EI GC-MS total ion chromatogram showing the matrix components present in a blank wool extract



EI GC-MS total ion chromatogram showing the matrix components present in a blank wool extract

APPENDIX D - AMOUNT OF EACH SYNTHETIC CANNABINOID PYROLYZED

Listed below is the amount of each controlled substance that was pyrolyzed in each trial. Also included is the amount of quartz wool that was used to 'sandwich' the chemical.

JWH-030

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	64.61 mg	75 µL	16.32 mg
Trial #2	59.62 mg	75 µL	25.39 mg
Trial #3	46.52 mg	75 µL	22.69 mg

Due to limit quantity of the JWH-030 standard, only 75 microliters of the 20 mg/mL standard were used per trial. Samples were allowed to air dry (overnight) before pyrolysis.

JWH-018

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	51.6 mg	5.46 mg	24.4 mg
Trial #2	51.0 mg	5.67 mg	26.1 mg
Trial #3	48.9 mg	7.34 mg	22.2 mg

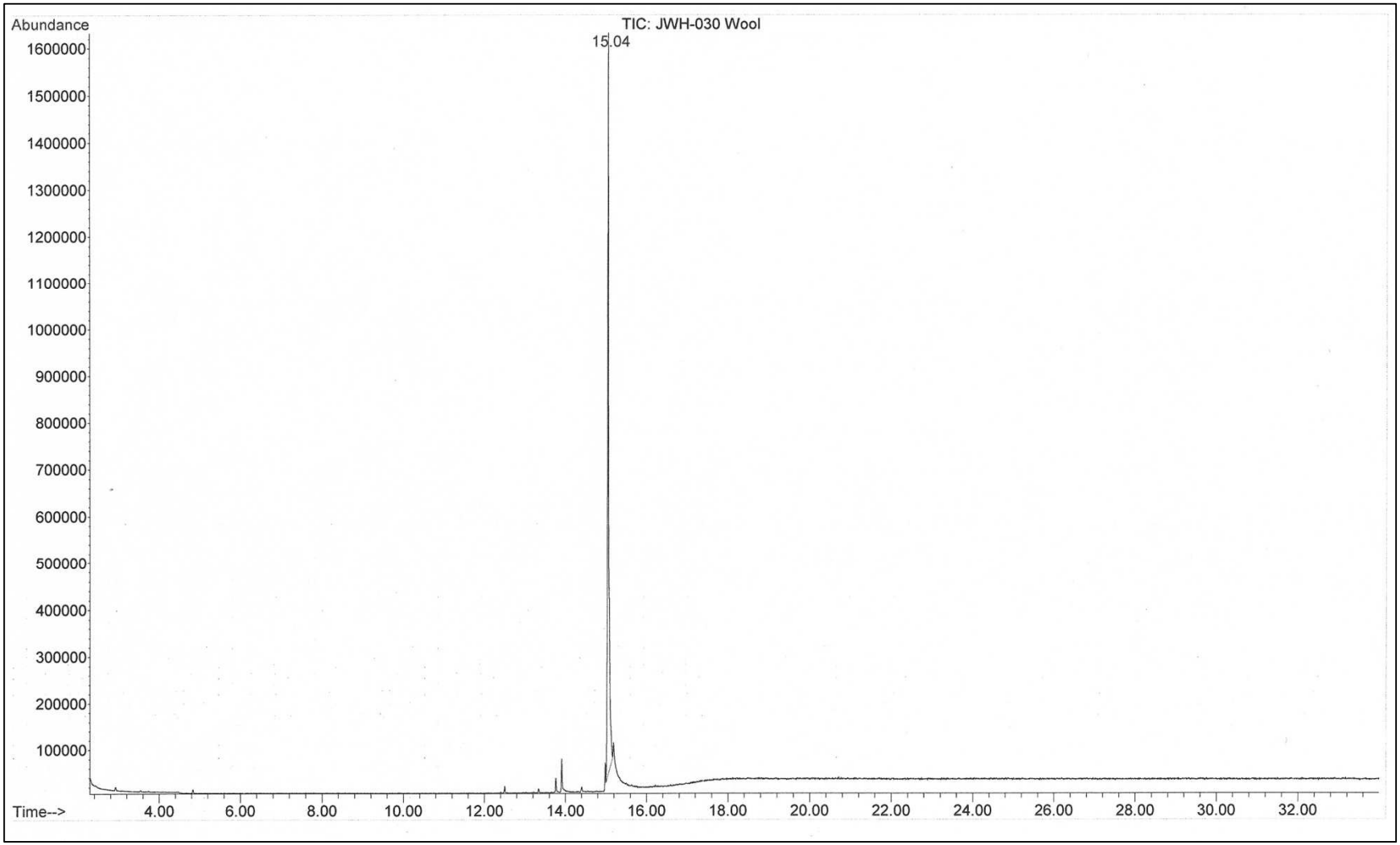
JWH-081

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	49.9 mg	5.0 mg	18.2 mg
Trial #2	51.5 mg	4.8 mg	18.9 mg
Trial #3	48.2 mg	5.6 mg	20.1 mg

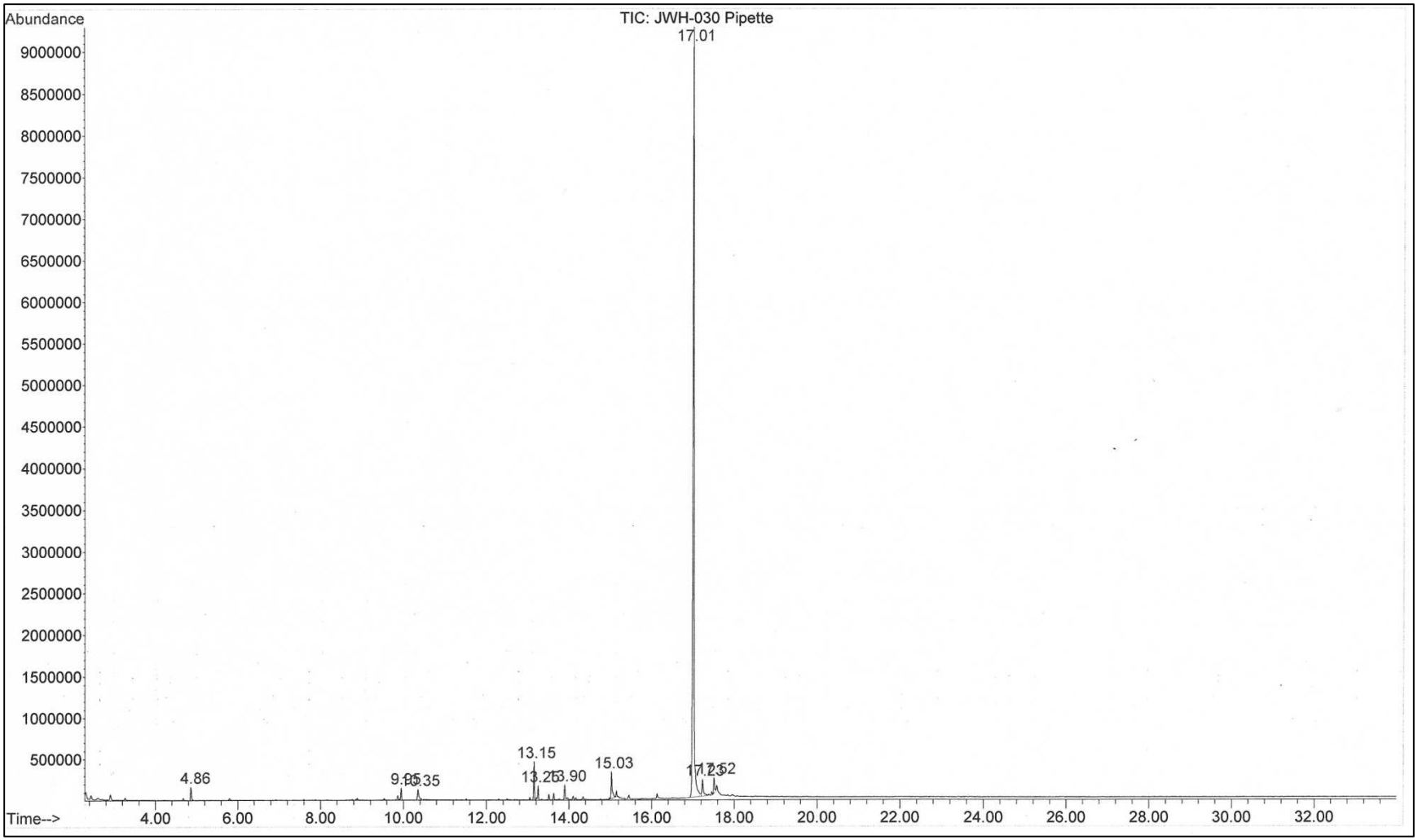
UR-144

	Bottom Plug of Wool	Chemical	Top Plug of Wool
Trial #1	50.6 mg	5.1 mg	23.5 mg
Trial #2	58.2 mg	5.4 mg	17.2 mg
Trial #3	49.9 mg	5.0 mg	20.9 mg

APPENDIX E - PYROGRAMS REPRESENTATIVE OF JWH-030

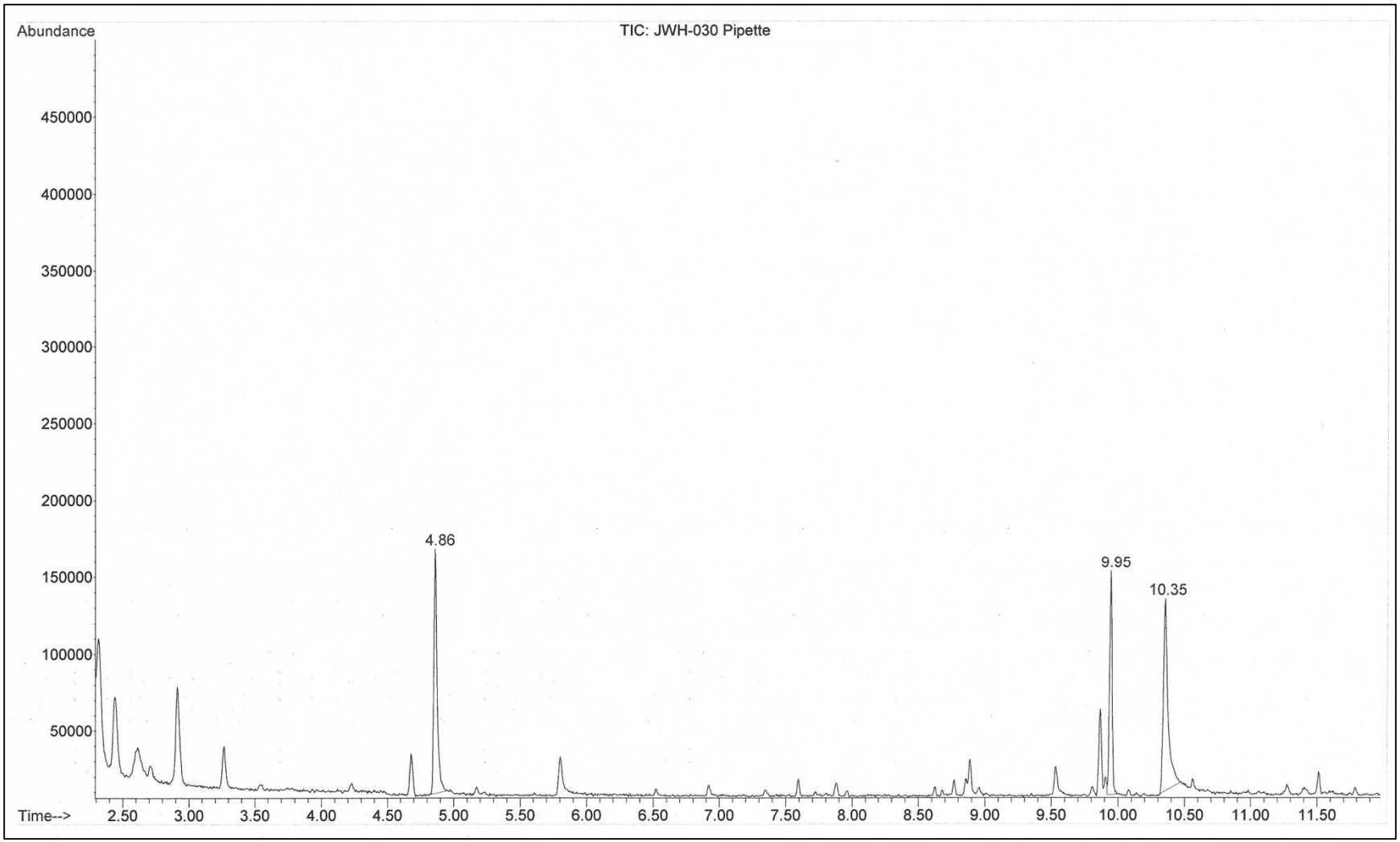


Pyrogram representative of the JWH-030 wool extract

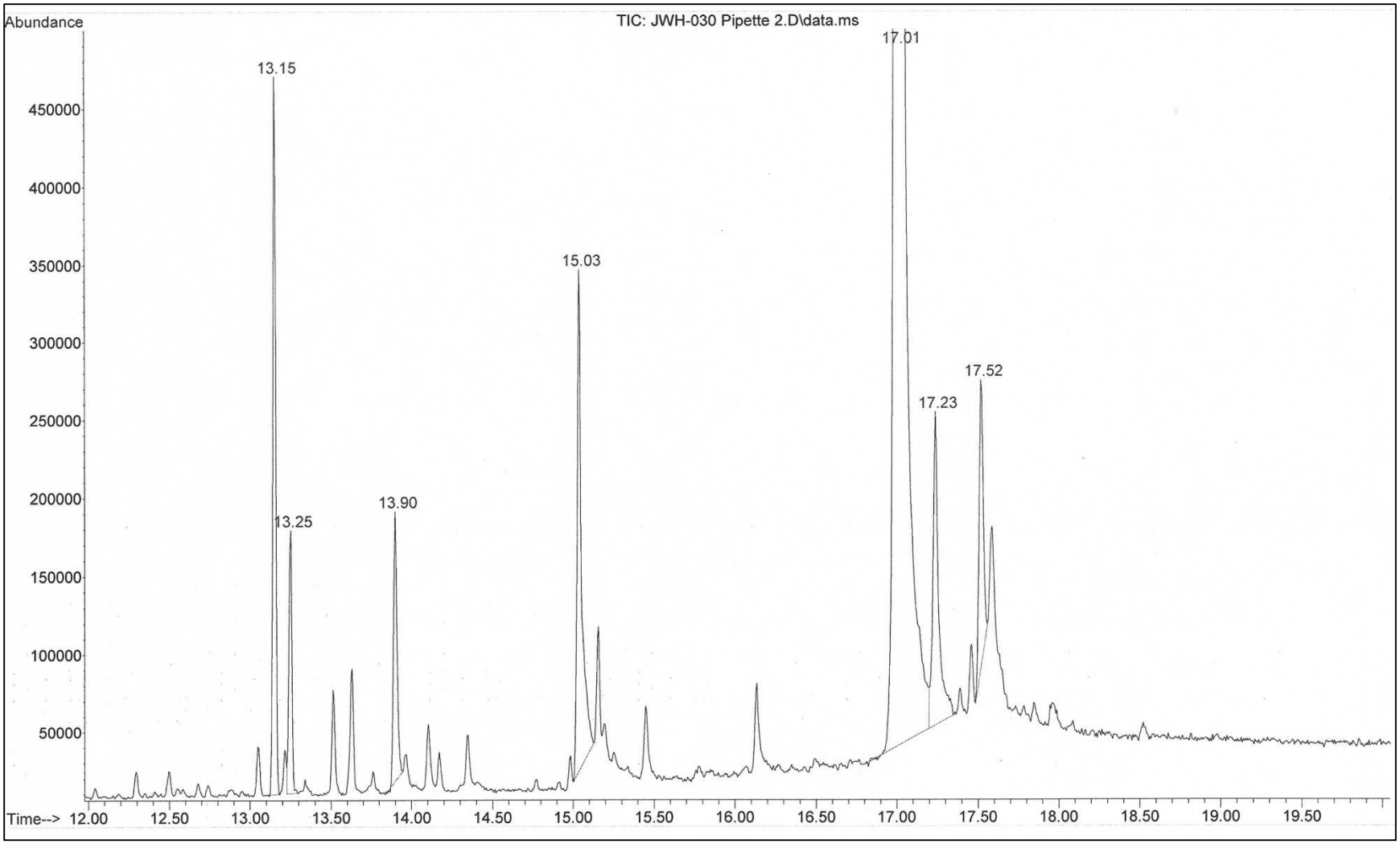


Pyrogram representative of the JWH-030 pipette extract

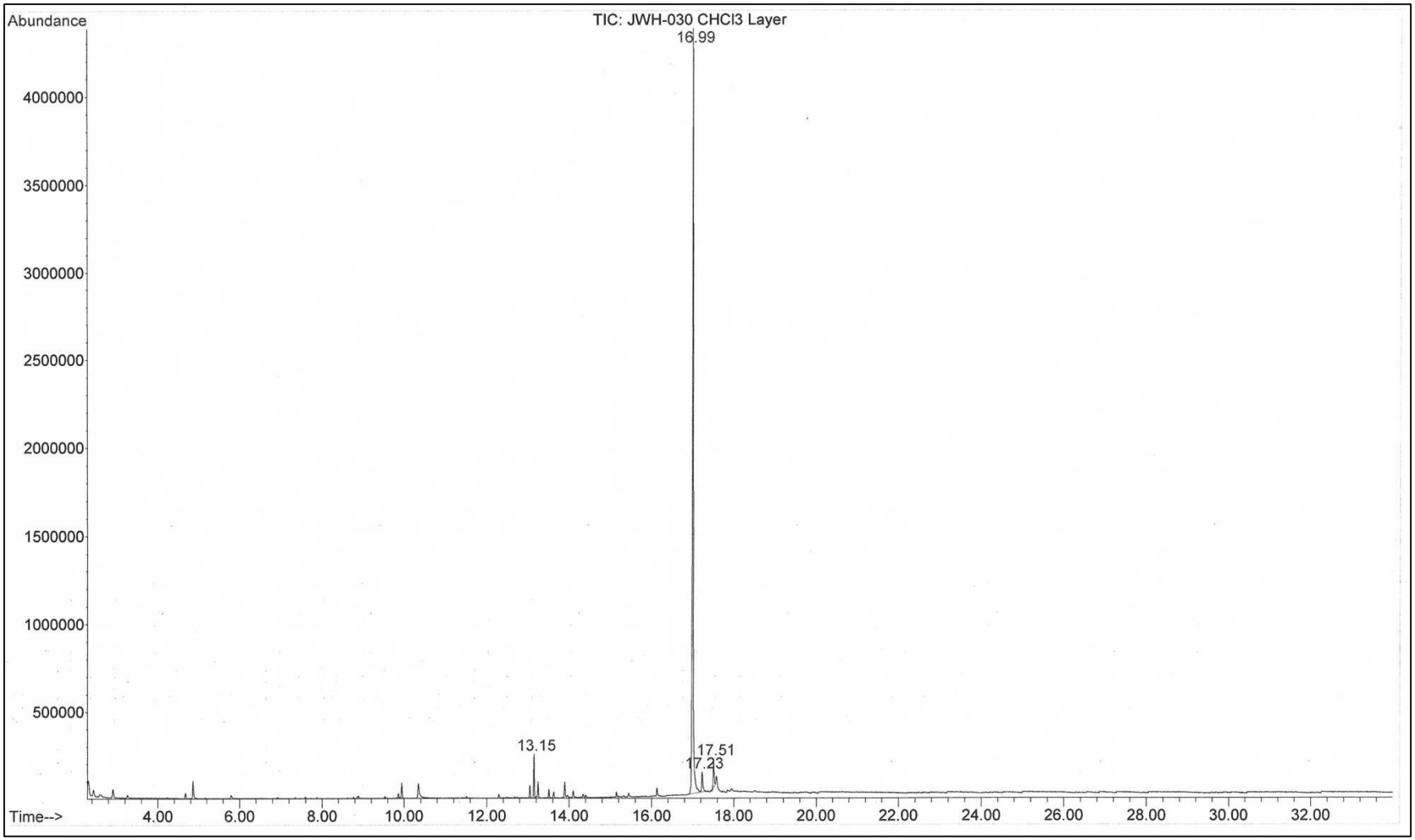
Zoomed in versions of this pyrogram can be found on the following two pages



