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IMPROVEMENT AND DEVELOPMENT OF ANALYTICAL FORENSIC METHODS:  
ARSON INVESTIGATION AND DRUG DETECTION IN FINGERPRINTS AND HAIR

A Thesis

Presented for the degree of Master of Science  
in the Department of Chemistry and Biochemistry  
The University of Mississippi

by

Sarah Anne Glenn

August 2017



## ABSTRACT

Forensic science is the cornerstone of criminal investigations. Due to the overexposure of forensic science in the media, the true nature of forensic science is often overlooked. Forensic science is a rigorous exacting science that applies methods developed in the wider scientific community to the analysis of evidence collected from crime scenes. As far back as the 6<sup>th</sup> century people were using forensics to solve crimes. Since then the science behind forensic science has increased exponentially with new methods being researched and developed. This research aims to address two of the larger subsections of forensic science, arson and drug detection. The number of arson cases each year continues to grow and cause hundreds of thousands of dollars of damage. The study conducted in this research aimed to create a preliminary database of evaporated gasoline samples using the internal standard di-n-decyl sulfide. This study shows that by using the internal standard, di-n-decyl sulfide, and the changes that occur in the gas chromatograms of gasoline samples that are evaporated, forensic chemists can assist arson investigators by determining fuel grades, sample origins (gas stations), and gasoline evaporation levels. The remaining studies performed in this research deal with the detection of drugs in biological matrices. A method was developed using matrix assisted laser desorption ionization, or MALDI, to detect the presence of caffeine in fingerprints, sweat, and saliva. An improved method for the detection of marijuana in hair was also performed in this research. The new method developed in this study required fewer analysis hours and detected comparable concentrations (120 pg/mg  $\pm$  10 pg/mg) of marijuana metabolites in hair compared to current methods found in

literature. Forensic science is an expanding field that requires the assistance from researchers to create new methods and improve old methods.

## DEDICATION

This work is dedicated to my harshest critic, myself.

## ACKNOWLEDGMENTS

I would like to acknowledge the help received from Dr. Murrell Godfrey, Cecily Reily, Ashley Veech, Madison Russel, Brandon Stamper, Caroline Spencer, and El Sohly Laboratories.

## TABLE OF CONTENTS

ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
CHAPTER 1: HISTORY AND IMPORTANCE OF FORENSIC SCIENCE.....	1
1.1 INTRODUCTION.....	1
1.2 WHAT IS FORENSIC SCIENCE?.....	1
1.3 FORENSICS AS AN APPLIED SCIENCE.....	2
1.4 HISTORY OF FORENSIC SCIENCE.....	3
1.5 FORENSICS BEFORE THE SCIENCE.....	8
1.6 FORENSIC SCIENCE NOW.....	16
1.7 ACCREDITED CRIME LABS.....	19
1.8 ACCREDITED ACADEMIC PROGRAMS.....	21
1.9 NATIONAL COMMISSION ON FORENSIC SCIENCE.....	22
1.10 FORENSIC SCIENCE OF TOMORROW.....	23
CHAPTER 2: GC-MS CHEMICAL FINGERPRINT COMPARISON OF.....	
EVAPORATED AND UNEVAPORATED FUEL SAMPLES USING.....	
THE INTERNAL STANDARD, DI-N-DECYL SULFIDE.....	24
2.1 ABSTRACT.....	24
2.2 INTRODUCTION.....	25
2.2.1 GAS CHROMATOGRAPHY.....	28
2.2.2 MASS SPECTROMETRY.....	30
2.2.3 INTERNAL STANDARD.....	31
2.3 MATERIALS AND METHODS.....	32
2.3.1 MATERIALS.....	32
2.3.2 SAMPLE PREP WITHOUT INTERNAL STANDARDS.....	32



2.3.3 SAMPLE PREP WITH INTERNAL STANDARDS.....	33
2.3.4 MANUAL INJECTION.....	33
2.3.5 AUTO SAMPLER INJECTION.....	34
2.3.6 INSTRUMENT PARAMETERS .....	34
2.4 RESULTS AND DISCUSSION .....	35
2.4.1 WITHOUT INTERNAL STANDARD .....	35
2.4.2 WITH INTERNAL STANDARD .....	38
2.5 CONCLUSION.....	51
CHAPTER 3: METHOD DEVELOPMENT FOR THE DETERMINATION OF.....	
CAFFEINE IN PRESSED FINGERPRINTS USING MATRIX .....	
ASSISTED LASER DESORPTION IONIZATION .....	53
3.1 ABSTRACT.....	53
3.2 INTRODUCTION .....	54
3.2.1 FINGERPRINTS IN FORENSICS.....	54
3.2.2 BIOLOGY OF FINGERPRINTS .....	55
3.2.3 SWEAT AND SWEAT SOLUBLE DRUGS.....	55
3.2.4 CAFFEINE .....	56
3.2.5 MATRIX ASSISTED LASER DESORPTION IONIZATION .....	57
3.2.6 CURRENT RESEARCH INTO DRUG DETECTION IN FINGERPRINTS .....	58
3.3 METHOD .....	60
3.3.1 SELECTION OF MALDI MATRIX.....	60
3.3.2 DETERMINATION OF IMAGE RESOLUTION .....	61
3.3.3 DETERMINATION OF MATRIX APPLICATION AND CALIBRATION CURVE.....	61
3.3.4 CALCULATION OF CALIBRATION CURVE .....	63
3.3.5 SAMPLE COLLECTION AND PREPARATION .....	63
3.3.6 MALDI DATA COLLECTION .....	64
3.4 RESULTS AND DISCUSSION.....	64