The first of two pyrograms from the JWH-030 pipette extract which have been magnified to enlarge details

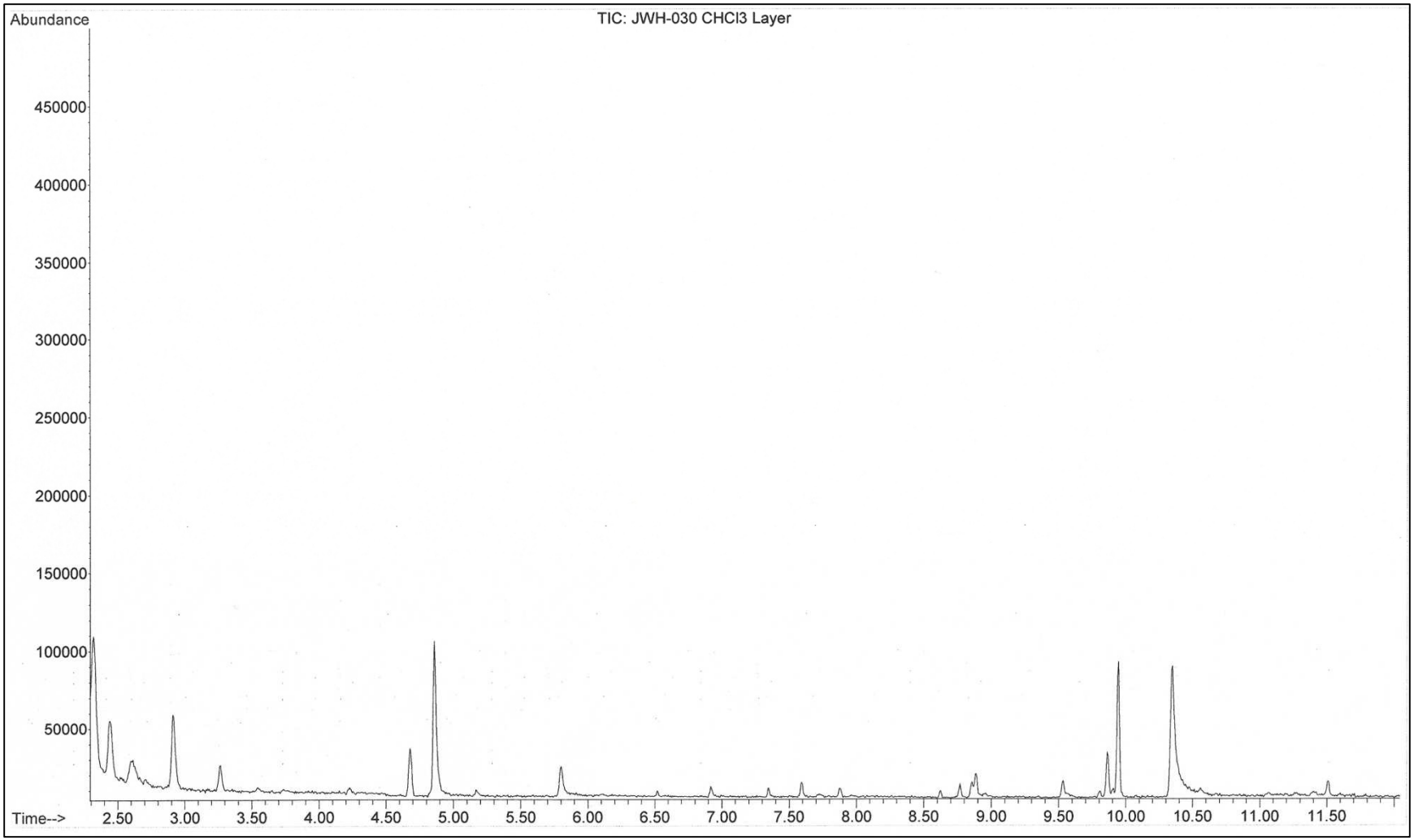


The second of two pyrograms from the JWH-030 pipette extract which have been magnified to enlarge details

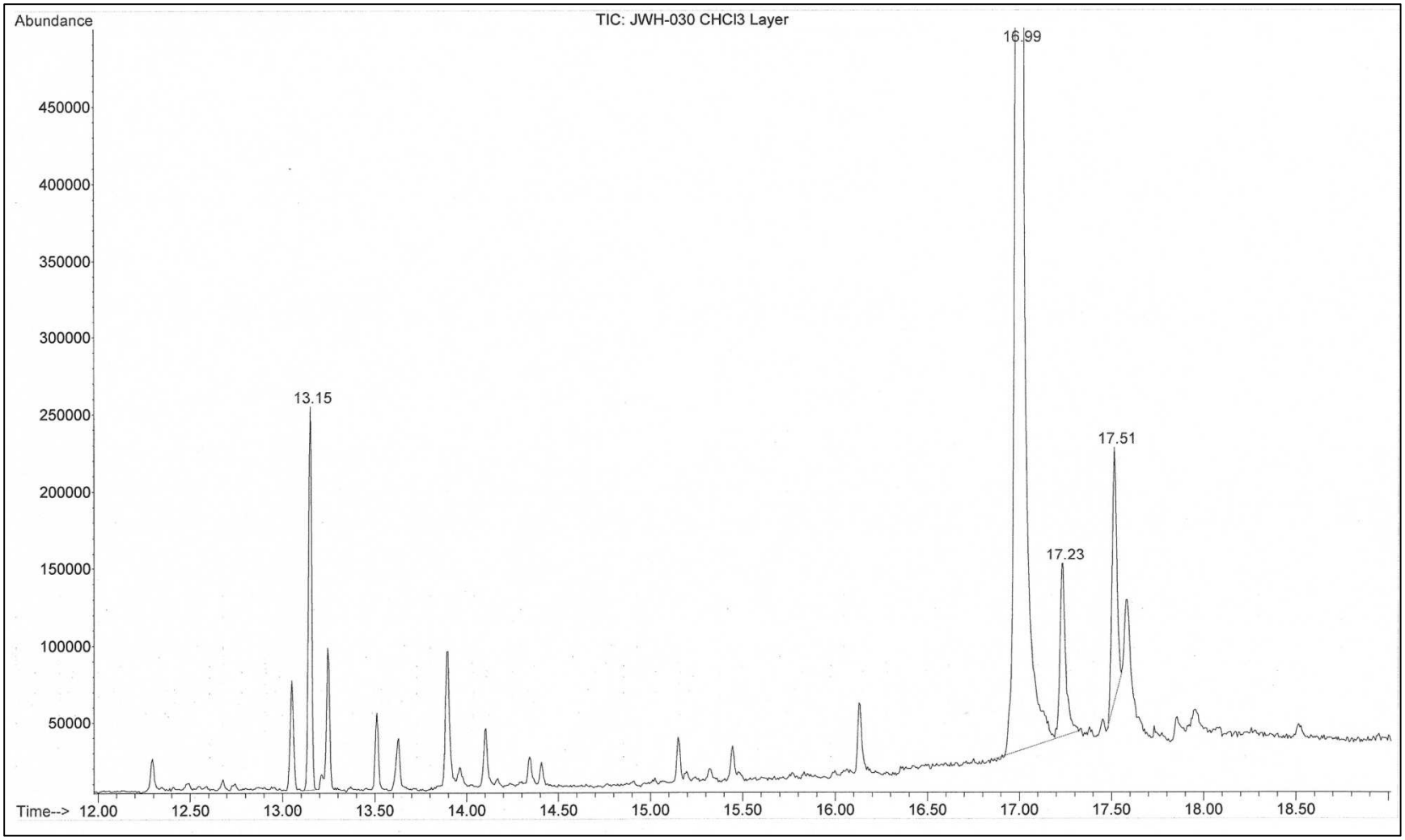


Pyrogram representative of the JWH-030 solvent layer extract

Zoomed in versions of this pyrogram can be found on the following two pages

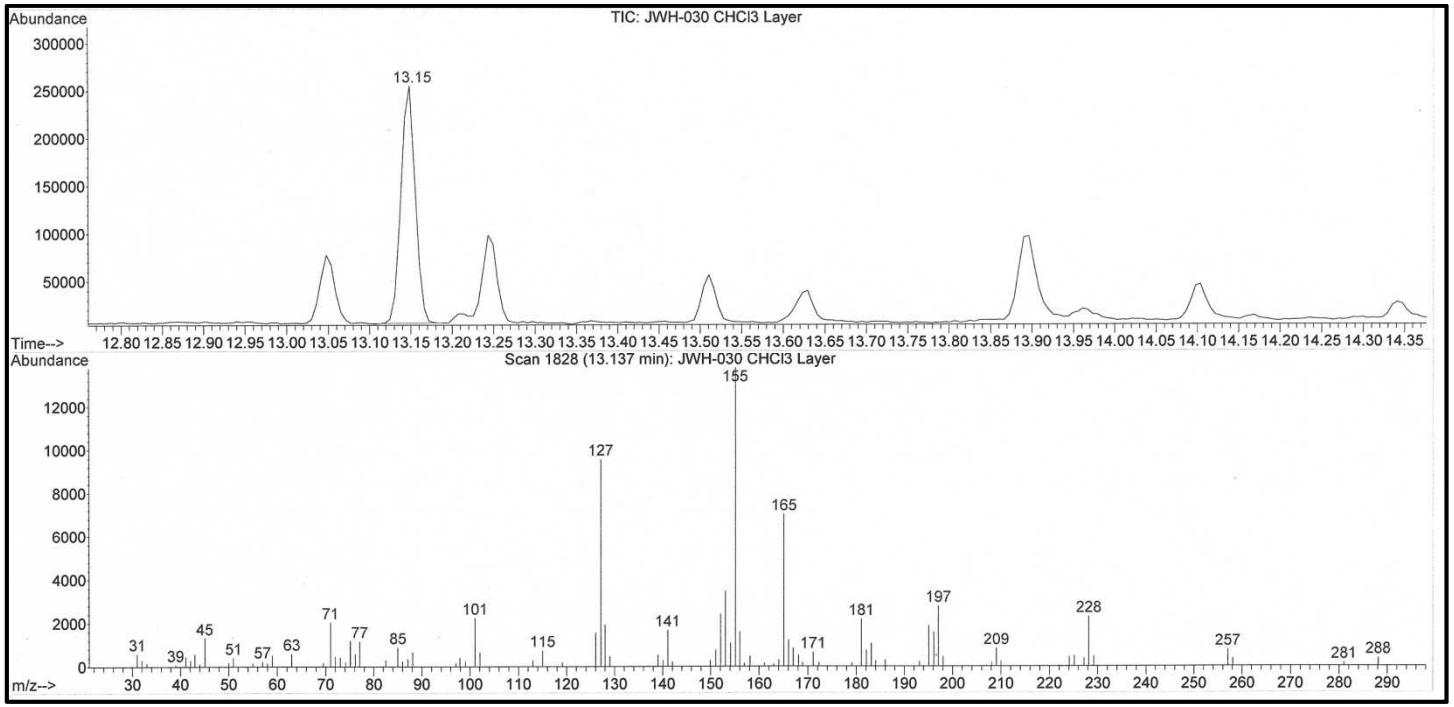


The first of two pyrograms from the JWH-030 solvent layer extract which have been magnified to enlarge details



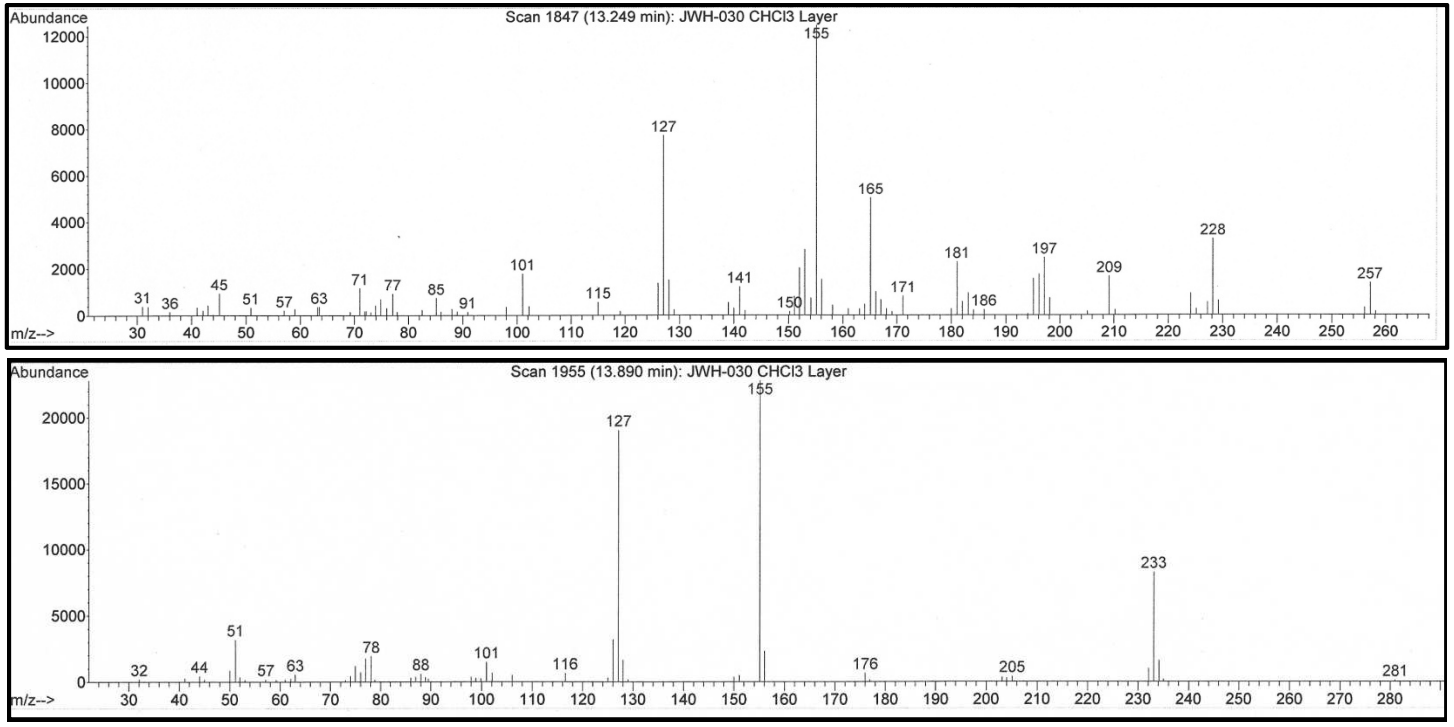
The second of two pyrograms from the JWH-030 solvent layer extract which have been magnified to enlarge details

APPENDIX F – CHROMATOGRAPHIC AND MASS SPECTRAL INFORMATION OF THE UNIDENTIFIED JWH-030 PYROLYTIC PRODUCTS

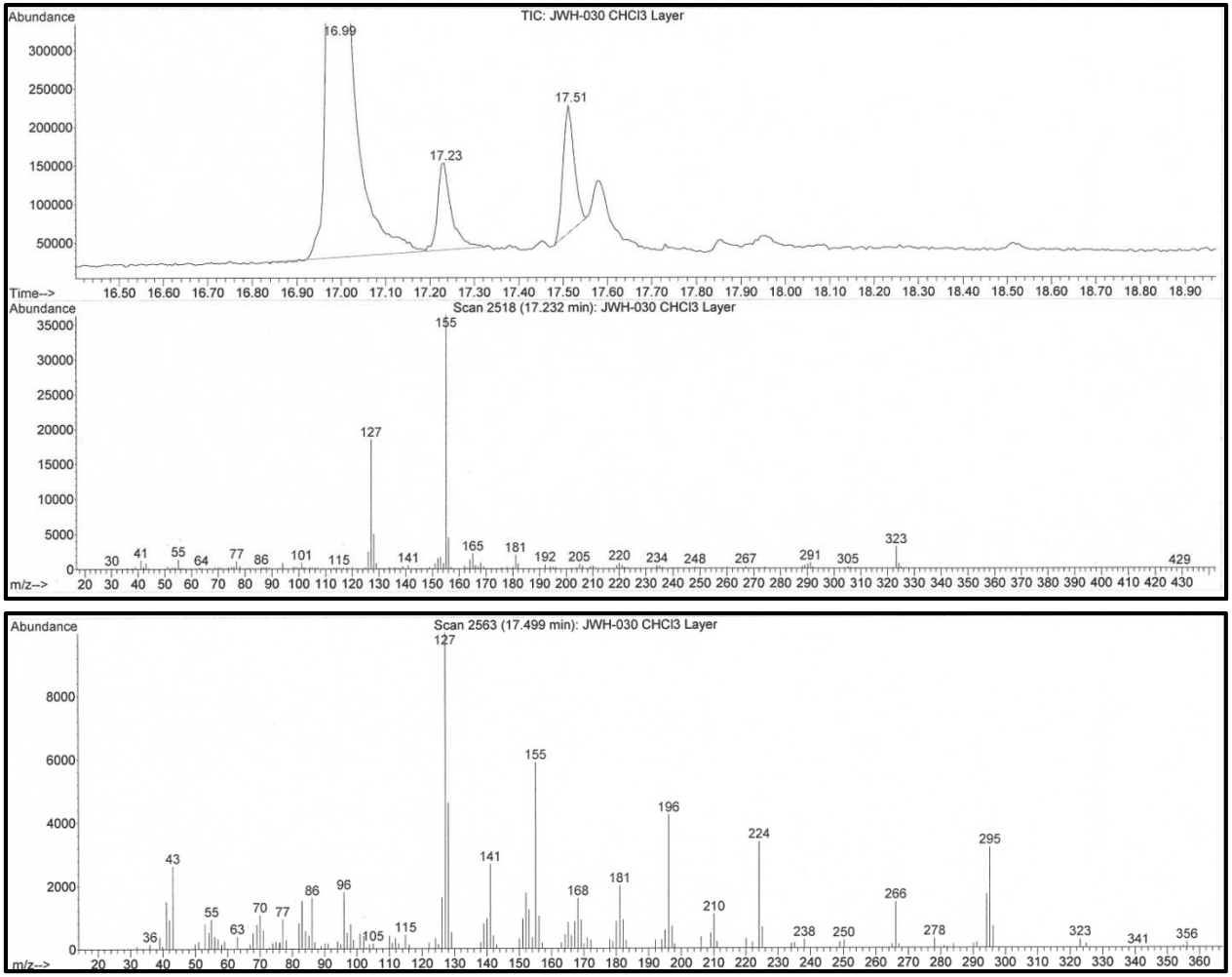


Chromatographic display (pyrogram; top) for the first three unidentifiable pyrolytic products at 13.15, 13.25, and 13.90 minutes; and mass spectra results of the unknown at 13.15 minutes

Spectra for the other four pyrolytic products are provided on the following two pages

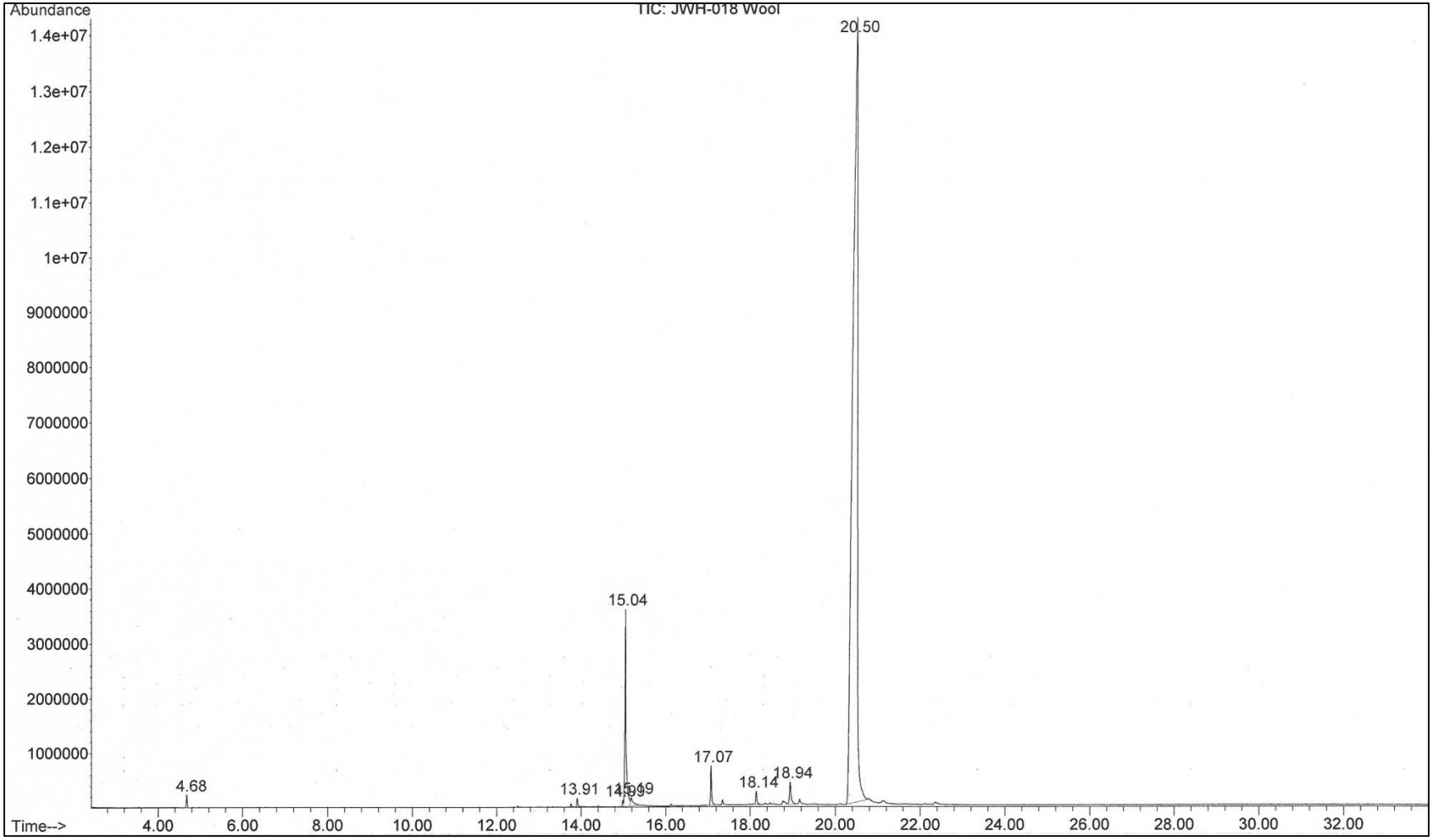


Mass spectra for the pyrolytic products at 13.25 and 13.90 minutes (see above page for magnified image of pyogram)



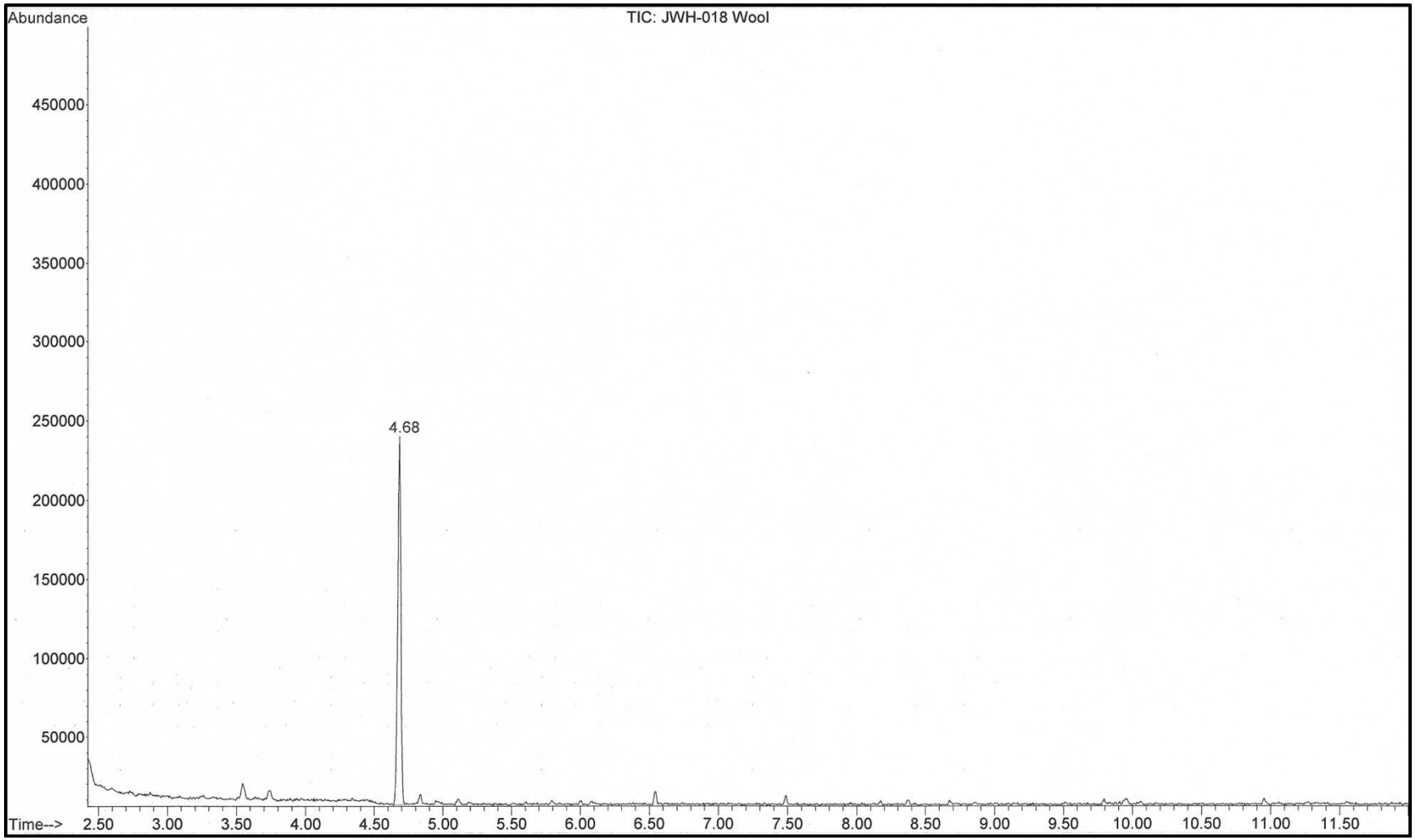
Chromatographic display (pyrogram; top) and mass spectral results of the two unidentifiable pyrolytic products at 17.23 minutes (top) and 17.51 minutes (bottom)

APPENDIX G – PYROGRAMS REPRESENTATIVE OF JWH-018

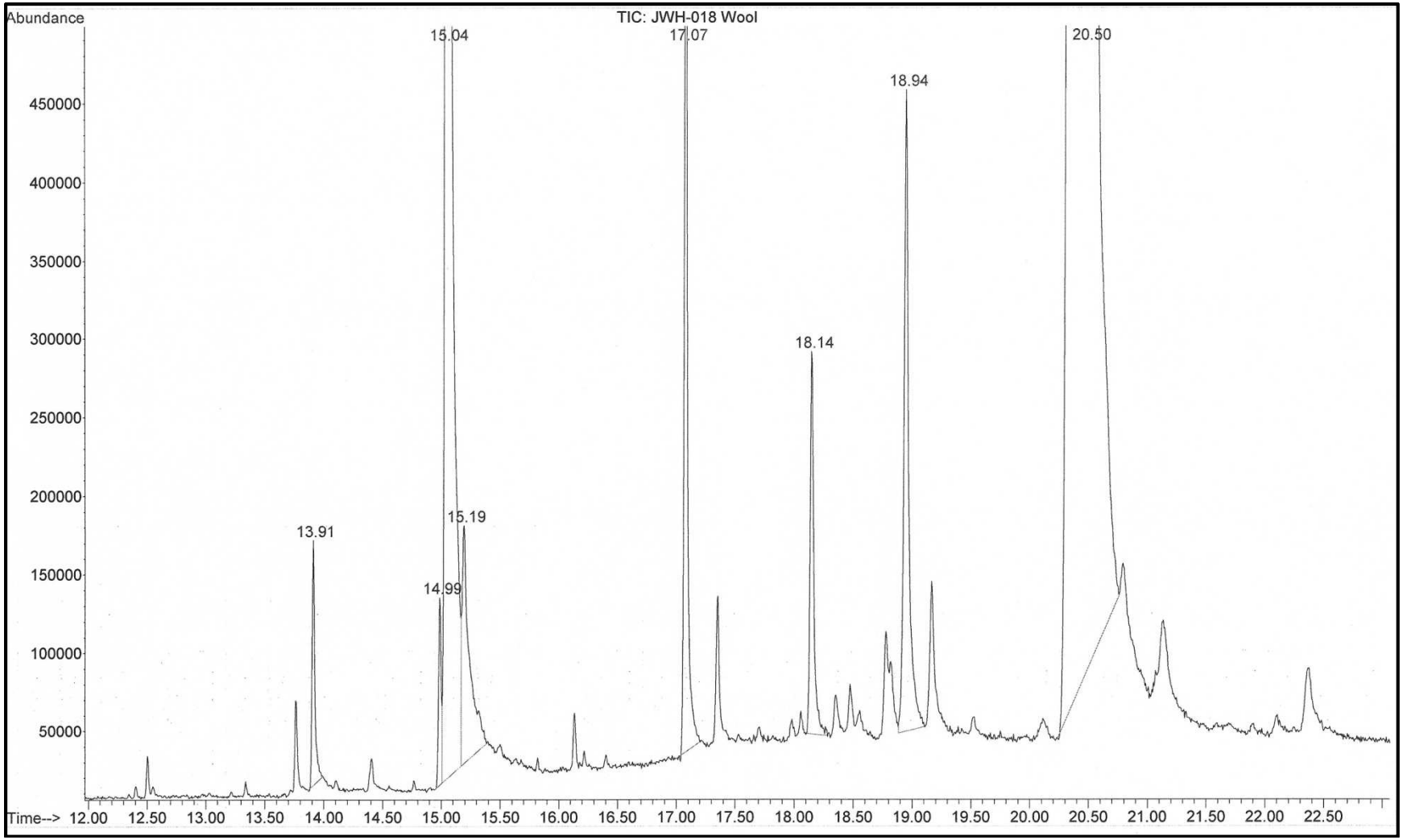


Pyrogram representative of the JWH-018 wool extract

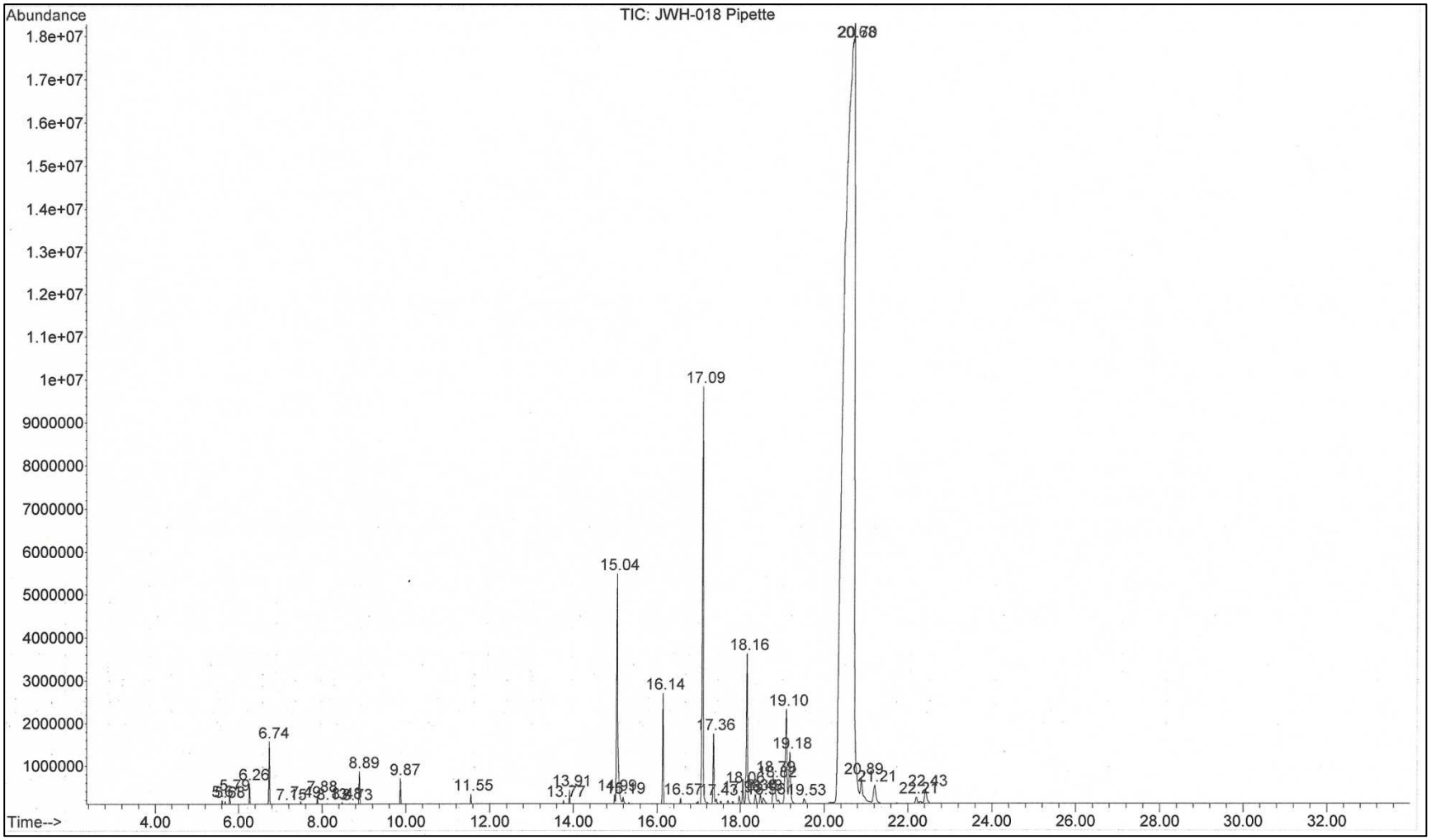
Zoomed in versions of this pyrogram can be found on the following two pages



The first of two pyrograms from the JWH-018 wool extract which have been magnified to enlarge details

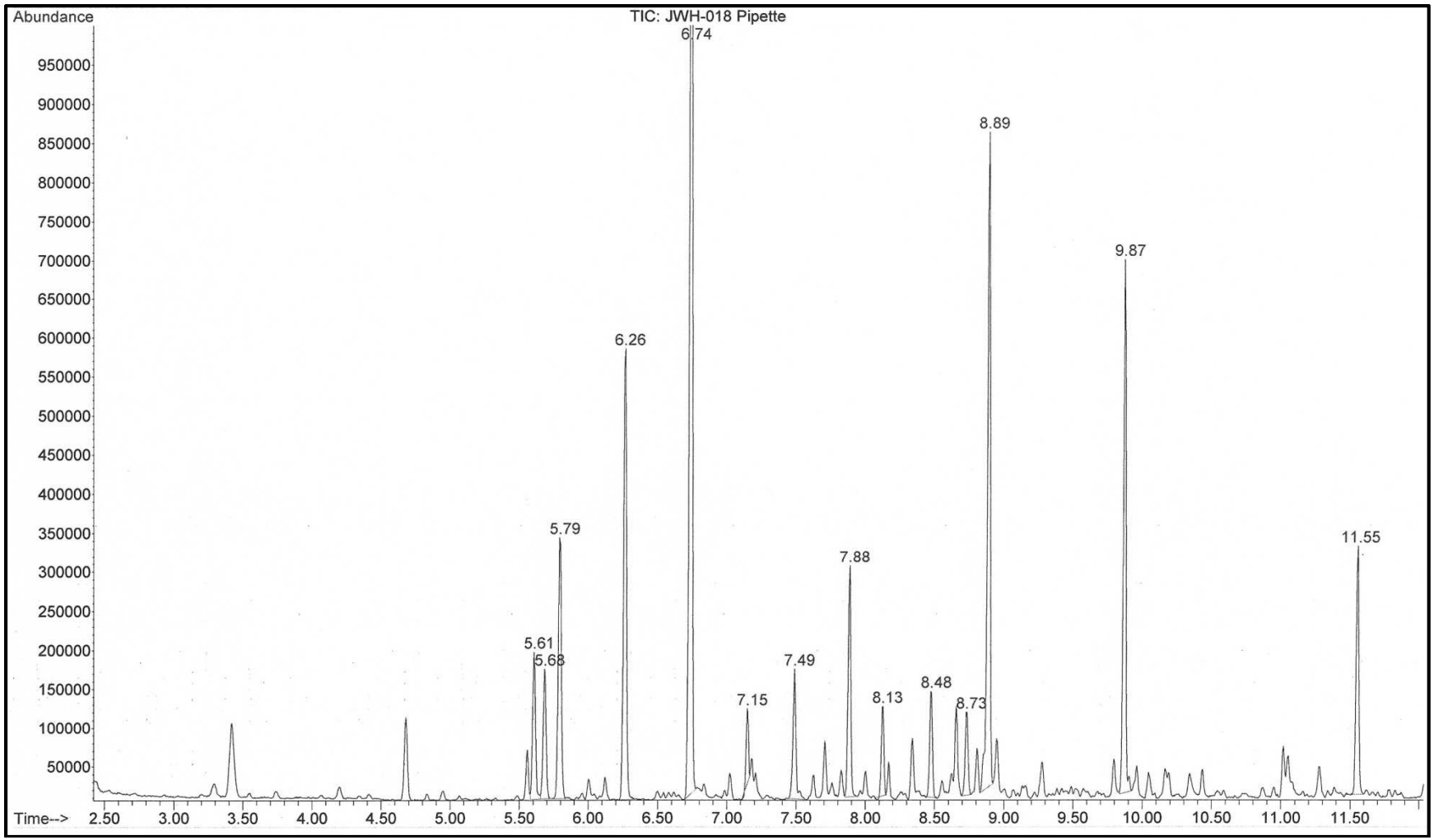


The second of two pyrograms from the JWH-018 wool extract which have been magnified to enlarge details