3.4.1 DETERMINATION OF IMAGE RESOLUTION .....	64
3.4.2 MATRIX APPLICATION METHOD .....	65
3.4.3 SAMPLE COLLECTION.....	72
3.5 CONCLUSION.....	74
CHAPTER 4: METHOD IMPROVEMENT FOR THE DETECTION OF.....	
DELTA 9-TETRAHYDROCANNABINOL IN HAIR.....	76
4.1 ABSTRACT.....	76
4.2 INTRODUCTION .....	77
4.2.1 WHAT IS CANNABIS?.....	77
4.2.2 HISTORY OF AMERICAN LAWS REGARDING CANNABIS .....	80
4.2.3 DETECTION OF THC IN BIOLOGICAL FLUIDS .....	82
4.2.4 DETECTION OF DRUGS IN HAIR .....	83
4.2.5 LIQUID-LIQUID EXTRACTION .....	84
4.2.6 LIQUID CHROMATOGRAPHY .....	84
4.2.7 TANDEM MASS SPECTROMETRY .....	86
4.3 METHODS .....	88
4.3.1 WASHING.....	88
4.3.2 EXTRACTION .....	89
4.3.3 LC/MS/MS.....	90
4.4 RESULTS AND DISCUSSION .....	91
4.5 CONCLUSION.....	97
BIBLOGRAPHY .....	99
VITA.....	108

## LIST OF TABLES

TABLE	PAGE
Table 1. Peak height/internal standard ratios of Texaco 87 at 0% and 99% evaporation .....	39
Table 2. Peak ratios of 2-Methyl-1-Nitropropane, Toluene, Xylene, and Tri-Methyl Benzene in Texaco 93 and Shell 93 gasoline. ....	44
Table 3. Peak ratios of 2-Methyl-1-Nitropropane, Toluene, Xylene, and Tri-Methyl Benzene in Texaco 87 and Shell 87 gasoline. ....	45
Table 4. Peak ratios of major peaks in Texaco 87-1 and Texaco 87-2 during evaporation. ....	51
Table 5. Eight Main Classifications of Cannabinoids and Their Base Structure .....	78
Table 6. The parameters for the gradient LC program during analysis of the hair samples. ....	90
Table 7. Summary of the parameters for tandem MS. ....	91
Table 8. Time comparison of the preparation time required for the various methods of hair preparation (THC) ...	92
Table 9. The concentration of the 11-nor-9-carboxy THC and Tetrahydrocannabinol detected .....	95

## LIST OF FIGURES

FIGURE	PAGE
Figure 1. Gas Chromatograph schematic .....	28
Figure 2. Schematic of a quadrupole mass spectrometer .....	31
Figure 3. Texaco 87 Unevaporated Gasoline without Internal Standard.....	36
Figure 4. Chromatogram of Texaco grade 87 gasoline at 0%, 75% and 99% evaporation levels without internal standard .....	37
Figure 5. Chromatogram of Texaco grade 93 gasoline at 0%, 75% and 99% evaporation levels without internal standard. ....	37
Figure 6. Chromatograms of Texaco grade 87 gasoline at 0% and 99% evaporation levels with internal standard.....	39
Figure 7. A comparison of unevaporated Texaco 87 gasoline with and without internal standard to show the internal standard does not chemically interact with the gasoline.....	40
Figure 8. Chromatogram comparison of Texaco 93 and 87 gasoline without internal standard .....	41
Figure 9. Chromatogram comparison of Texaco 87 and 93 gasoline with internal standard .....	42
Figure 10. Chromatograms comparing Texaco 93 and 87 without large propane .....	42
Figure 11. Chromatogram comparison of Shell 87 and 93 gasoline with internal standard.....	43
Figure 12. Chromatogram comparison of Texaco 93 and Shell 93 gasoline with internal standard .....	44
Figure 13. Chromatogram comparison of Texaco 87 and Shell 87 gasoline with internal standard .....	45
Figure 14. Comparison of all evaporation levels of Texaco 87 with internal standard .....	45
Figure 15. Comparison of all evaporation levels of Shell 87 with internal standard .....	47
Figure 16. Comparison of all evaporation levels of Texaco 93 with internal standard.....	48
Figure 17. Comparison of all evaporation levels of Shell 93 with internal standard. ....	49
Figure 18. A graph of the major peaks in gasoline as the sample evaporated fully for Texaco 87-1 and Texaco 87-2 .....	51
Figure 19. Molecular structure of caffeine .....	57
Figure 20. A schematic of the Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) instrument.....	58
Figure 21. Visual Representation of MALDI Imaging Analysis.....	61
Figure 22. MALDI Imaging Resolution Test.....	65
Figure 23. MALDI imaging results of matrix application techniques and calibration curves .....	65
Figure 24. GC/MS Results of caffeine using in this study .....	67
Figure 25. Distribution analysis of calibration curve for spotted standards with matrix added to standards .....	67

Figure 26. Distribution summaries of calibration curve for spotted standards with matrix added to standards.....	68
Figure 27. Distribution analysis of calibration curve for spotted standards with no water added.....	69
Figure 28. Distribution summaries of calibration curve for spotted standards with no water added .....	70
Figure 29. Distribution analysis of calibration curve for spotted standards with water added.....	71
Figure 30. Distribution summaries of calibration curve for spotted standards with water added .....	71
Figure 31. Calibration curves of the MALDI data. ....	72
Figure 32. Analysis of Saliva, Sweat, and Fingerprints using MALDI.....	73
Figure 33. Timed Analysis of Caffeine Metabolism Using MALDI .....	74
Figure 34. Major pathway of tetrahydrocannabinol (THC) metabolism. ....	79
Figure 35. A visual representation of liquid-liquid extraction. ....	84
Figure 36. Schematic of a liquid chromatograph .....	85
Figure 37. Schematic of a tandem quadrupole mass separator.....	88
Figure 38. The mass spectrums from quadrupole 1 of THC-COOH for the different methods of hair preparation. ..	93
Figure 39. The mass spectrums from quadrupole 1 of THC for the different methods of hair preparation .....	94
Figure 40. Calibration curve of Standard 11-nor-9-carboxy-THC that produced data for 345m/z (11-nor-9-carboxy THC) and 315m/z (THC) molecules. ....	95
Figure 41. The mass spectra from quadrupole 1 of THC for the different of hair preparations. ....	96

## CHAPTER 1

### HISTORY AND IMPORTANCE OF FORENSIC SCIENCE

#### 1.1 INTRODUCTION

This chapter has been written for those people that don't appreciate or know what forensic science really is. It will define forensic scientists and examine its evolution from as early as the 6<sup>th</sup> century to the present. This chapter will further touch on some of the famous court cases, policy changes, and accrediting bodies that have helped influence people's trust in the criminal justice system.