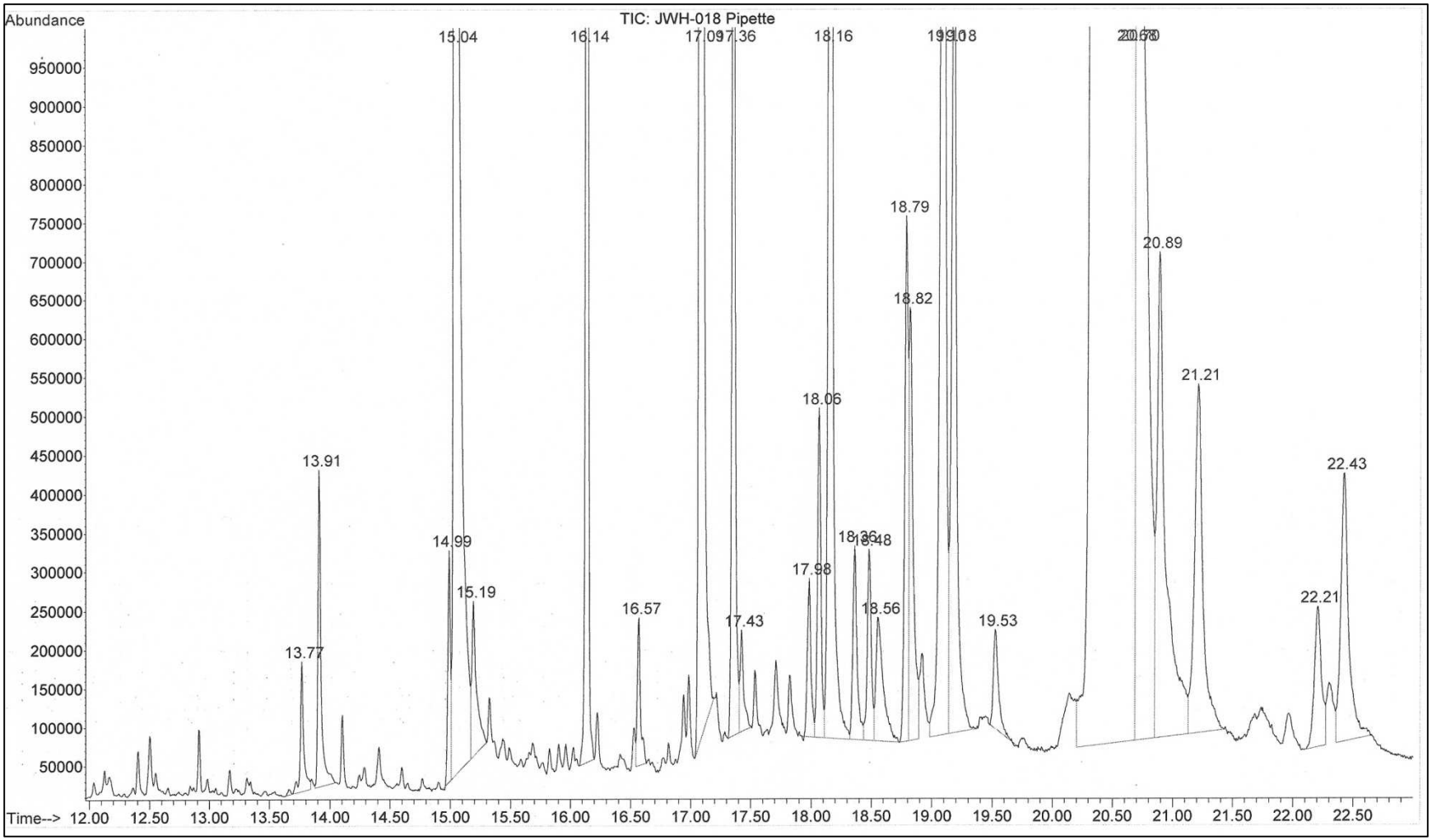


Pyrogram representative of the JWH-018 pipette extract

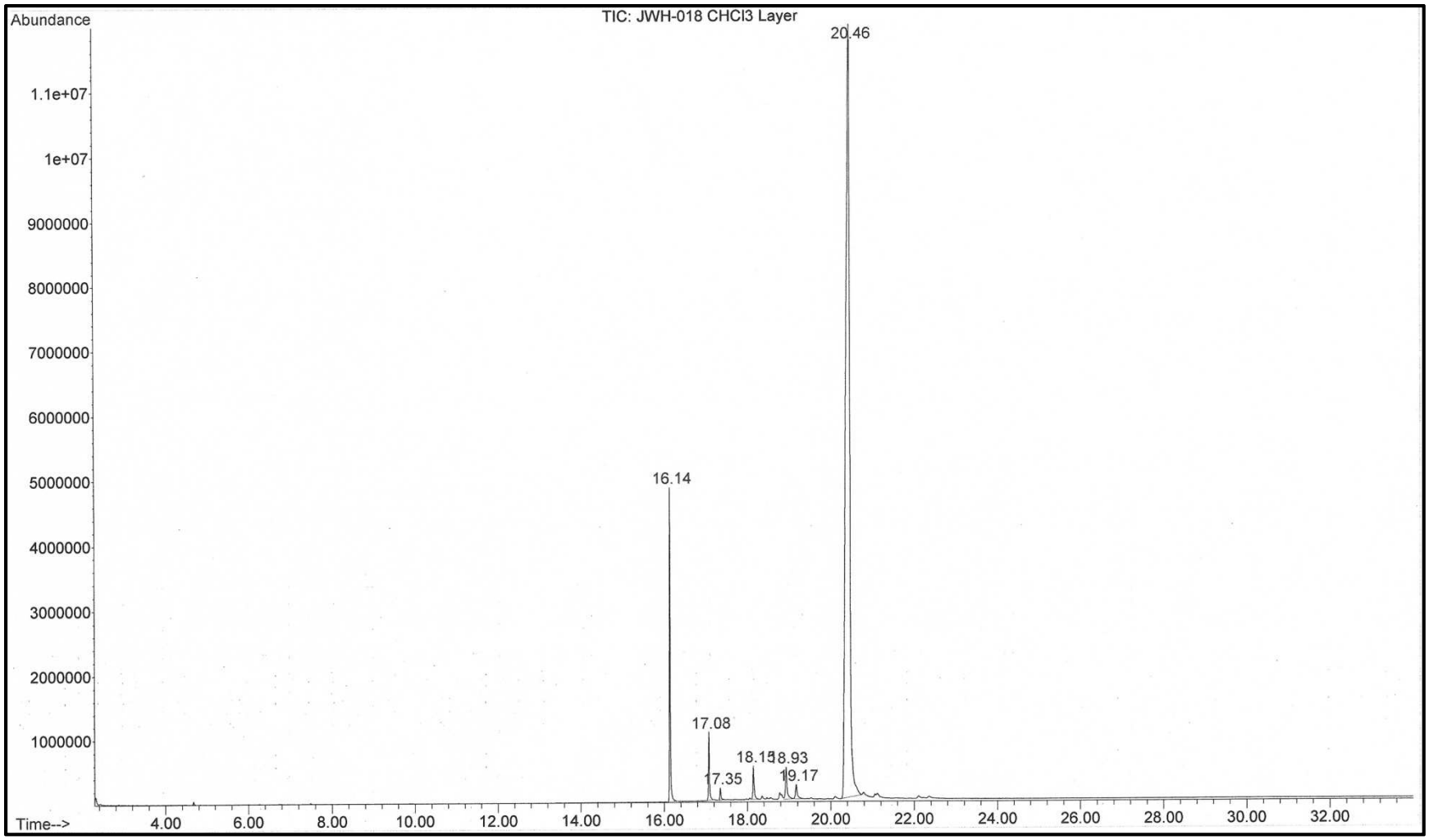
Zoomed in versions of this pyrogram can be found on the following two pages



The first of two pyrograms from the JWH-018 pipette extract which have been magnified to enlarge details

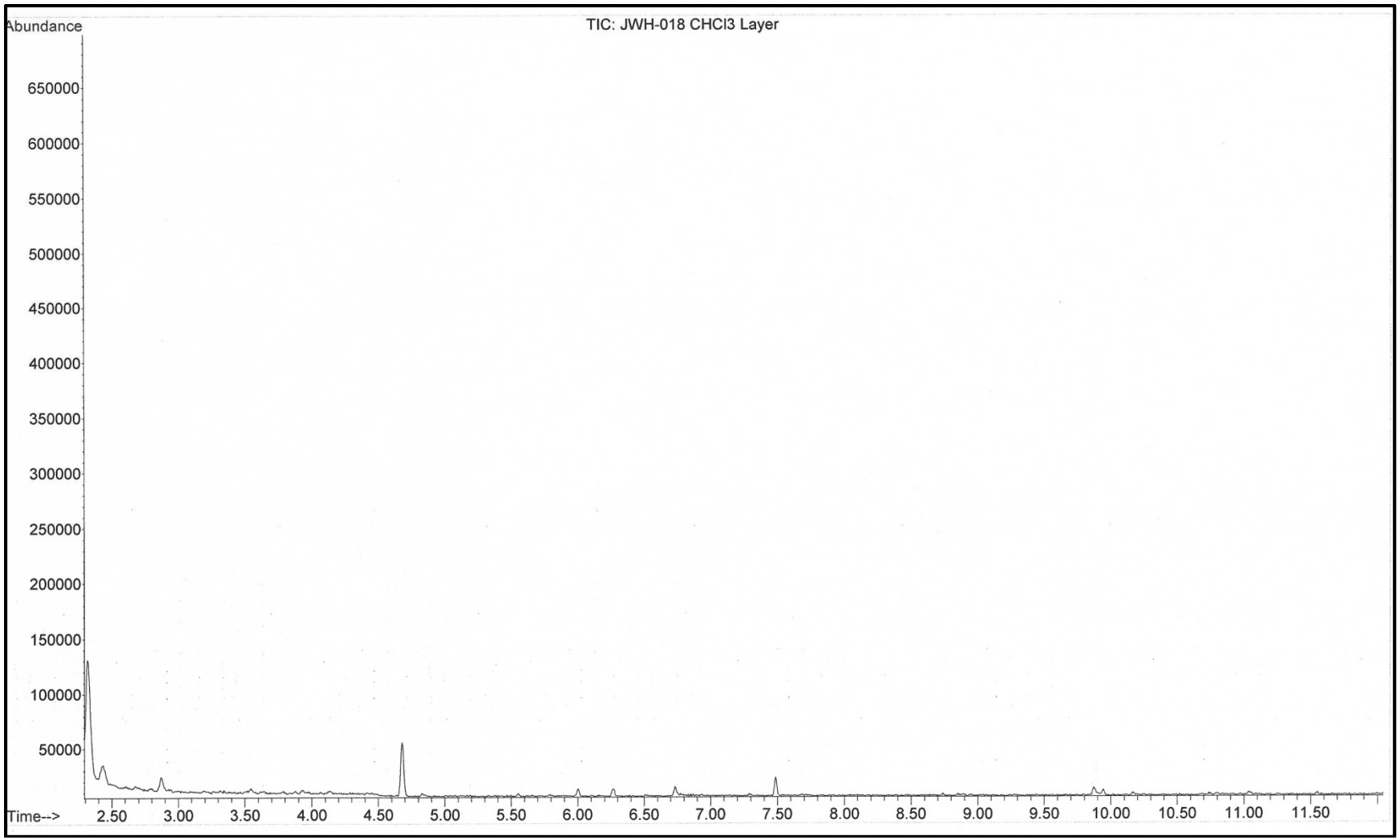


The second of two pyrograms from the JWH-018 pipette extract which have been magnified to enlarge details

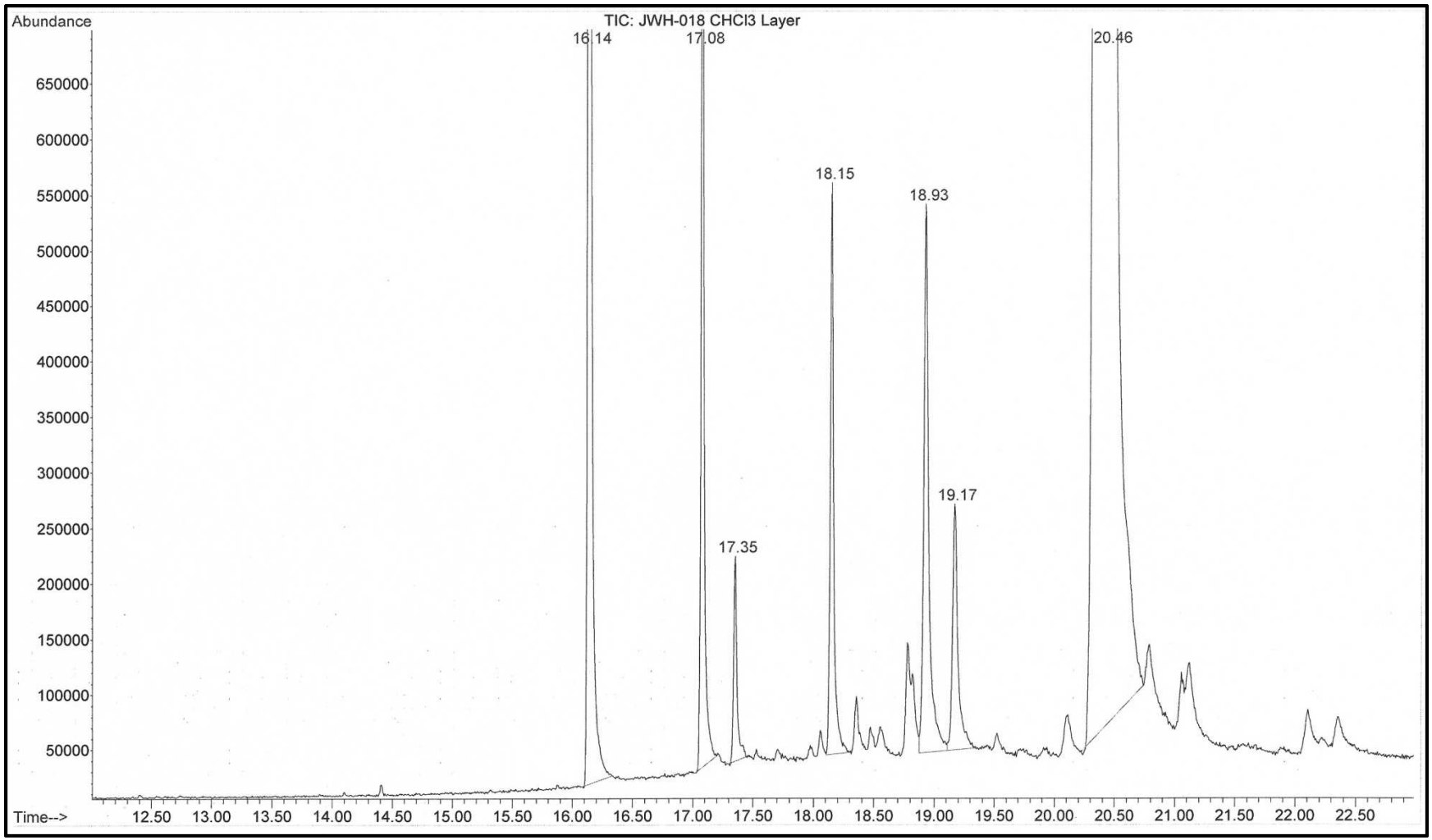


Pyrogram representative of the JWH-018 solvent layer extract

Zoomed in versions of this pyrogram can be found on the following two pages

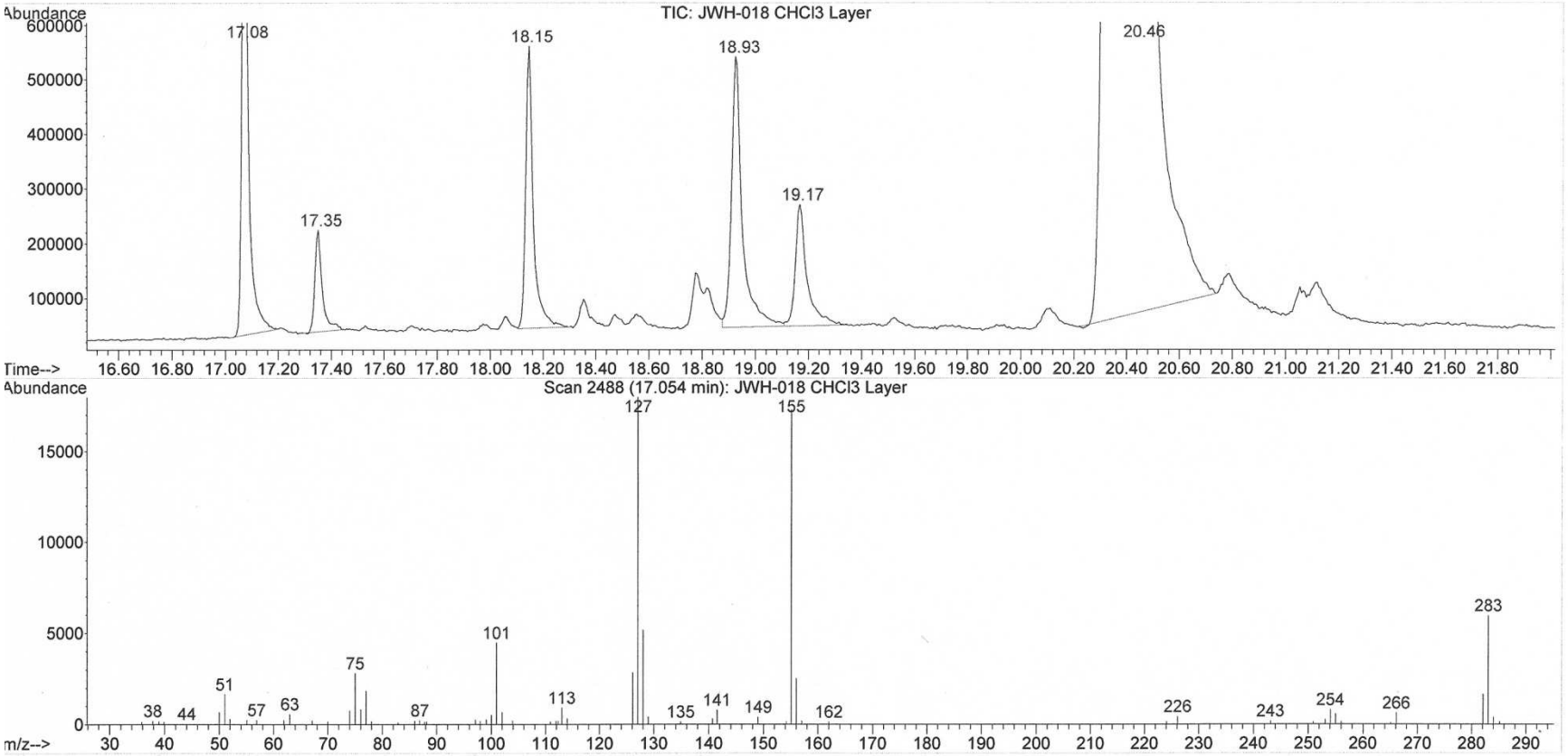


The first of two pyrograms from the JWH-018 solvent layer extract which have been magnified to enlarge details



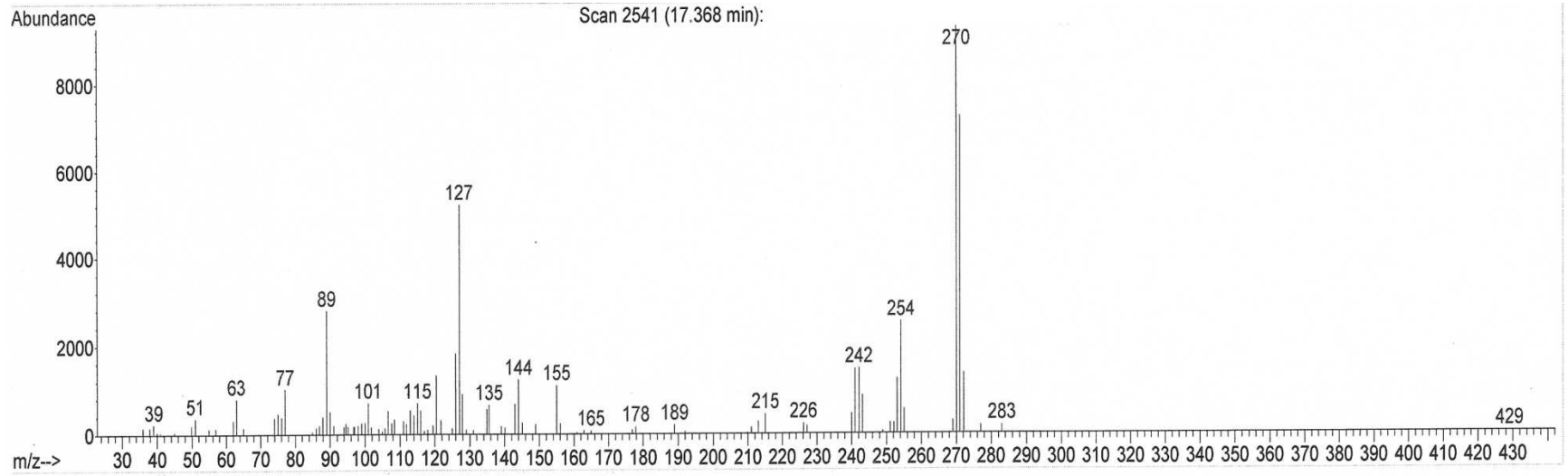
The second of two pyrograms from the JWH-018 solvent layer extract which have been magnified to enlarge details

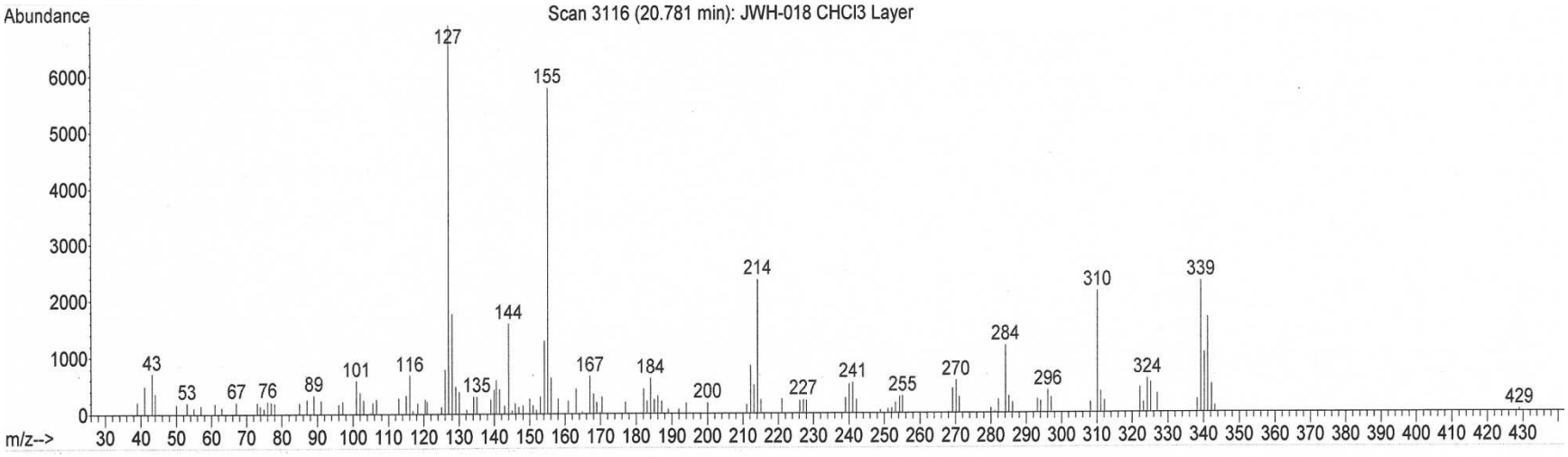
APPENDIX H – CHROMATOGRAPHIC AND MASS SPECTRAL INFORMATION OF THE UNIDENTIFIED JWH-018 PYROLYTIC PRODUCTS



Chromatographic display (pyrogram; top) for the first unidentifiable pyrolytic product at 17.05, 17.37, and 20.78 minutes and mass spectra results of this unknown at 17.05 minuets

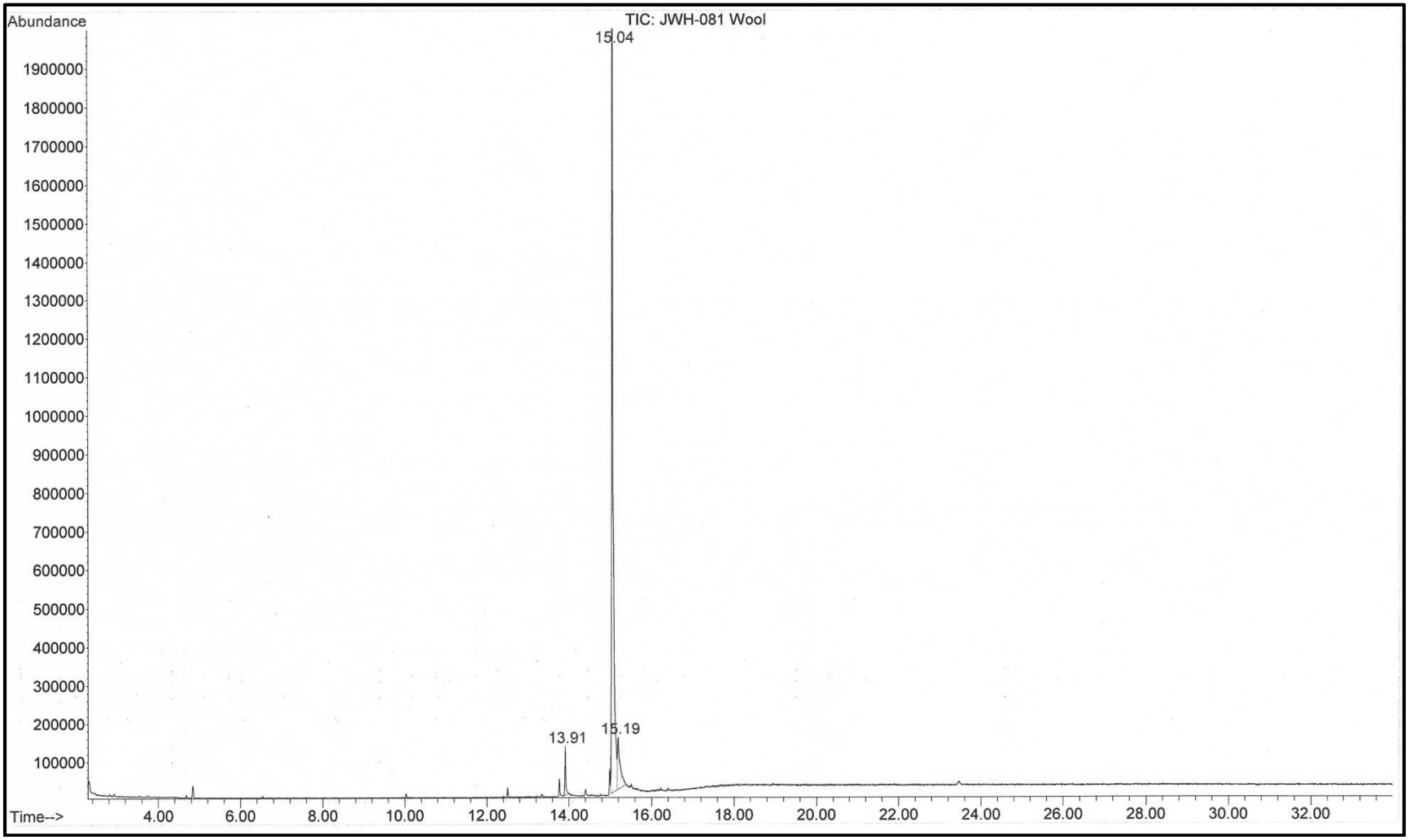
Data for the other two pyrolytic products are provided on the following page



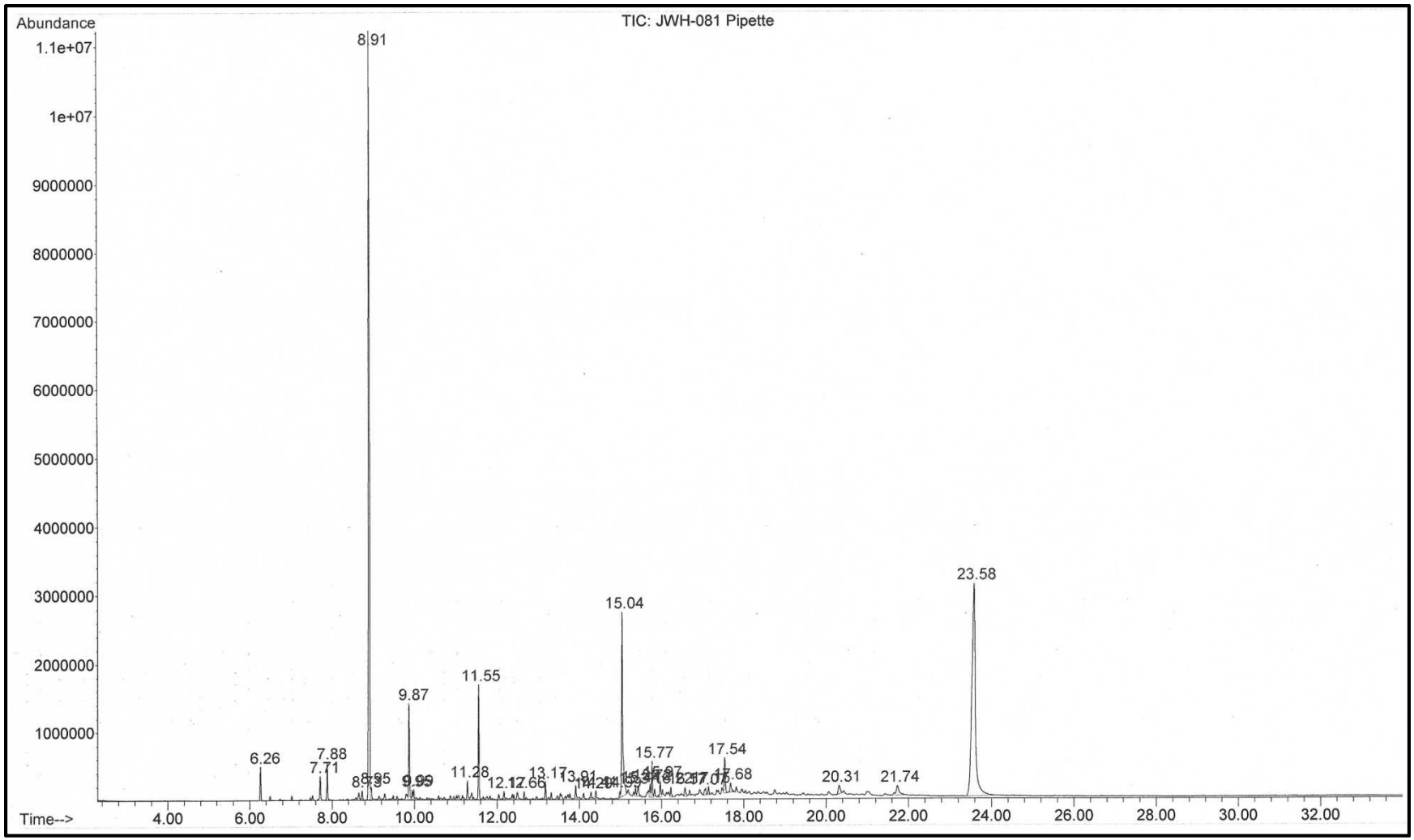


Mass spectra for the pyrolytic products at 17.39 and 20.78 minutes (see above page for magnified image of pyogram)