#### 1.2 WHAT IS FORENSIC SCIENCE?

The term forensics has various meanings. The earliest definition, "of the forum," originated from Rome. Criminal cases were presented to the public in the forum. The accuser and accused were allowed to introduce their cases to the public. The person with the best argument would win the case. Although modern forensics has evolved significantly from Roman times, there are aspects in common.

In modern times, the word forensics is an accepted shortened term of forensic science. It is the use of science to determine the guilt or innocence of those who are accused of breaking the laws. Forensic scientists use a wide range of scientific disciplines to do their jobs including, pathology, chemistry, biochemistry, physics, and mathematics.

A major commonality that modern day forensics shares with its Roman counterpart is the requirement of presenting evidence in front of the public. Forensics scientists are required to appear before the judge and defend the evidence that they have analyzed. Evidence can vary widely, from blood spatter patterns, to a deceased body, or even potentially illicit substances. Forensic scientists are required to have a firm knowledge of their specialty and are required to use science to support their methods. Failure to use scientifically sound techniques could result in the conviction of the innocent. [1]

### 1.3 FORENSICS AS AN APPLIED SCIENCE

Forensics is a multi-disciplinary science that encompasses nearly all sub categories in the wider scientific community. Physicists, biologist, chemists, archeologist, geologists, and even mathematicians make up the ranks of a forensic laboratory. These scientists apply the knowledge and specialized training of their specialties in order to effectively analyze evidence.

Trace evidence is a forensic division with a wide range of duties. Trace evidence is comprised of all the small traces that are transferred from one object to another during contact. Trace evidence includes hair, fibers, gunshot residue, soil, wood, paint, and many more. This division of forensics uses skills from biology, chemistry, geology, and other disciplines to analyze the evidence and connect it to the person or object that left it behind.

Forensic DNA experts continually stay abreast of their ever-improving field. They adopt newly validated testing reagents, testing methods, DNA genetic markers, and other advances made in their field. DNA related research is a rapidly expanding field that works to sequence genomes, clone animals, discover the genetics of cancer and more. Applying DNA advancements to forensics has resulted in an increase in the conviction rate of criminals, a decrease in the conviction

rate of the innocent, and an increase in the exoneration of those falsely incarcerated. [1]

Drug chemists or forensic chemists make up a majority of many crime laboratories. These chemists are tasked with identifying and classifying unknown drugs found at a crime scene or on a suspect. There are wide varieties of techniques that make up a drug chemists' arsenal. Forensic chemists use color tests, chromatography, spectrometry, spectroscopy, and spectrophotometry among others to analyze the vast number of cases that come their way. Due to the high number of drug cases, drug chemists do not have the time to conduct research to advance their field. They rely on research scientists to develop new or advanced methods in order to improve their craft.

Arson analysis is a current changing field of forensic science. In previous years, arson investigation was one of the least scientific fields. Investigators used intuition and 'rules of thumb' passed down over the years to analyze crime scenes. Early arson investigators used instinct and non-science based logic to determine how fires behaved and what marks it left on the crime scene. Recent high profile mistakes are causing the arson division of the forensic laboratory to receive a personnel update. It is has been recently recognized that scientists with chemistry or physics degrees perform more accurate fire analysis than arson investigators. It is through the application of knowledge on how fire behaves and how materials can affect fires that will elevate arson investigation from a science light division to a science full division.

#### 1.4 HISTORY OF FORENSIC SCIENCE

Forensic science has a vast and rich history. As early as the 6<sup>th</sup> century archeologists have been able to find evidence of civilizations, those in Egypt and the Mesopotamia, that used forensics. Also in the 6<sup>th</sup> century, the Chinese developed and wrote a book on forensic medicine. Despite the lack of modern day advances, those civilizations were able to use observation and logic



to determine cause of death.

Unfortunately, not all civilizations were as advanced. Before forensics became widely accepted and used peacekeepers and police, some societies had less sophisticated methods of determining guilt. When a person was accused of a crime, they were taken in for questioning. Questioning in those days was a violent and brutal affair. For capital crimes such as murder, thievery, or arson, the accused were tortured. If they confessed, they were found guilty, but if they held out and didn't confess, they were found innocent. It wasn't until the 18<sup>th</sup> century that forensics started to play a larger role in determining the guilt or innocence criminals.

It is said that one of the forefathers of modern day forensics is none other than Sherlock Holmes, a character in a popular detective series authored by Sir Arthur Conan Doyle in the late 19<sup>th</sup> century. Sherlock utilized his knowledge of science and inductive reasoning to reach his conclusions. Inductive reasoning is the extrapolation of observed information used to arrive at conclusions about unobserved events. Forensics is based around these principles. Forensic scientists gather information from crime scenes and use those facts to paint a picture of what occurred. The main difference between Sherlock and modern day forensic scientists is the advancements in science.

During the mid-19<sup>th</sup> to the mid-20<sup>th</sup> century, the field of forensics saw a rapid expansion of scientific techniques at its disposal. Mathieu Orfila is one of the first contributors to the advancement of science that spurred the forensic approach to investigating. In the early 19<sup>th</sup> century Orfila, a native of Spain, published the first treatise on poison detection and their effect on animals. Orfila was a renowned medical professor and is considered the father of forensic toxicology. His rigorous scientific study of poisons, their effect on the body, and how to detect them in the deceased started the process of legitimizing the use of science in criminal cases. [2]

While around the same time that Orfila was characterizing poisons, two men were working on characterizing human identification. In 1879 Alphonse Bertillon, a French criminologist and anthropologist, started developing the field of anthropometry. Anthropometry is a systematic recording of specific body measurements. Bertillon believed that an individual's measurements were unique and the person could be identified by them. There were several flaws in his logic, but when combined with emerging photographic technology it was considered the most accurate technique for nearly twenty years. [3]