APPENDIX I – PYROGRAMS REPRESENTATIVE OF JWH-081

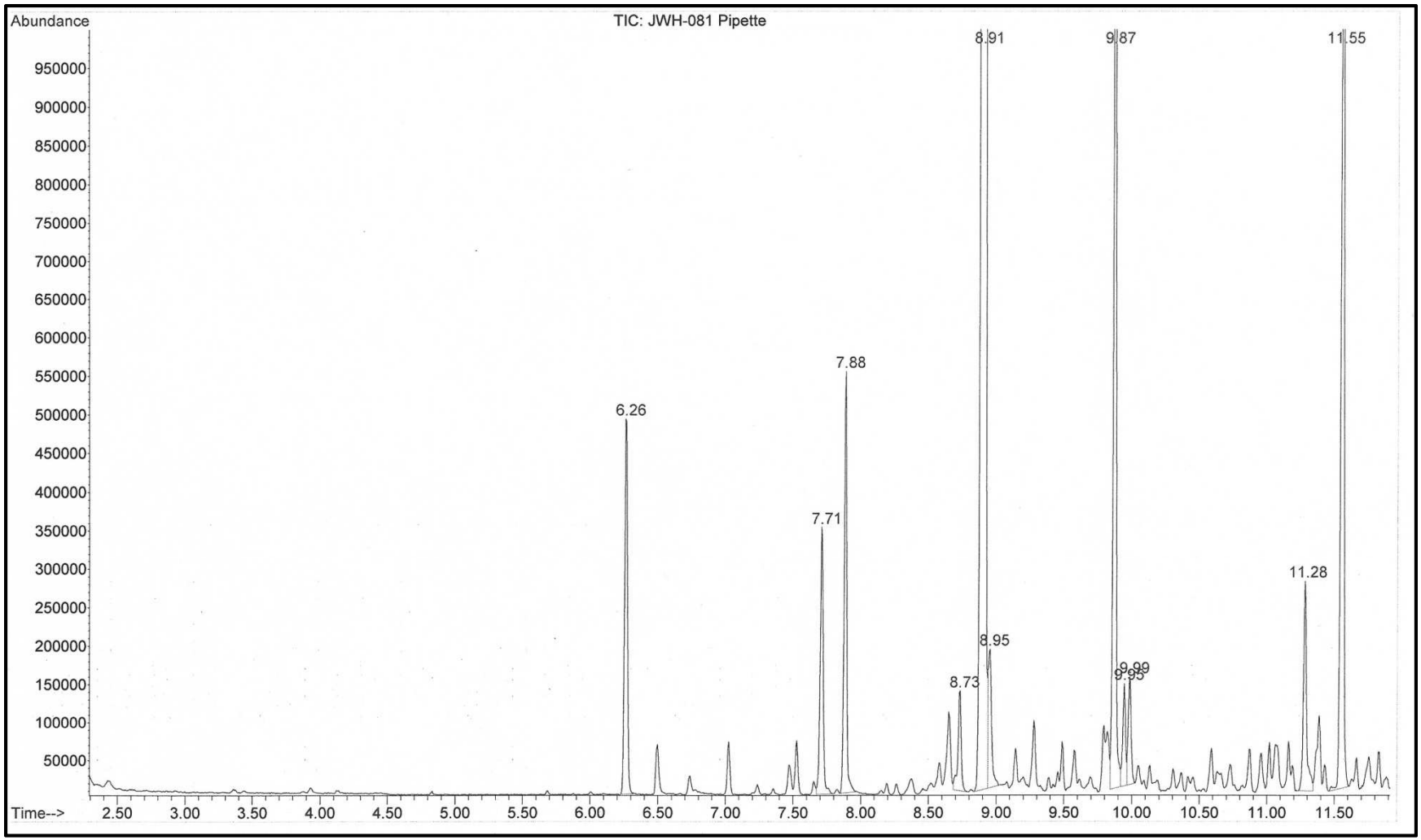


Pyrogram representative of the JWH-081 wool extract

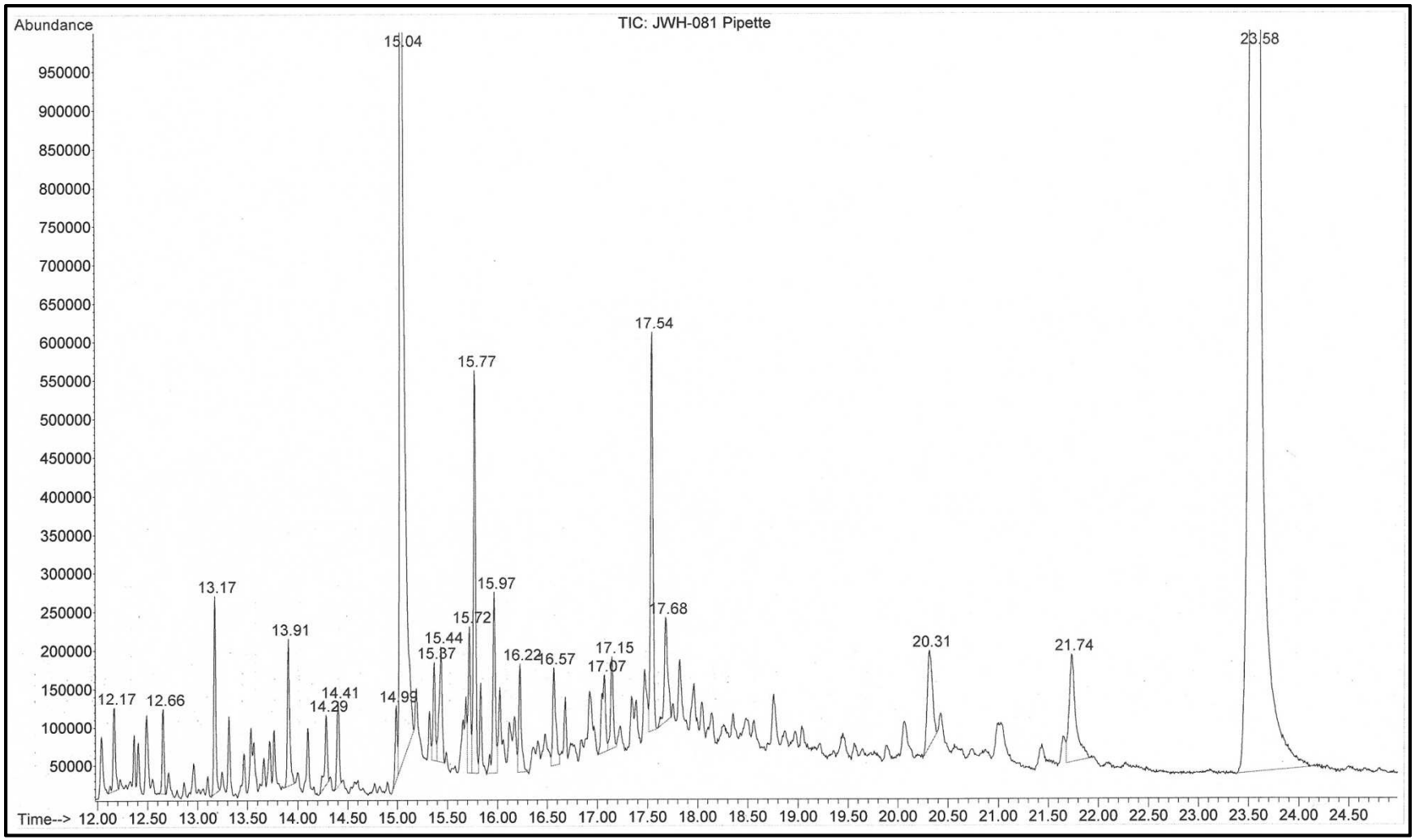


Pyrogram representative of the JWH-081 pipette extract

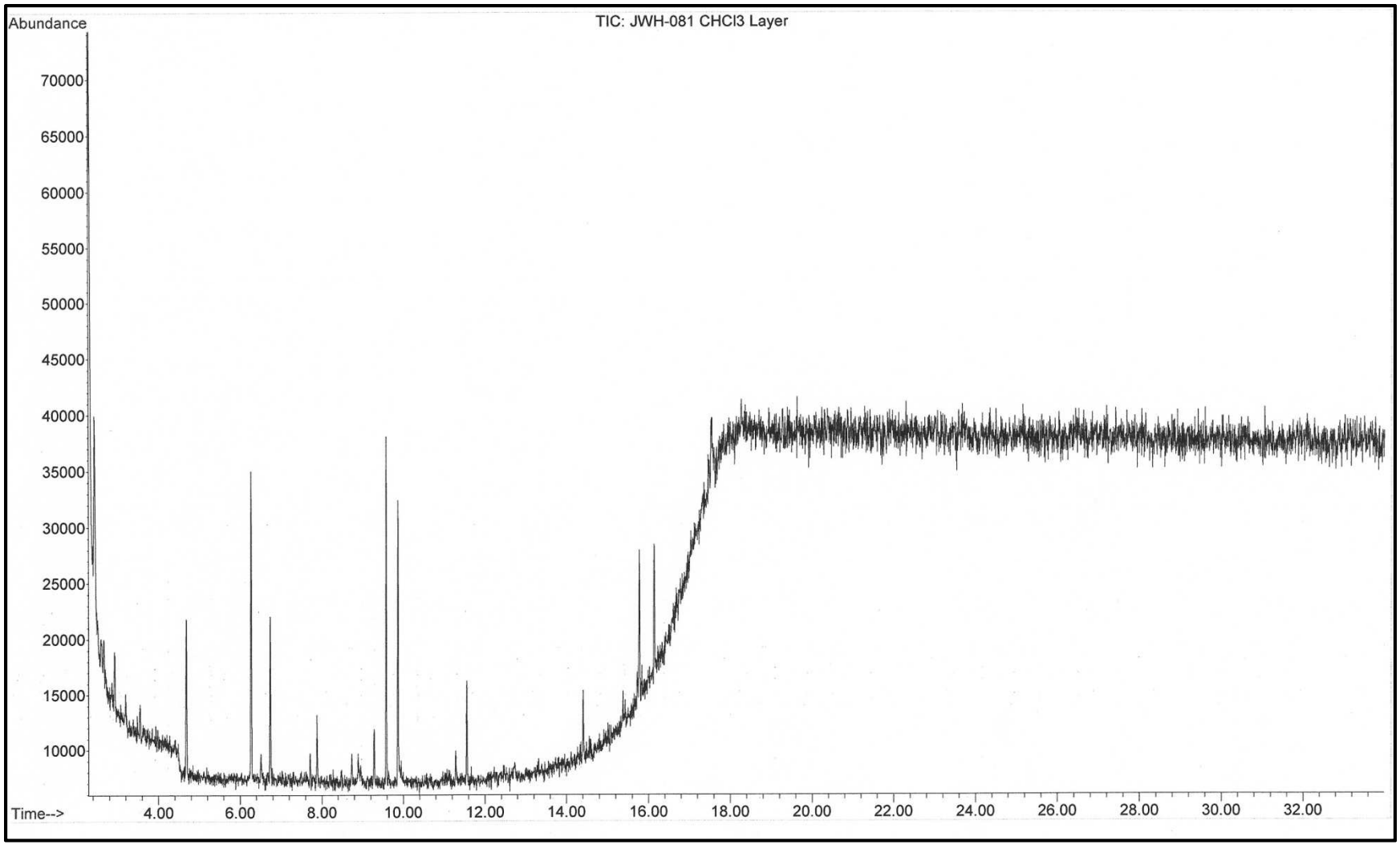
Zoomed in versions of this pyrogram can be found on the following two pages



The first of two pyrograms from the JWH-081 pipette extract which have been magnified to enlarge details



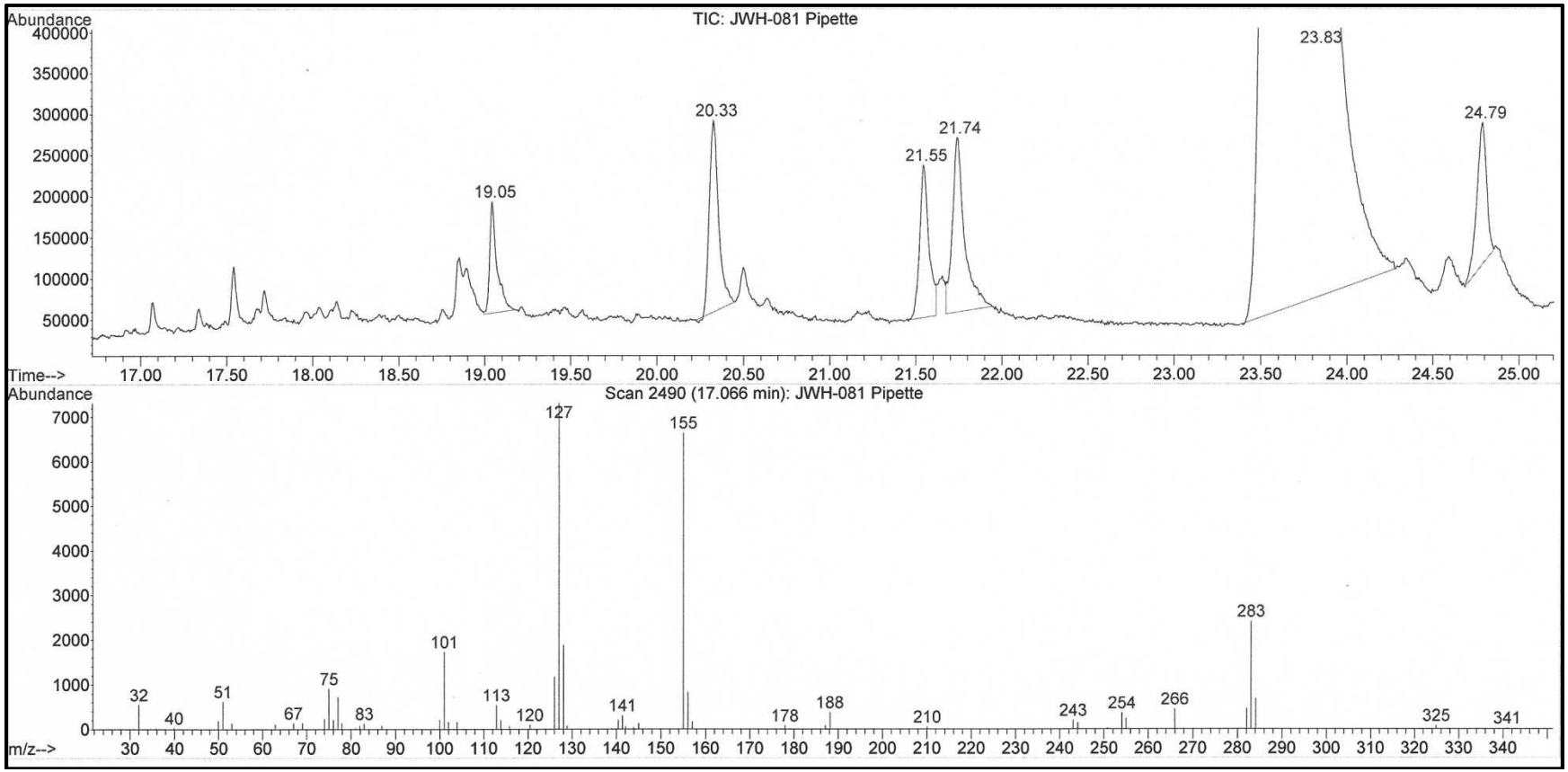
The second of two pyrograms from the JWH-081 pipette extract which have been magnified to enlarge details



Pyrogram representative of the JWH-081 solvent layer extract

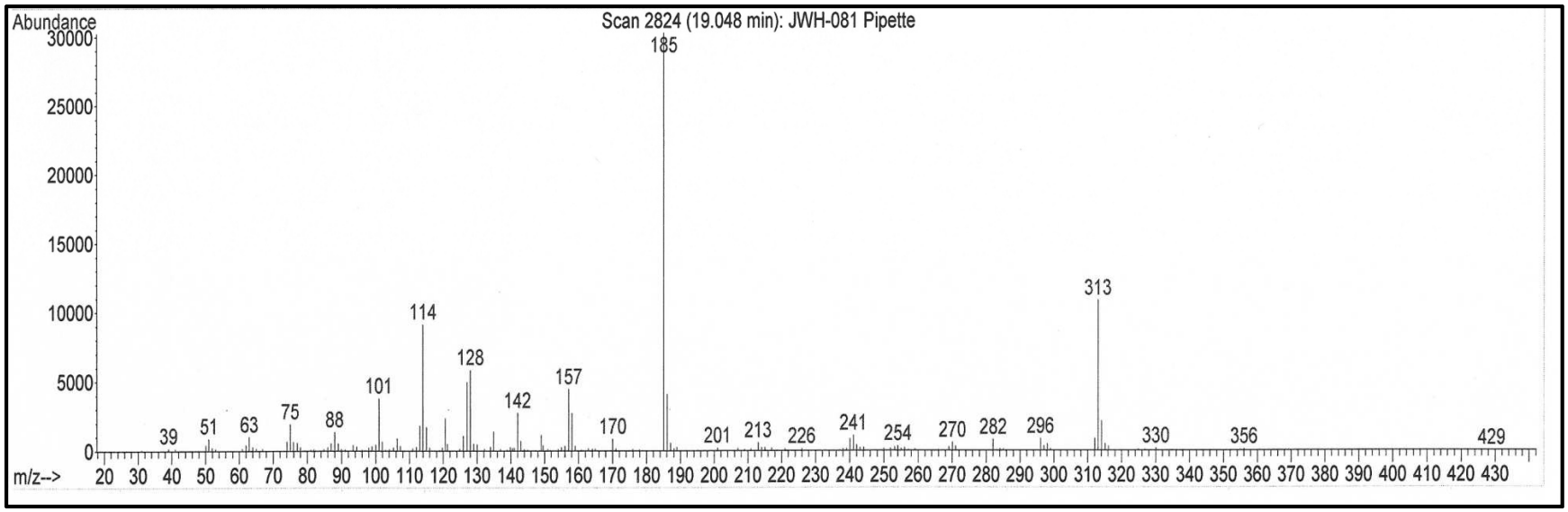
Note: two of the three solvent layer extracts for JWH-081 showed significantly lower levels than the other synthetic cannabinoids

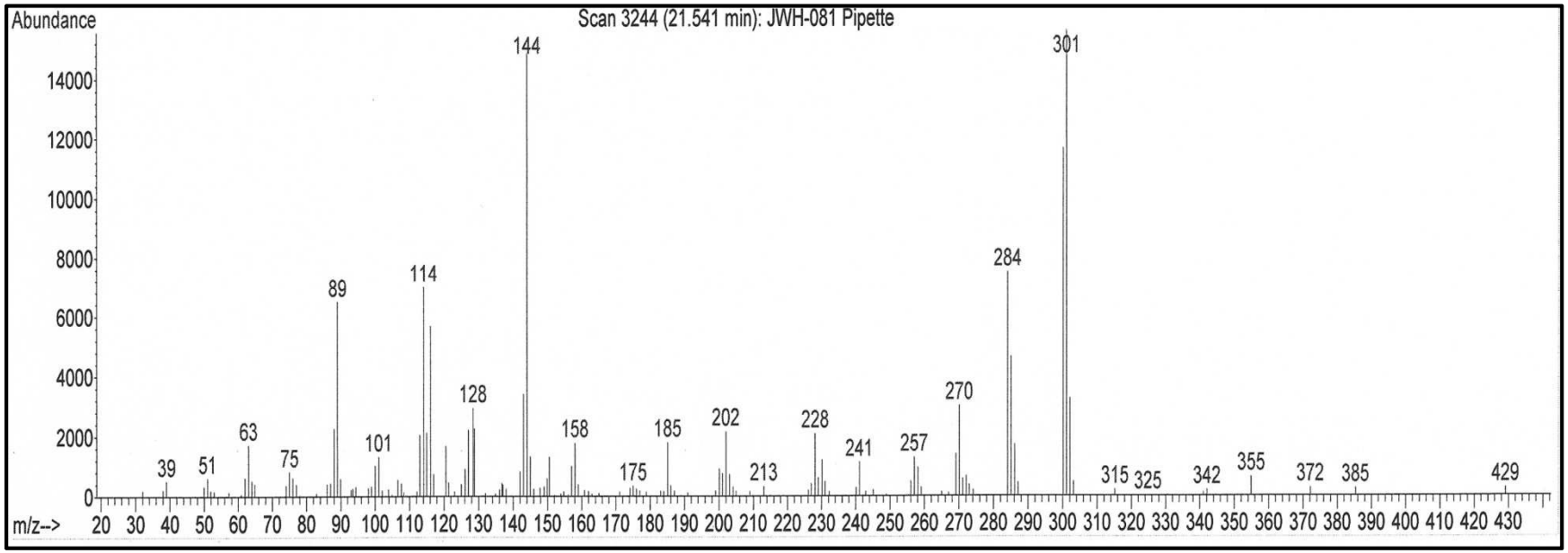
APPENDIX J – CHROMATOGRAPHIC AND MASS SPECTRAL INFORMATION OF THE UNIDENTIFIED JWH-081 PYROLYTIC PRODUCTS



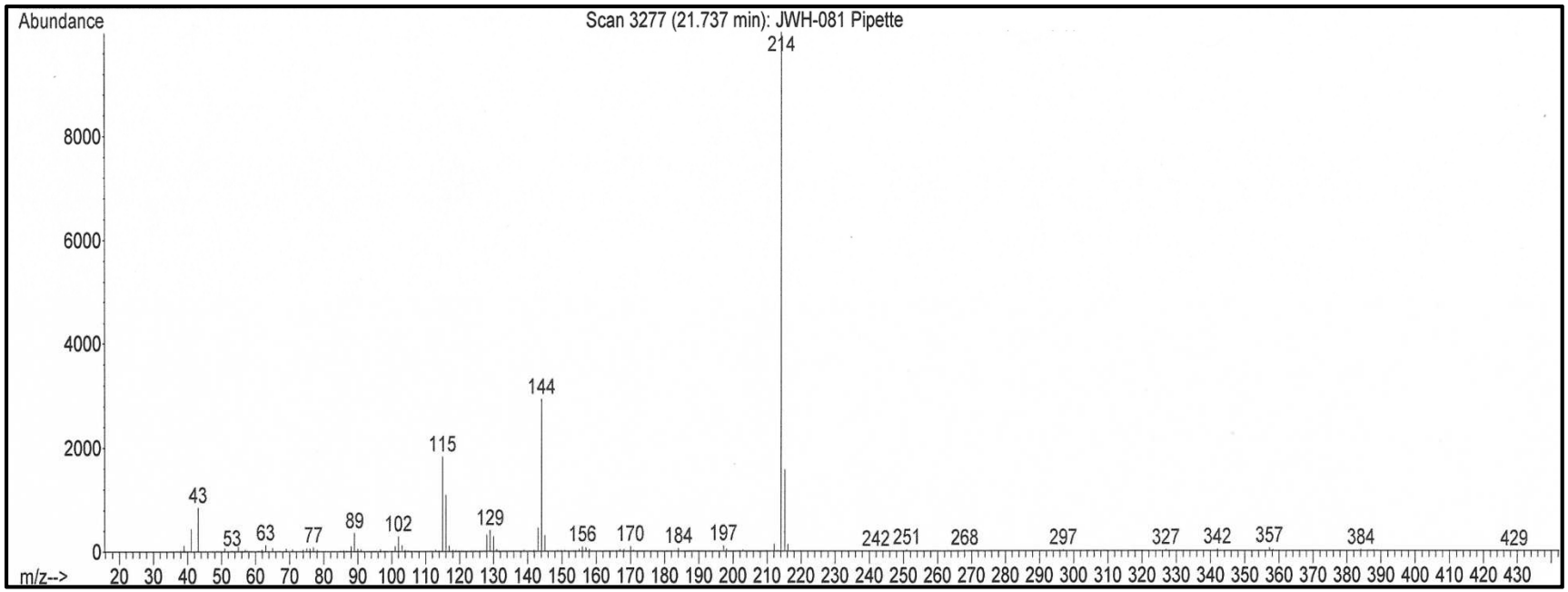
Chromatographic display (pyrogram; top) for the four unidentifiable pyrolytic products at 17.07, 19.05, 21.54, and 21.74 minutes; the mass spectra of the unknown at 17.07 minutes has also been provided above

Data for the other three pyrolytic products are provided on the following two pages