In the early 20<sup>th</sup> century three separate men, Sir William James Herschel, Dr. Henry Faulds, and Sir Francis Galton, took a different approach to individual identification than Bertillon. Herschel and Faulds pioneered the development of fingerprints for identification. Herschel, a British officer in India, used fingerprints for contracts. Twenty years later Faulds a Scottish scientist proposed that fingerprints could be used for forensic work. Sir Francis Galton compiled all of the knowledge and understandings of fingerprints and wrote them into a book. He also proposed a technique that identifies and compares common patterns in fingerprints, forming a classification system, which is still used today. For his research into and classification of fingerprints, Galton is known as the father of forensic fingerprints. [4]

In 1925 Calvin Goddard, a U.S. army colonel, turned a medical degree and a gun hobby into one of the most known forensic fields. Goddard developed the techniques for bullet comparison that are used today. He determined that the markings, commonly referred to as striations, left on a bullet from the barrel of the gun left distinct patterns that could be traced back to a gun. Using a bronchoscope and cystoscope from his medical days, Goddard was able to see inside the barrel of a gun and confirm that the markings made on the gun came from defects within the barrel itself. Along with the characterizations of striations on the bullets, Goddard also

modified the comparison eyepiece for the microscope to allow for the simultaneous analysis of two separate bullets. This contribution is a cornerstone of many comparison forensic disciplines including, ballistics and hair and fiber analysis. [5]

Dr. Karl Landsteiner made an important discovery in 1901. He discovered that blood could be grouped in to different categories; A, B, AB, and O. Dr. Leone Lattes, a professor at the Institute of Forensic Medicine in 1915, expanded on Landsteiner's work and applied it to the growing field of forensics. He developed a simple test that could determine the blood type of a dried bloodstain. Testing for blood type is still done today in order to rule out suspects who have the incorrect blood type. [6]

Despite all the advances going on and the leaps in forensic disciplines, the police had to seek out qualified assistance on their own. For the larger more metropolitan cities with a larger budget, this wasn't an issue. They were able to seek out and hire these pioneers of the field and their students. Smaller towns with less money had to rely on applying these new methods themselves, without an expert present.

In 1910 Edmond Locard, a French doctor and criminalist started a process that would drastically increase the number of experts and the areas they could serve. Locard began his career assisting a criminologist and professor, Alexandre Lacassagne, before collaborating with Alphonse Bertillon. During the First World War, Locard worked with the French Secret Service as a medical examiner. After the war, Locard started the first forensic laboratory in Lyon France. He was given two attic rooms of the police stations, two assistants, a microscope, and a rudimentary spectrometer. With his great enthusiasm for forensics, Locard was able to make lasting impressions that still govern forensics today. [7]

Locard's Exchange Principle is the governing principle of present day forensics and the

first thing learned when studying the subject. The principle states that when a person interacts with an object they leave a trace of themselves behind while taking a trace of the object with them. This principle sparked the formation of the trace evidence unit in the forensic laboratory. This unit deals exclusively with finding and characterizing the miniscule traces left behind at crime scenes. It can also be described as the principle behind crime scene analysis. [7]

In the early 1900s, Americans started to embrace the forensics movement. The first crime lab was created in Los Angeles, California in 1923. In 1930, Goddard founded the Scientific Crime Detection Laboratory at Northwestern University's School of law in Chicago, Illinois. The Federal Bureau of Investigation (FBI), in 1932, organized a national crime laboratory in Washington DC to provide forensic services to all the law enforcement agencies in the nation. The FBI succeeded and their forensic laboratory is now one of the largest forensic laboratories in the world. [5]

The largest single advancement in modern forensics had nothing to do with forensics. In 1953, Watson and Crick were able to determine the structure of DNA leading to rapid advancements in that field. In 1984 Alec Jefferies, a British geneticist developed genetic fingerprinting. This technique allowed DNA to be used as a form of identification for an individual. Two years later Kary B. Mullis developed polymerase chain reaction (PCR). It allows for the rapid multiplication of small amounts of DNA. This advancement allowed for the testing of trace amounts of DNA by forensic laboratories on blood smears or single blood droplets. [8]

Advancements in the science that drives forensic analysis is an ongoing process. In 200 years, the world went from torturing confessions out of suspects, to the sophisticated DNA testing done today. The foundation of forensics has been laid by several of the leading scientists. Scientists of today can only continue the trend, creating stronger and more reliable forensics for the future.

## 1.5 FORENSICS BEFORE THE SCIENCE

Forensics is a powerful force in the criminal justice system. It has the power to convict a person or set them free. Forensic science and scientists are not infallible. Each needs continual improvement in order to make forensic science and scientists more reliable. The following cases illustrate what happens when forensic science or scientists fail to properly do their job.

On June 30 1987, a fire broke out on the second floor of an apartment complex in Columbus Grove, Ohio. Cynthia Collins, a two-year-old girl died of asphyxia due to smoke inhalation during the fire. Her mother Hope Collins was spending the night with her boyfriend after a late night party. Ms. Collins told police that she asked Kenny Richey, and he agreed to watch her daughter in exchange for staying the night at her apartment.

When the Fire Chief initially investigated the scene, he declared the fire accidental. He blamed it on an electrical fan, which was removed from the Collins apartment. Since the death of a small child resulted from the fire, the Fire Chief ordered the Assistant State Fire Marshal Robert Cryer to also look at the scene and form his own conclusions. Cryer looked over the crime scene and declared the fire intentional.

The prosecution believed that Kenny Richey was the arsonist and began building a case against him. It turned out the Kenny Richey had an ex-girlfriend who lived in the apartment below Hope Collins. The prosecution alleged that in a fit of anger over his ex-girlfriend dating someone new, Richey set fire to the apartment above hoping that the fire would spread downwards and injure the new couple. They claimed that Richey stole gasoline and paint thinner from a nearby greenhouse, climbed up to Hope Collins apartment, poured the gasoline and lighter fluid in the living room, and set it on fire all before escaping back over the balcony with the empty cans.

The prosecution questioned witnesses from the party Hope Collins attended who claimed

Richey, his ex-girlfriend, and her new boyfriend were at the party. Richey met his ex-girlfriend when she moved into the apartment complex around June 15, fifteen days before the fire. On June 24, nine days later, Richey caught his then girlfriend sleeping with another man. Richey attacked this man with a knife and a fight occurred which resulted in Richey breaking his hand.

Witnesses at the party claimed that the ex-girlfriend brought a new man to the party, and asked him to date her in front of Richey. The witnesses were divided on what happened next. Some witnesses said that Richey became upset over the new relationship, and others said that the three discussed it and that Richey accepted the situation. Other witnesses claimed they overheard Richey, or Richey spoke directly to them, state if he couldn't have his ex-girlfriend then no one could. He then threatened to burn down his ex-girlfriend's apartment complex.

Hope Collins claimed that Richey asked to stay the night in her apartment around 2AM and she refused. At 3AM Hope Collins' boyfriend drove up and asked if she wanted to go with him. Hope granted Richey's request to stay in her apartment if he would watch her daughter. At 4:15 AM neighbors called police about flames coming out of the Collins' apartment. Richey was seen coming out of the apartment and firefighters had to restrain him from going back into the apartment. The same witnesses that claimed that Richey was planning on burning down the apartment complex, told investigators that Richey drank a beer and laughed over the fire while boasting about doing a good job.

Richey admitted to the police that he had stolen two plants from a nearby greenhouse. A witness at the party told police that Richey offered to steal her a couple of plants from the same greenhouse. The greenhouse owner told police that he kept paint thinner and gasoline in the greenhouse, but wouldn't be able to tell them if anything was missing.

During the investigation, the State retrieved six samples of debris from the fire. The first

issue with the samples was that they were not taken immediately from the crime scene. A piece of carpet from the crime scene was obtained two days after the fire from the local dump. The carpet was then left in the sheriff's parking lot for three weeks before being tested, where it sat close to gasoline pumps. The state also obtained a wood chip sample from inside Hope Collins apartment three weeks after the fire.

The Assistant State Fire Marshal, Robert Cryer, concluded from his assessment of the Collins apartment that burn patterns indicated an accelerant was poured on the deck and living room carpet. An expert for the prosecution agreed with Cryer that the fire was arson and accelerants were used. A piece of the carpet retrieved also contained traces of gasoline, and the wood chips from the deck contained paint thinner as confirmed by gas chromatography.

Police immediately suspected 18-year-old Kenny Richey and he was arrested on July 1, nearly three weeks before any testing was done. In his statement to police, Richey claimed he was drunk and did not know how the fire started. He also claimed that he knew two-year-old Cynthia Collins was in the apartment, and had checked on her during the party. Richey also made threatening statements toward the prosecution and witnesses.

The defense in the case claimed that no arson had taken place, and that conflicting circumstantial evidence and faulty forensic evidence and practices lead to a false conclusion. The forensic analysis of the evidence used a chromatographic technique that had not been reviewed by any of his peers or published in the wider scientific community. Unfortunately, for Richey, the defense in his first trial was incompetent and it wasn't until his later retrial that he received competent representation.

During a retrial in 2008, a specialist in fire reconstruction refuted Cryer's claim that accelerant had been used. The specialist stated that the burn patterns occur naturally in areas with

plenty of oxygen beneath the fire, such as the deck where the fire burn the hottest, and that ten gallons of fuel would have been needed to produce the burn pattern. Experts for the defense also asserted that there was no accelerants found on Richey's clothing, and that Cryer initially declared that the fire was accidental and allowed the owner to gut the apartment instead of sealing the scene and preserving vital evidence. The defense also claimed that Richey was in a plaster cast for a broken wrist and would have found it difficult to climb into and out of the Collin's apartment. The defense also learned, and presented it at retrial, that the fire chief had been called to Collin's apartment three times in the preceding weeks because of mysterious smoke. The last hole in the prosecution's case came from their star witness recanting her statements.

Ohio Prosecutor Dan Gershutz told reporters 'Even though this new evidence may establish Mr. Richey's innocence, the Ohio and United States constitutions nonetheless allow him to be executed because the prosecution did not know the scientific testimony offered at the trial was false and unreliable'. The state of Ohio denied all attempts made by Richey to appeal his case until ordered to by the Ohio Supreme Court. Richey plead 'no contest', neither innocent nor guilty, for his retrial and was released from prison. This case was a horror show of faulty evidence collection and analysis techniques, along with a strong bias where the prosecution believed that Richey was guilty from the onset of the investigation. [9]

Faulty techniques and ineffective sample gathering and storage are not the only issues that a forensic laboratory can face. On occasion, it is the forensic scientist who cause issues, and it is usually not limited to a single case. Instead, the problems are linked to every case the forensic scientist was responsible for analyzing.

From 2005 to 2015, New Jersey State Police employed lab technician Kamalkant Shah. Shah's job was to analyze seized drug evidence to determine the identity and legality of the



evidence. In 2015, Shah was observed faking test results for drug evidence. Shah was immediately removed from his job, but now all cases in which he worked are being questioned.

The New Jersey State Police now has to address the major issue of retesting evidence that Shah ‘analyzed’. In many of these cases, there is insufficient or no samples available for reanalysis. Currently, New Jersey has no policies in place to deal with the above-mentioned issue. It is likely that those convicted in cases with no or insufficient evidence remaining will be released regardless of actual guilt or innocence. At this time, no charges have been filed against Shah. [11]

In 2012, it was discovered that Annie Dookhan, a Massachusetts state lab drug analyst, forged results for analyses that she performed. It is alleged that Dookhan would forge results for prosecutors she was friendly with and for offenders she believed were guilty and should go to jail. During her employment for the Massachusetts state crime lab, Dookhan analyzed over 40,000 samples in thousands of cases. The result from each of the 40,000 pieces of evidence are now being questioned.

Dookhan was known throughout the state crime lab and prosecution offices as being able to analyze samples three times the speed of her colleagues. In 2010, Dookhan was audited due to her fast analysis times. No wrongdoing was found during the investigation. However, a year later, she was observed forging a coworker’s signature and improperly handling evidence. Dookhan was eventually suspended from her job and then fired after all of her misdeeds became known. Her mishandling of evidence, forging results, and blatant bias has earned Dookhan a jail sentence. Over 300 prisoners have had their convictions overturned because Dookhan ‘analyzed’ the evidence in their case.