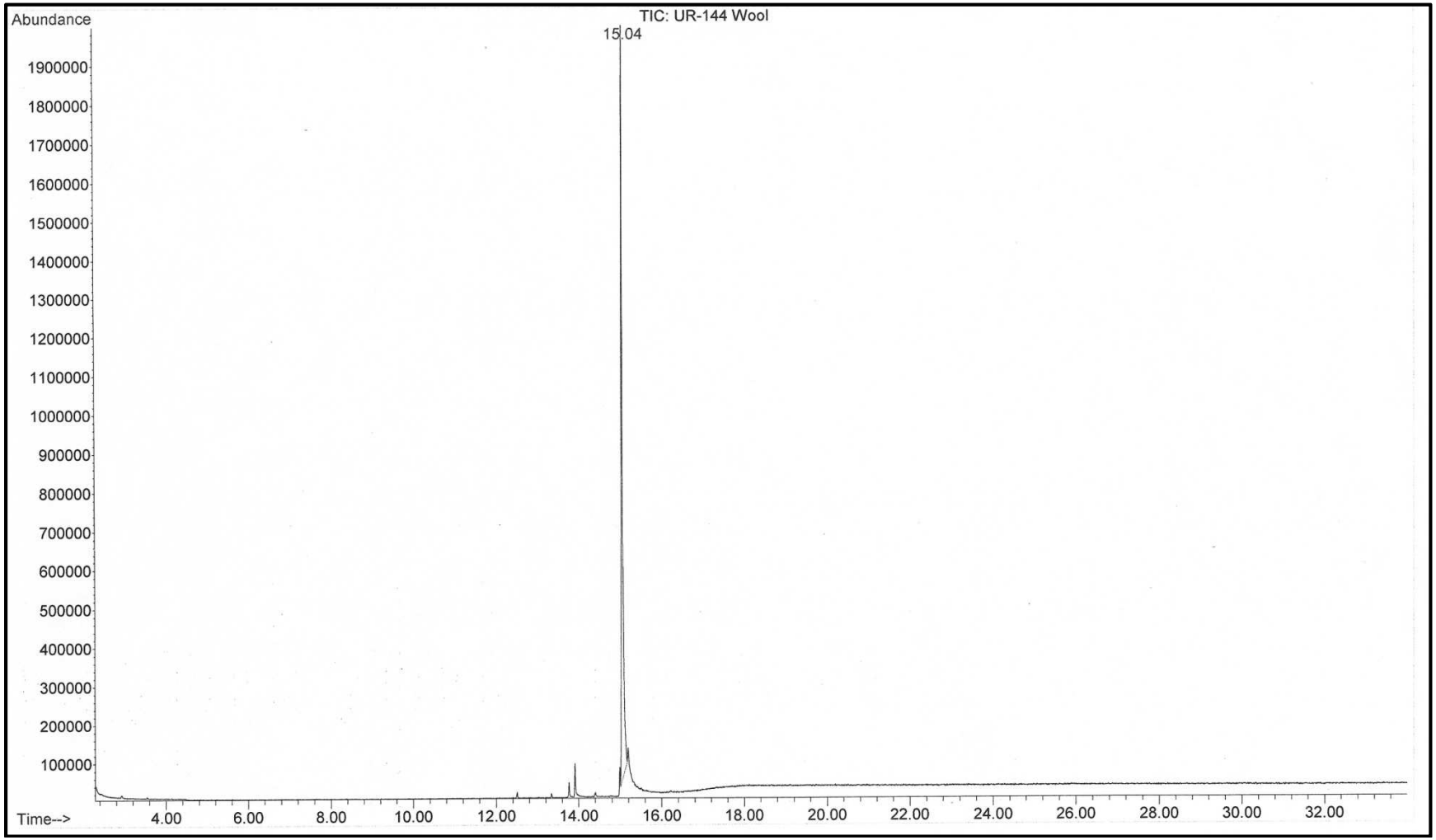


Mass spectra for the pyrolytic products at 19.05 and 21.54 minutes (see previous page for magnified image of pyrogram)

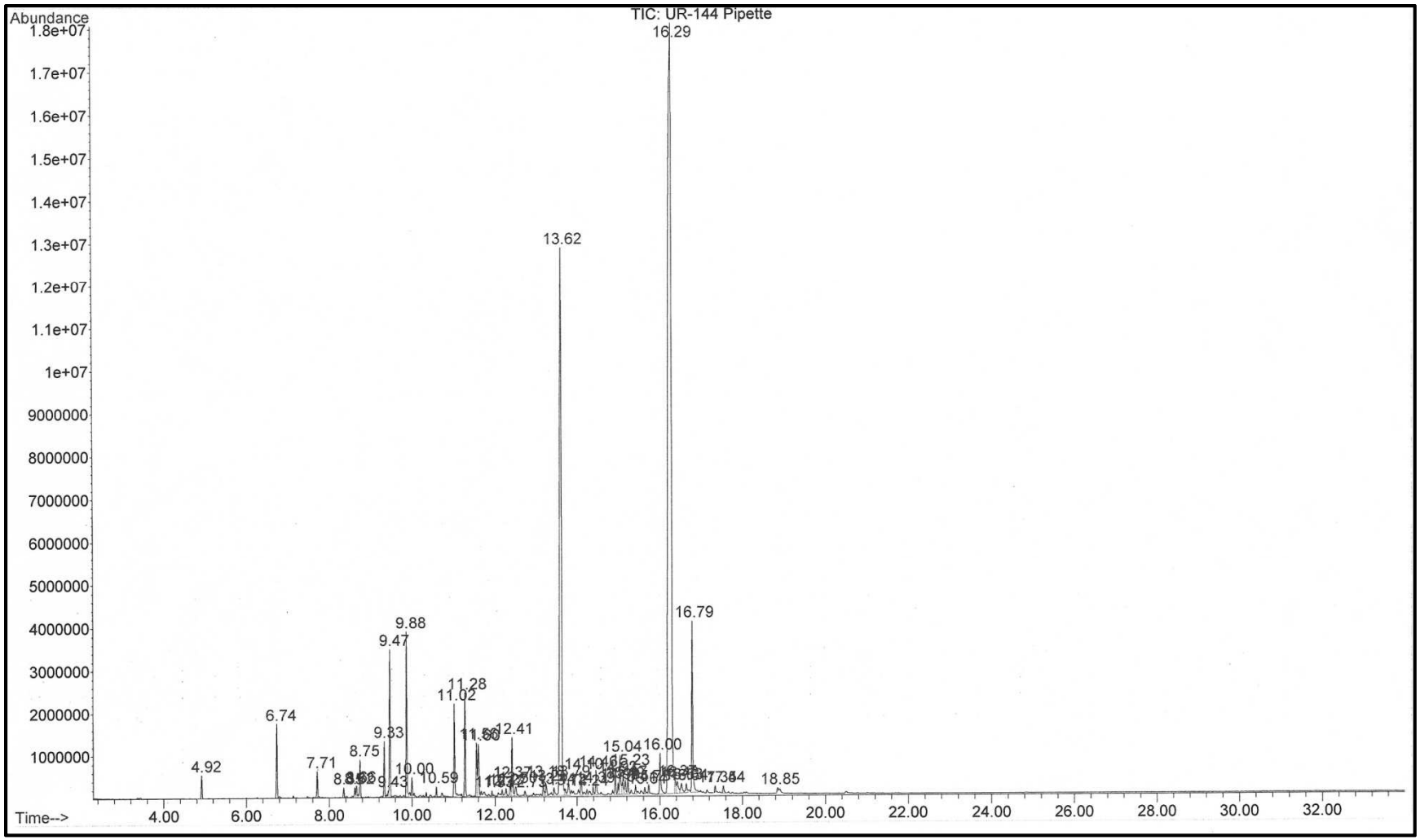


Mass spectra for the pyrolytic product at 21.74 minutes (magnified image of pyrogram provided above)

APPENDIX K – PYROGRAMS REPRESENTATIVE OF UR-144

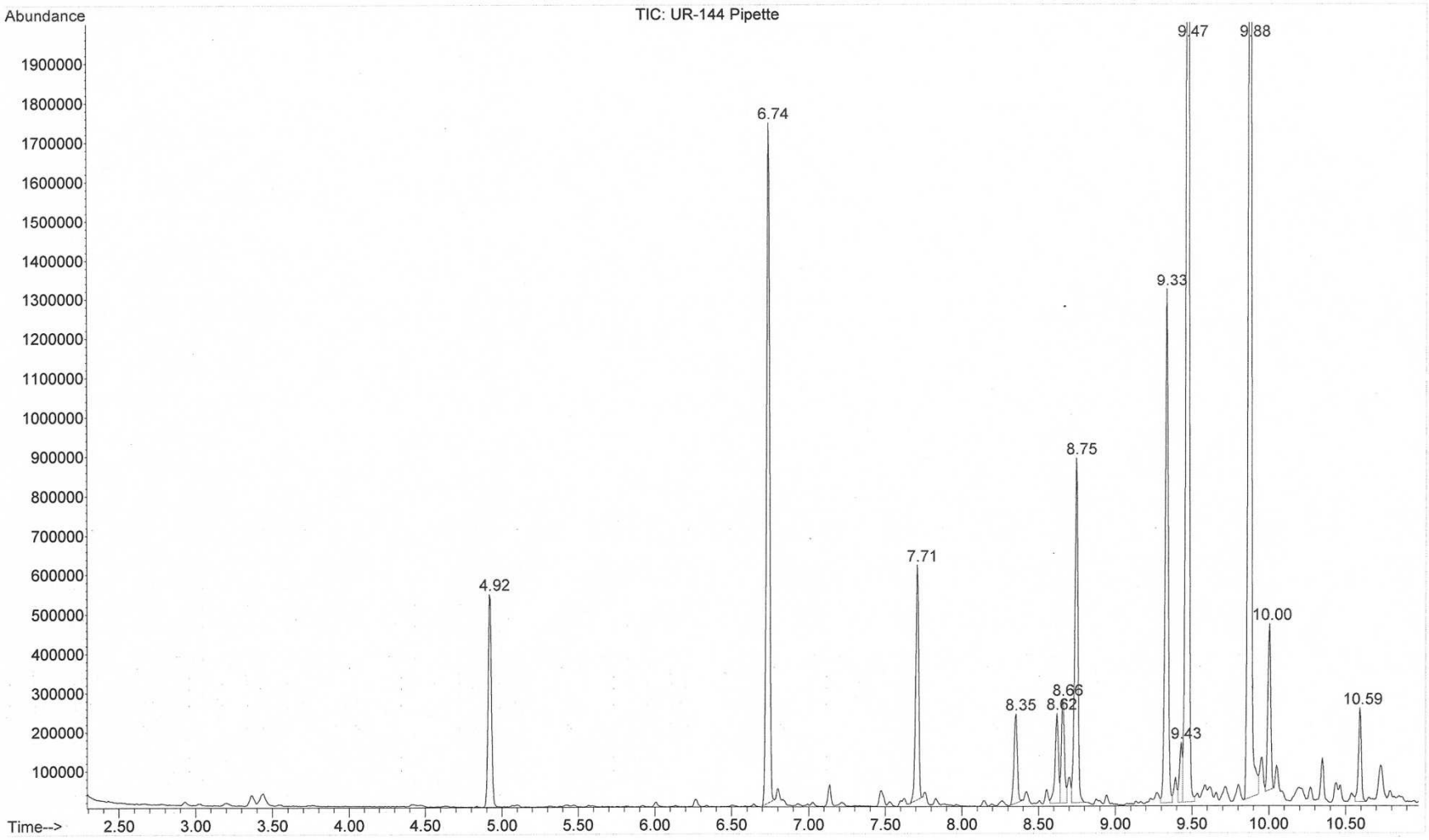


Pyrogram representative of the UR-144 wool extract

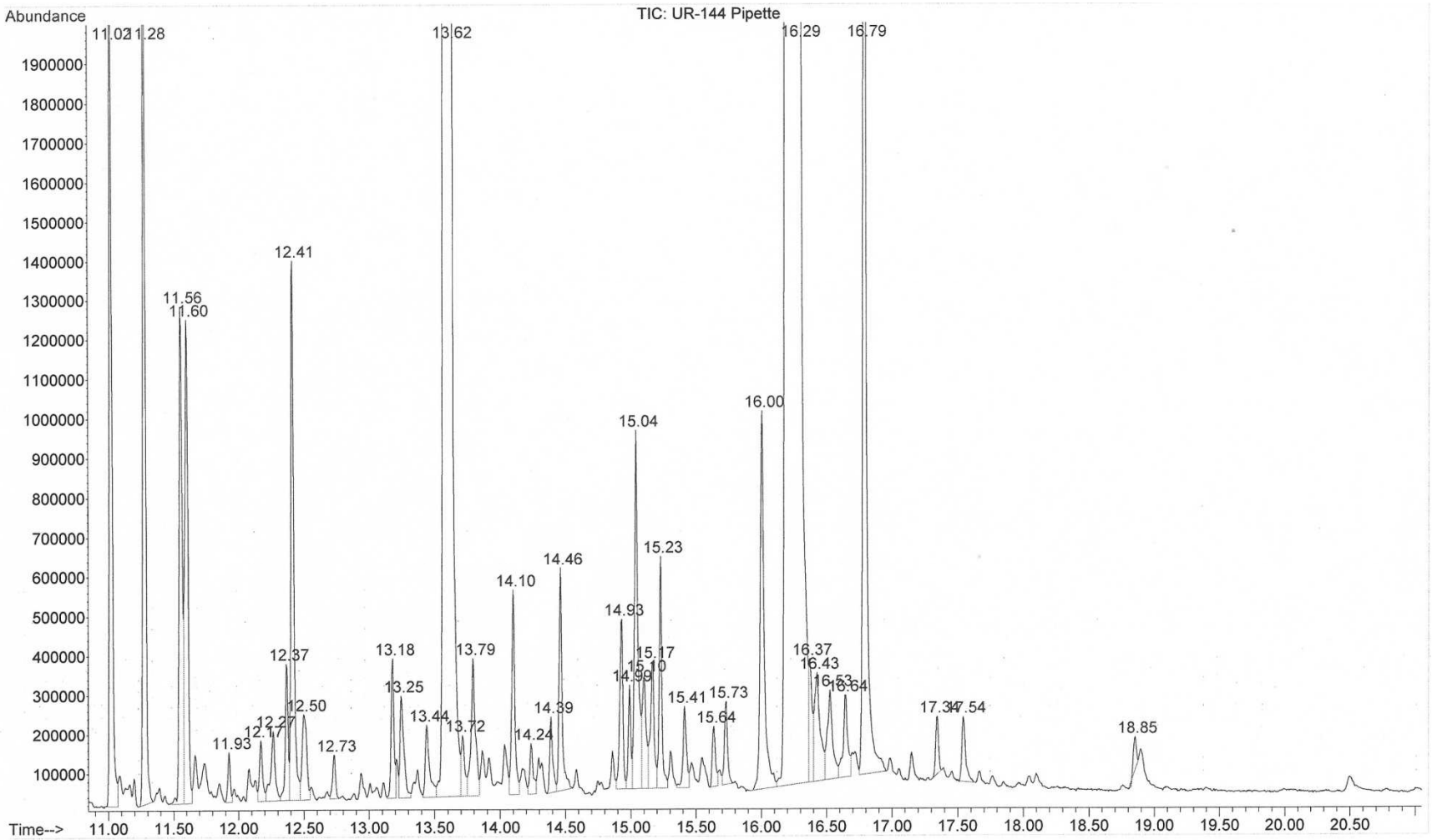


Pyrogram representative of the UR-144 pipette extract

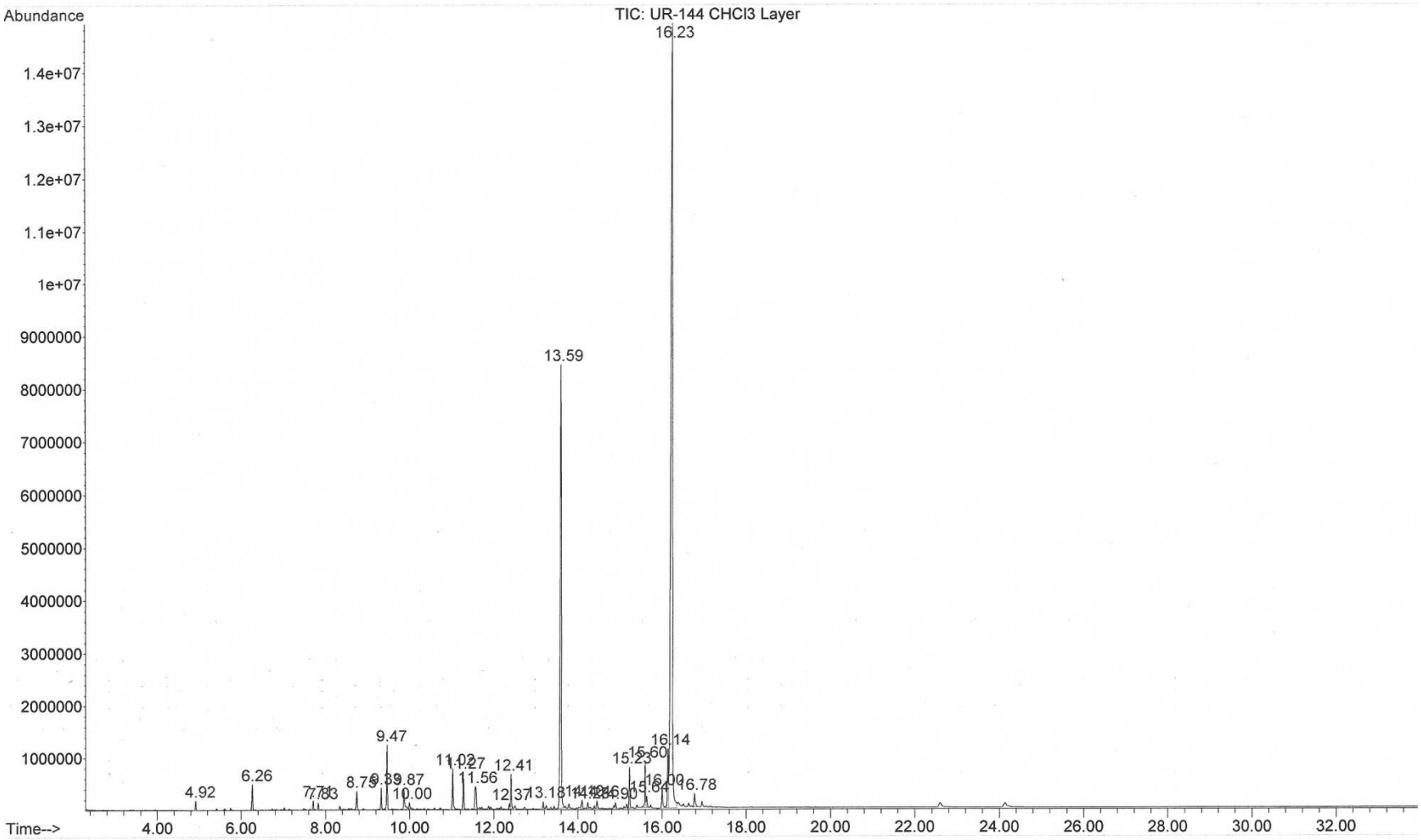
Zoomed in versions of this pyrogram can be found on the following two pages



The first of two pyrograms from the UR-144 pipette extract which have been magnified to enlarge details