After Dookhan’s deceptions were found, a thorough examination of the Massachusetts state crime lab was conducted. An analyst in the same lab as Dookhan was found to have forged

her college transcripts. She graduated as a sociology major, but claimed that she was a chemistry major. She gave expert testimony on an additional 190,000 cases when she was not properly qualified resulting in an audit of her cases as well as Dookhan's cases. [12]

In a different lab, but still in the Massachusetts state crime lab, another analyst was found who tampered with evidence. Unlike Annie Dookhan, Sonja Farak did not forge evidence results to convict drug dealers; she tampered with drug evidence in order to steal the drugs. Farak was a drug addict who used her job to supply her habit. She was arrested and convicted of tampering with evidence and received an 18-month jail sentence. [13]

Massachusetts and New Jersey aren't the only states facing issues with their forensic scientists. Texas has also shared in the misery when they discovered a forensic scientist who was forging results in 2013. South Carolina had a forensic scientist who was forced to resign after her training and work quality were called into question in August of 2014. In February of 2014, Florida dealt with a supervisor who would replace illicit drugs with over the counter pills. Continuing the forensic laboratory issues of 2014 New York is facing an audit of cases due to an analyst inaccurately weighing a drug sample. [13,14,15]

It is cases like these, where forensic scientists fail to live up to the high standards necessary for the positions, which calls for more stringent oversight and faster analytical tests. Forensic laboratories are inundated with cases at a rate far faster than they can process. This overload of cases causes laboratories to hire less qualified scientists and to have less supervision over the scientists. Steps need to, and are being undertaken to address the underlying causes to prevent more bad scientists from making their way into forensic laboratories.

A case that highlighted major issues within the forensic science community, the police departments, and the interaction of the two was the high profile case of OJ Simpson in 1994. On

June 12 1994, Nicole Brown Simpson and her friend Ronald Goldman were found murdered outside of Nicole's home.

When police began investigating the crime, OJ Simpson, Nicole's ex-husband, was the main suspect. The prosecution built what they thought was a strong case against Simpson. Simpson had bought a midnight flight to Chicago the night of the murder. Once Simpson returned, investigators found a bloodied glove at the crime scene, blood in Simpson's car, droplets leading into his home, and blood on a sock inside his house. DNA analysis of the various blood evidence was able to link OJ Simpson, Nicole Brown Simpson, and Richard Goldman to all pieces of evidence.

The prosecution thought they had an easy case with solid evidence. Unfortunately, for them problems resulting from the beginning of the investigation and continuing throughout case left the defense plenty of opportunities to question the validity of the evidence. The defense claimed that the collection and analysis of the blood samples led to invalid or tampered results.

Sloppy documentation and collection of the evidence started with the first officer on scene. A bloody fingerprint was found on the gateway leading to Nicole's house and was described by the first officer on scene but never photographed or collected by latter crime scene technicians. The fingerprint was never collected and it's only mention comes from the initial officers report. The fact that a crucial piece of evidence was neglected and lost to the elements was just a small part of the defense's case.

The defense alleged that the evidence used to build the prosecution's case was sloppily collected and mishandled during analysis. Technicians who documented the crime scene did not use scales or position markers in the photographs. These failings in proper protocol made it difficult to ascertain where the photos were taken and make measurements from the photos.

Different pieces of evidence was gathered and placed in the same bag leading to cross contamination. Wet items were prematurely bagged and not allowed to dry causing possible chemical changes or mold growth to occur. Even the body of Nicole Brown Simpson was contaminated when someone covered it with a blanket found in her house. On top of all the blunders caused by the forensic technicians, the Los Angeles police officers compounded the situation by trampling through the scene leaving bloody footprints in their wake.

Issues with the evidence did not stop after it was collected. Investigators improperly handled the evidence before turning it over to the forensic laboratory. Evidence was carried around in pockets where it could degrade instead of being turned over immediately to the forensic laboratory for proper storage and preservation. Known blood samples provided by OJ Simpson were spilled in the same laboratory where additional DNA evidence from the crime scene was analyzed shortly thereafter. The forensic laboratory even 'lost' 1.5mL of Simpson's known blood sample. The person who collected the blood from Simpson estimated 8 mL had been taken, but the forensic laboratory could only account for 6mL.

The defense even accused investigators of planting DNA evidence that linked back to OJ Simpson. With the careless gathering of evidence and lack of proper documentation, many pieces of evidence were not fully logged or described when collected. The sock collected at Simpson's home that contained DNA evidence linked to his ex-wife was not noted to have blood on it at the time it was collected. Investigators even went back to the scene weeks after the crime occurred and collected more blood evidence that was linked back to Simpson.

Though it could not be proven that evidence had been planted or tampered with, the defense was able to plant a seed of doubt in the jury's minds. They were able to show that the investigators and forensic scientists who worked on the case used improper handling and analytical techniques when

processing the evidence. The lack of standards and approved procedures led to a not guilty verdict in the OJ Simpson double murder case.

One of the largest issues in the Simpson case was the lack of accreditation of the forensic laboratory that processed the DNA. At the time of the case, only a handful of labs around the country were accredited for DNA analysis. , The Los Angeles forensic laboratory and their police department did not have the proper procedures or training in place to adequately handle DNA. This case is a clear example of why training, procedures, and reviews are necessary to keep a forensic laboratory creditable and working at maximum efficiency. [16,17]

## 1.6 FORENSIC SCIENCE NOW

Crime is a fascinating subject. It has spawned books, movies, and television shows. A genre of television shows are made of forensic driven crime procedurals. These shows draw in millions of people and allows them to experience “forensics” in the comfort of their home. This elevates forensics to a household term, but spreads misconceptions that have wide spread consequences.

Forensic science is an exacting science that requires immense attention to detail and rigorous application of proven scientific methods. Forensic scientists need to be methodical and detailed in their approach for evidence as their work is intended to be used in court and could be the deciding factor in a case. Because of this, courts hold forensic scientists to high standards.

The techniques found in forensic examinations come from the wider science community. They are thoroughly examined and peer reviewed before making their way into a forensic laboratory. Only established methodology that has been proven to work consistently is implemented in the forensic laboratory.

When forensic scientists run into a problem that their established protocols can't solve,

they turn to research scientists and the work that they do. Forensic scientists comb the literature looking for peer-reviewed methods that they can apply in their lab. Issues with this problem-solving strategy can often arise during court testimonies. It is the responsibility of the scientist to convince the attorneys and judge that the method used is a scientifically sound and accepted method in the science community. If the method isn't accepted by the judge, the evidence could be deemed inadmissible.

Forensics hinges on convincing a judge or jury that the facts presented are true and reliable. When put in a courtroom forensic scientists become expert witnesses. The courts in the U.S. have worked at defining what an "expert witness" truly is.

In 1923, a young man by the name of James Alphonzo Frye was tried for murder. Frye underwent a lie detector test, also known as a systolic blood pressure test. It's believed that when a person lies it causes a physical reaction that can be picked up by monitoring blood pressure. Frye's expert witness tried to testify that the lie detector test administered showed that Frye was telling the truth when he claimed innocence. The courts, however, refused to allow the testimony. The courts declared that evidence must be analyzed using "well recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs." [18]

Frye was used by the courts to try to add structure to what expert testimony could consist of and eliminated most of the lengthy hearings that validated techniques prior to this ruling. A failing of this ruling is that it did not define what "general acceptance" was or how to define a "particular field." Rapidly changing or innovative methods were also ill defined. Scientists and others found the Frye rulings ill-equipped to deal with the rapid changes in forensics and susceptible to judicial interpretations. [18]

Congress adopted the Federal Rules of Evidence in 1975. This document set out to define what could and could not be used as evidence in a court of law. Rule 702 states: “If scientific technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training or education, may testify thereto in the form of an opinion or otherwise.” [19]

Opinions of the new rules were once again clouded with confusion. It could not be determined if these rules were a reiteration of the Frye ruling, a new standard altogether, or a hybrid Frye standard. One of the main similarities between the Frye standard and Rule 702 is that the qualifications of the expert and the admission of evidence was not clearly defined.

In order to settle the disagreements between scholars, litigations, and judges, the federal court stepped in in 1993 to review a Ninth Circuit Court of Appeals decision, a step below the Supreme Court, in *Daubert v. Merrell Dow Pharmaceuticals*. This case involved two mothers who claimed that the medicine given to them from Merrell Dow Pharmaceuticals to help reduce morning sickness had caused birth defects in their children. [20]

The prosecution had an expert who claimed that the structure of the drug was similar to a teratogen, a compound that causes birth defects and that their reanalysis of published studies showed a statistical correlation between the drug and children born with birth defects. The expert witness for Merrell Dow Pharmaceuticals argued that the published studies that the prosecution had reanalyzed did not show a significantly greater rate of birth defect in women taking the drug versus those not taking the drug.

The lower courts ruled that evidence based on unpublished reanalysis of data that was not subjected to peer review was not acceptable evidence. The Ninth Court affirmed the decision of the lower court and upheld the verdict that the data did not have the general acceptance of its

scientific community and was therefore inadmissible.

The Supreme Court ordered the Ninth Court of Appeals to hand over all the documents of the case and formed a committee of unbiased sources including the American Association for Advancement of Science, The National Academy of Sciences and other groups of scientists and law professors to review the case. The ultimate finding of the Supreme Court was that Rule 702 did not incorporate the Frye general acceptance standard and the Rule 702 incorporated a flexible reliability standard instead.

The Daubert case firmed up some of the guidelines of Rule 702 for admitting scientific expert testimony. The Supreme Court decided that the judge was the gatekeeper for expert testimony. The decision on whether the testimony was scientifically sound or not would be decided by the judge of the case. This means that the judge is required to make sure that the expert's testimony remains relevant to the case and has scientific backing from the community. The also ruled that results will be considered scientific knowledge if the expert can demonstrate that they were produced from sound scientific methods. [20,21]

Today forensic science is becoming more regulated and assimilated across the country in order to provide better service to the police districts they serve. The forensic landscape has changed from one in which the experts were the pioneers of the field, into one in which more and more scientists are needed to analyze all the evidence gathered. This has led to regulatory agencies being formed to ensure that forensic laboratories meet certain basic guidelines. [21]

## 1.7 ACCREDITED CRIME LABS

Around the same time that Congress was considering the Federal Rules of Evidence, the FBI held a meeting with forty-seven of the nation's forensic laboratory directors. The meeting was



a large success and plans were made to organize an association of directors. A year after the first meeting the American Society of Crime Laboratory Directors (ASLCD) was formed. [22]

At the same time, the Forensic Science Foundation created a voluntary proficiency test that measured the competence of forensic scientists. The results of this test showed distressing results. The competency levels of test takers were not as high as anticipated and led to the questioning of the quality of work performed at crime laboratories across the nation.

One of the first duties of the newly formed ASCLD was to address this concerning problem. The Committee on Laboratory Evaluation and standards was formed to form and implement a program that would insure a minimum standard of work for all forensic laboratories. The committee decided that individual certification, self-assessment, and an external peer review was adequate to determine the competency of a laboratory.

In 1982, the first eight labs were accredited in Illinois. Since that date, 392 labs have been accredited by ASCLD. Of those laboratories, 349 are local, state, or federal government laboratories, 18 are international laboratories, and 25 are private laboratories [22].

The guidelines that ASCLD follows for accreditation of its laboratories and forensic scientists allow for ethical scientist, competent and proficient labs, who are good at communication. An ethical forensic science is one who is unbiased and fair, who analyzes the evidence without trying to prove the guilt or innocence of a suspect. A good forensic scientist also does not make conclusions or testify on evidence outside of their expertise. Forensics is a massive field, and impossible for one person to be an expert of everything.