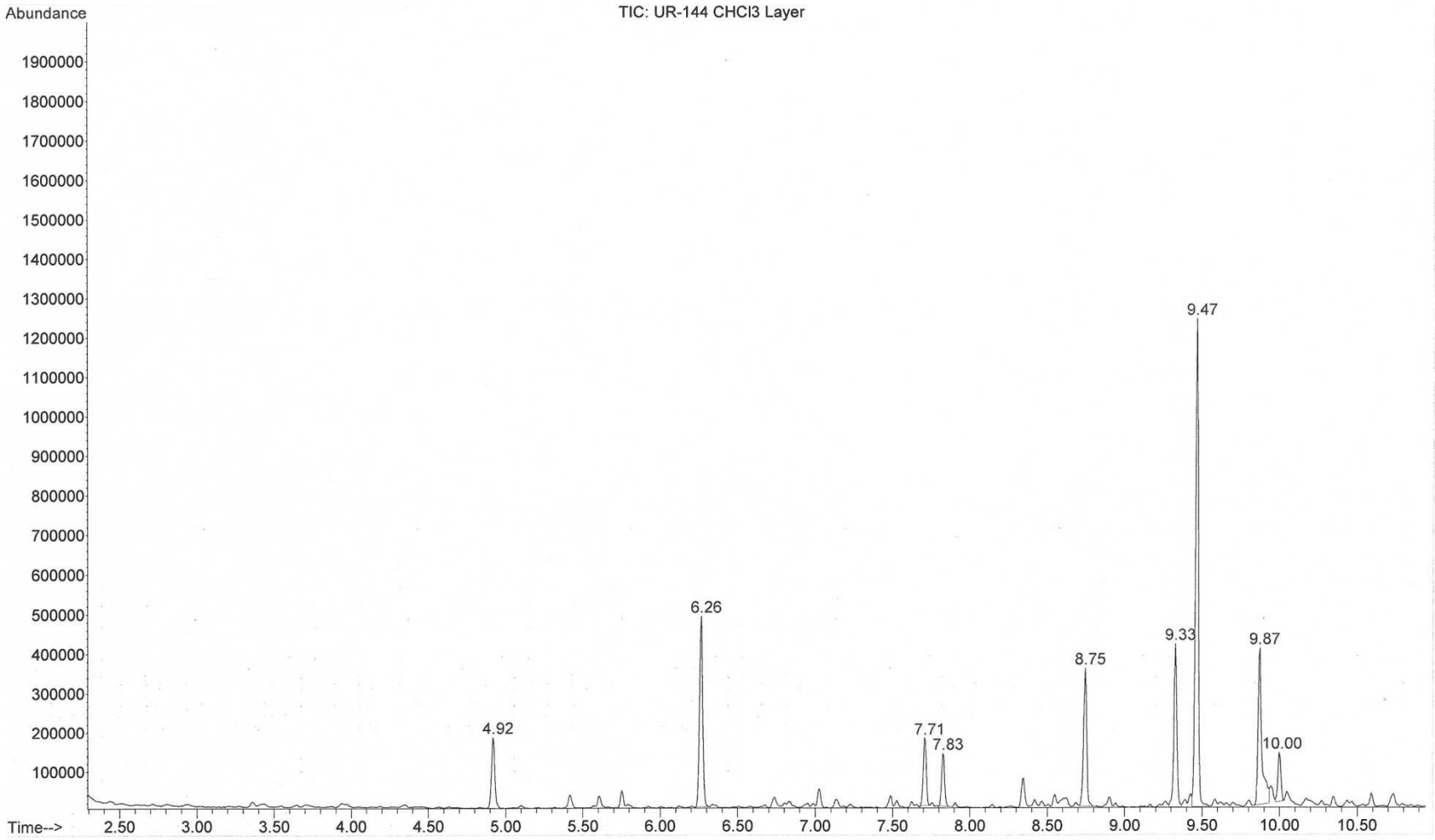


The second of two pyrograms from the UR-144 pipette extract which have been magnified to enlarge details

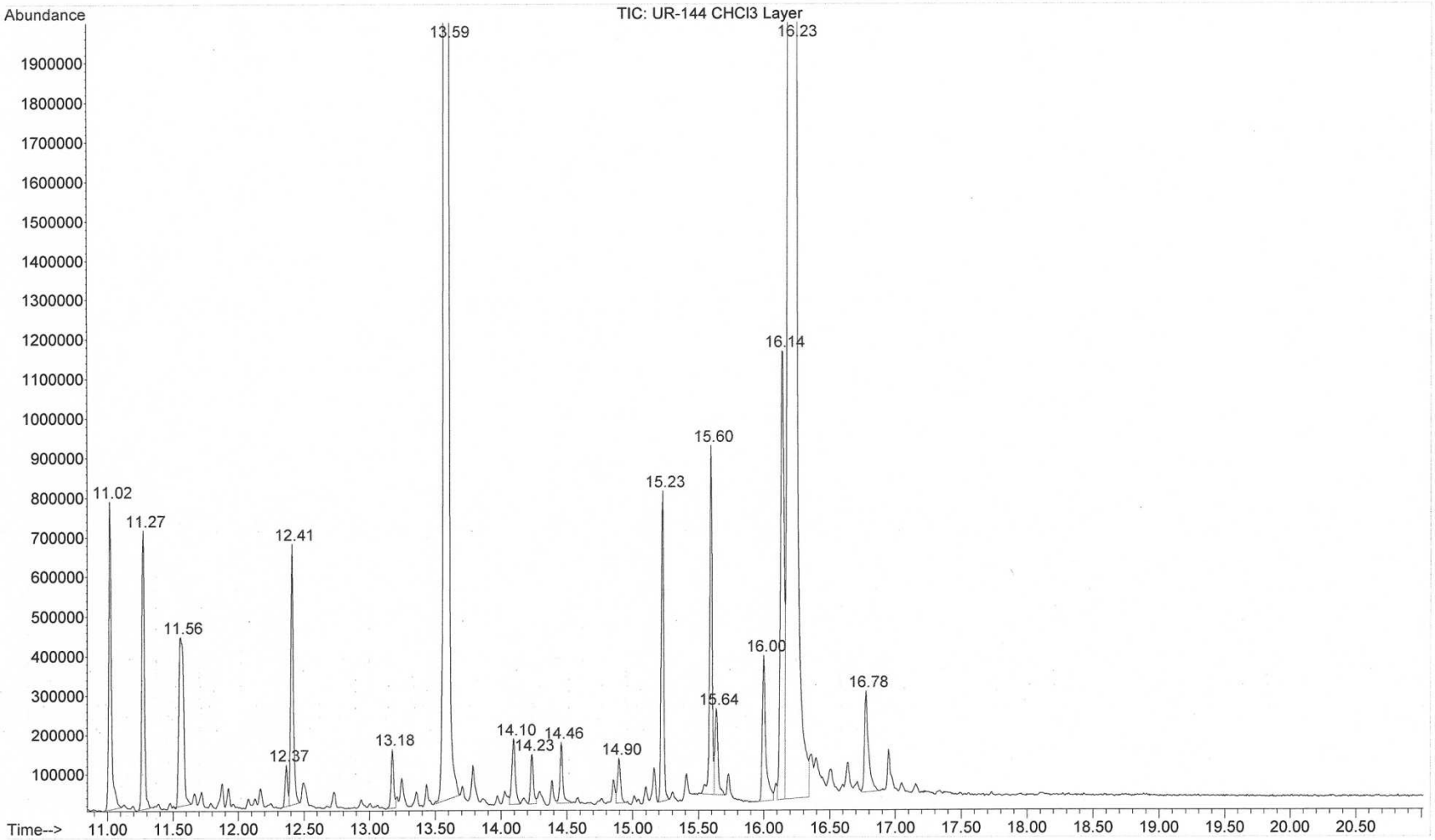


Pyrogram representative of the UR-144 solvent layer extract

Zoomed in versions of this pyrogram can be found on the following two pages

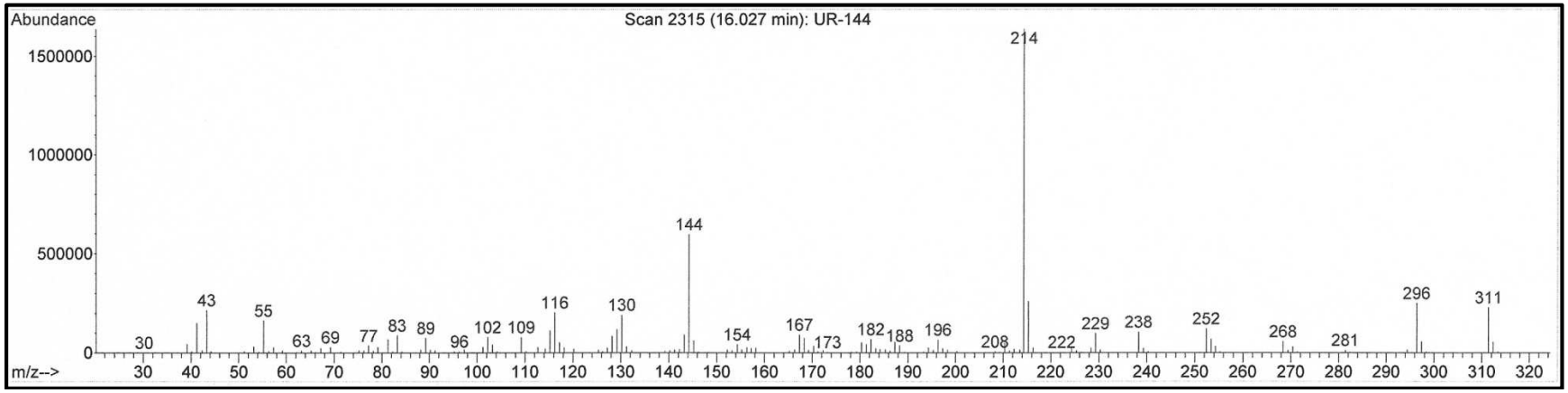


The first of two pyrograms from the UR-144 pipette extract which have been magnified to enlarge details

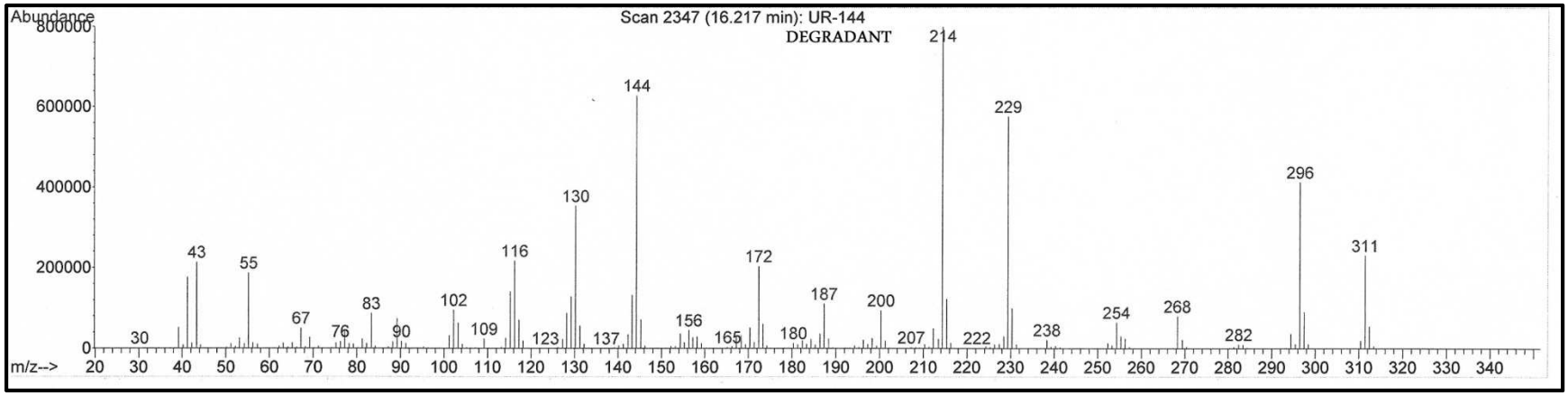


The first of two pyrograms from the UR-144 pipette extract which have been magnified to enlarge details

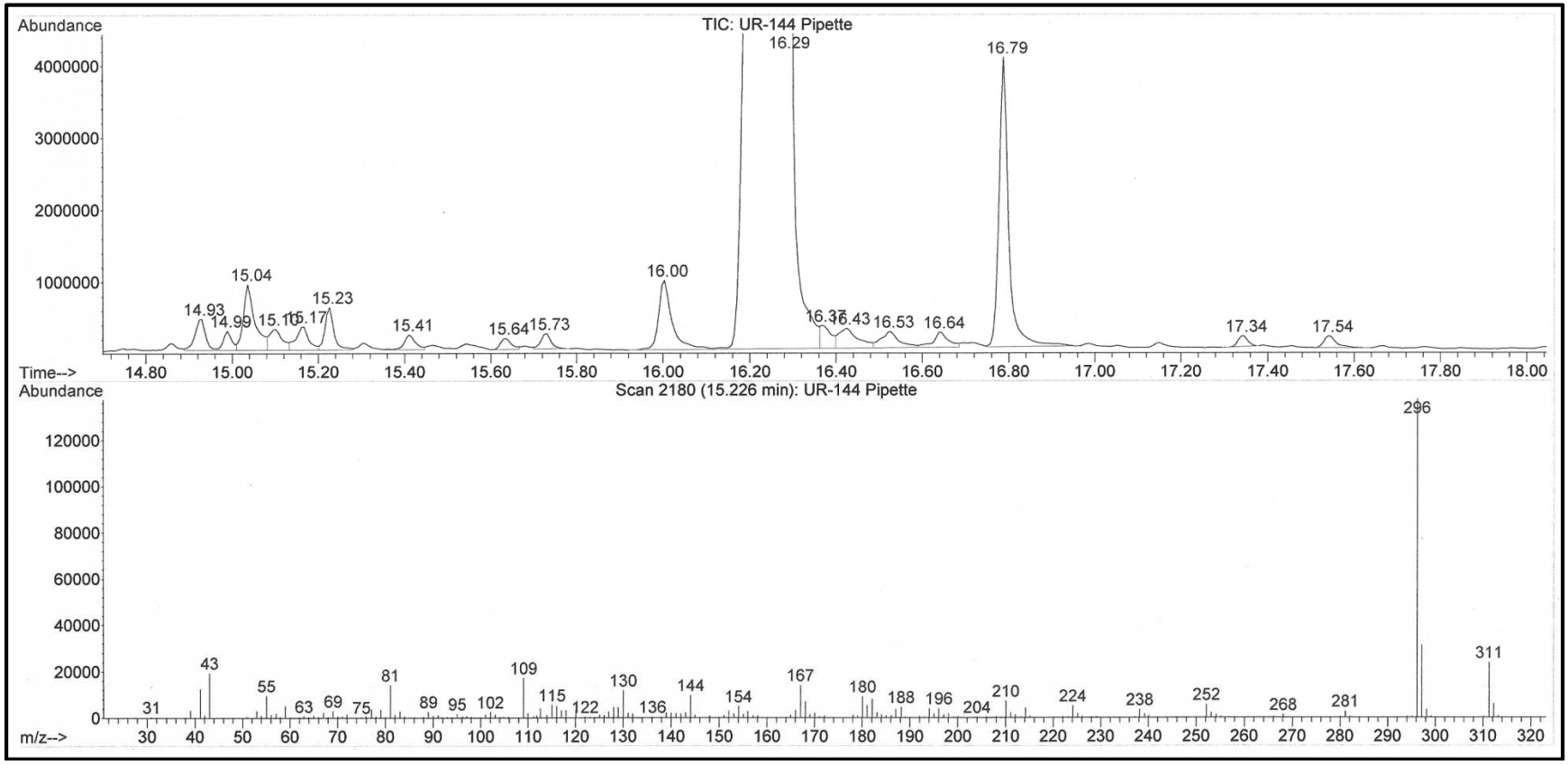
APPENDIX L – CHROMATOGRAPHIC AND MASS SPECTRAL INFORMATION OF THE UNIDENTIFIED UR-144 PYROLYTIC PRODUCTS



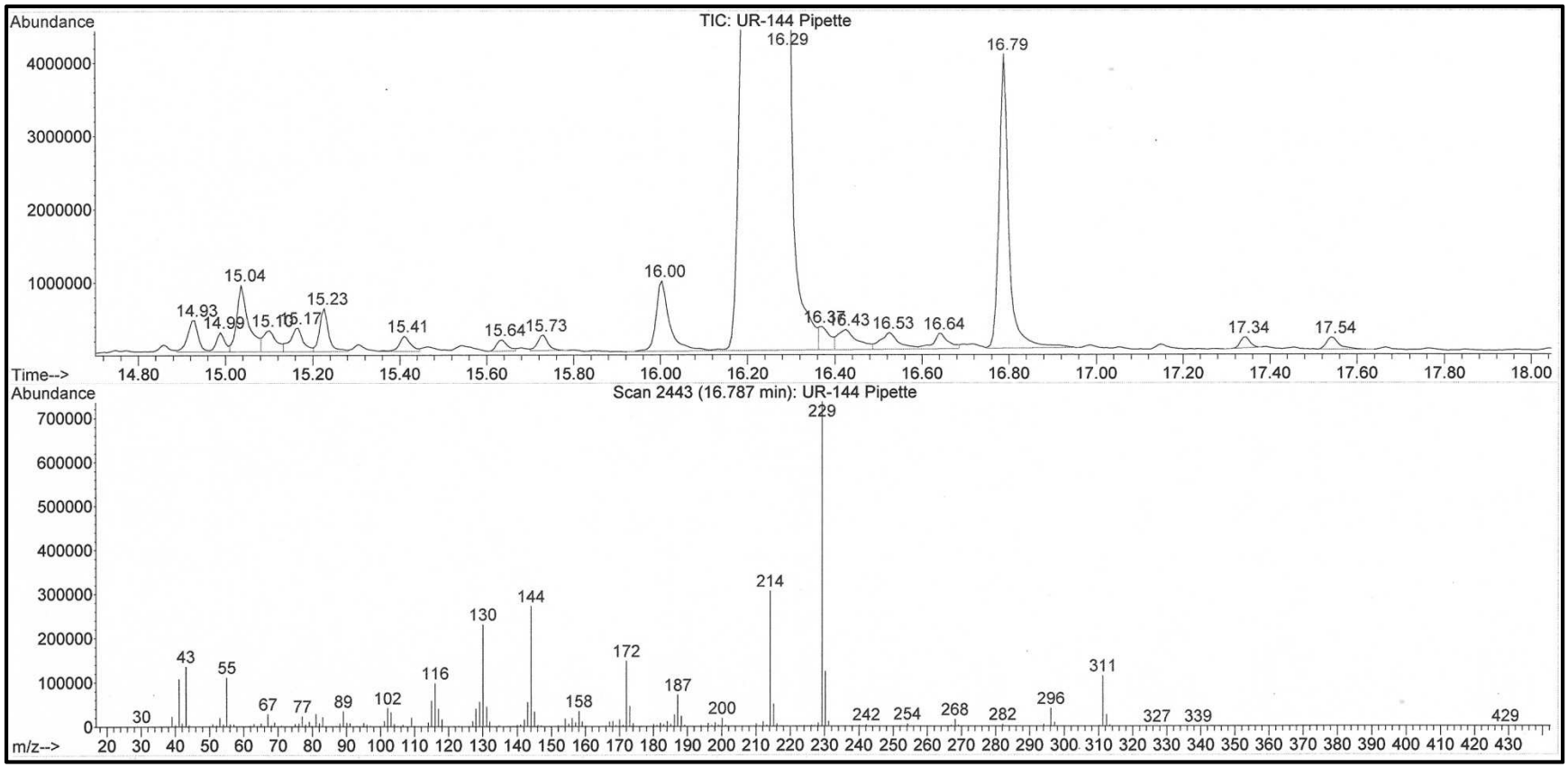
Mass spectrum for the UR-144



Mass spectrum for the UR-144 Degradant



Chromatographic display (pyrogram; top) of the unidentified UR-144 pyrolytic product (UPP3) at 15.23 minutes; the mass spectra of the UPP3 has also been provided



Chromatographic display (pyrogram; top) of the unidentified UR-144 pyrolytic product (UPP4) at 16.78 minutes; the mass spectra of the UPP4 has also been provided