ASLCD accreditation is important to crime labs around the world. With their stamp of approval lawyers, judges, and juries can feel safer accepting their testimony as it is backed up by a stringent accreditation process to ensure that crime labs are staffed and run properly. [22]

## 1.8 ACCREDITED ACADEMIC PROGRAMS

In 1999, the National Institute of Justice (NIJ) published an article titled Forensic Science: Review of Status and Needs. This article laid out the needs, educational and training, that the forensic scientists needs before stepping in to a laboratory. The list of needs was large and detailed, and the NIJ put it to the science community that there needed to be standards in forensic science education. The NIJ took it one-step further and set up a group that outlined sample curriculums and guidelines for universities with a forensic program in 2003.

In 2004, the Forensic Science Education Programs Accreditation Commission (FEPAC) was established by the American Academy of Forensic Sciences (AAFS). FEPAC performs a similar job as the American Society of Crime Laboratory Directors (ASLCD). The program works with universities around the United States who are interested in offering or maintaining an undergrad forensic science program. FEPAC provides a uniform and rigorous accreditation process that ensures quality education for the graduates. [23]

While crime labs do not require its applicants to graduate from accredited schools, those who do are considered a step ahead of the game. Graduates from accredited programs come with a grounding in chemistry, biology, biochemistry, genetics, and math that are often requirements for the various forensic laboratory sections. Accredited programs have emphases in forensics, and are required to cover topics such as an introduction to law, courtroom testimony, ethics, quality assurance, and a survey of forensics science. Along with the academic topics required, accredited schools are also required to require an internship at an accredited forensic laboratory.

Through accreditation, universities can begin properly training forensic scientists before they

step into the workforce. The goal of FEPAC and ASCLD is to hire and maintain a higher quality forensic scientist. These higher standards will work towards reducing the number of wrongful convictions by ensuring that high standards are required and met in forensic laboratories.

## 1.9 NATIONAL COMMISSION ON FORENSIC SCIENCE

In 2013 the Department of Justice (DOJ) and the National Institute of Standards and Technology (NIST) banded together to form a commission, the National Commission on Forensic Science (NCFS), to improve forensic science by improving the intersections between forensic science and the criminal justice system. This new program meant to ensure the quality of forensic science includes forensic scientists at the local, state, and federal level, along with prosecutors, defense attorneys, and judges from across the nation. [24]

According to its charter the objectives of the NCFS includes providing “recommendations and advice to the Department of Justice (DOJ) concerning national methods and strategies for: strengthening the validity and reliability of the forensic sciences (including medico-legal death investigation); enhancing quality assurance and quality control in forensic science laboratories and units; identifying and recommending scientific guidance and protocols for evidence seizure, testing, analysis, and reporting by forensic science laboratories and units; and identifying and assessing other needs of the forensic science communities to strengthen their disciplines and meet increasing demands generated by the criminal and civil justice systems at all levels of government” (NCFS Charter 2013). [25]

The National Commission on Forensic Science joins the American Society of Crime Laboratory Directors and the Forensic Science Education Programs Accreditation Commission as another group dedicated to improving the quality of forensic science and its reliability to the

criminal justice system. Forensic science continually looks to its past, looking for ways to improve itself. The forensics of the future is a better forensics thanks to these programs that insure that the quality and competency of forensic scientists is maintained and continually improved. [25]

#### 1.10 FORENSIC SCIENCE OF TOMORROW

From humble beginnings of detecting poison in a dead body and using fingerprints as identification, forensics has exploded in the twenty first century. Forensics is now recognized worldwide as an authority on determining the facts of a crime. Specialized forensics now deal with art, computers, ear prints, botany, engineering, linguistics, and even social work. These are only a few areas that forensics has impacted.

How forensics will progress from here comes down to research preformed. Forensics is an ever changing, ever growing field that implements new and improved techniques to more accurately analyze evidence. Without research into forensics, the field would have never reached the point where it is today. The future of forensics rests in the hands of today's scientists.

## CHAPTER 2

### GC-MS CHEMICAL FINGERPRINT COMPARISON OF EVAPORATED AND UNEVAPORATED FUEL SAMPLES USING THE INTERNAL STANDARD, DI-N-DECYL SULFIDE

#### 2.1 ABSTRACT

Due to the variety of methods used to commit arson, identifying the accelerant used poses a challenge for investigators. In this study, gasoline samples taken from gas stations in the Oxford, MS area were analyzed to understand the effect evaporation has on the chromatograms of samples evaporated to predetermined levels. To quantitatively measure the concentration of compounds in the samples, the internal standard di-n-decyl sulfide was chosen for this study since it 1) allows for important peak ratios to be calculated 2) has a retention time outside of the target peak range 3) is relatively inexpensive 4) and is less hazardous than other internal standards studied. Three grades of gasoline from two different gasoline stations with octane ratings of 87, 89, and 93 were analyzed in duplicate. All samples were measured with and without the internal standard. The gasoline samples were evaporated to 25, 50, 75, 83, 91, and 99% levels to investigate a broad range of states of evaporation. Without an internal standard, the composition of each gasoline sample chromatogram appears to change significantly as gasoline is evaporated. The internal standard concentration of the lighter compounds reduces while the heavier compounds increase relatively

before reducing at 99% evaporated. There is little change in spectral data when gasoline sample spectra alone (no internal standard) are compared to the results with the internal standard. Since the concentrations are measured in relative abundance, the heavier molecules appear on the chromatogram as the lighter molecules (those that elute first from the GC column) disappear from the chromatogram as they are evaporated from the samples. The internal standard also allows for the comparison of compound abundance in samples that can lead toward origin identification. Each gas station uses a different makeup of gasoline during production, and analysis of the chromatograms indicate these differences. This study proves that by using the internal standard, di-n-decyl sulfide, and the changes that occur in the gas chromatograms of gasoline samples that are evaporated, forensic chemists can assist arson investigators by determining fuel grades, sample origins (gas stations), and gasoline evaporation levels.

## 2.2 INTRODUCTION

This research was designed to initiate the creation of a database of chromatograms of various grades and brands of gasoline using gas chromatography-mass spectrometry (GC/MS). This study also sought to identify an internal standard that would allow for quantitative analysis of gasoline with the intent to determine the origin of unevaporated gasoline samples. Results from this study can be used to help solve future arson cases.

Arson, as defined by the Uniform Crime Reporting program under the FBI, is “any willful or malicious burning or attempting to burn, with or without intent to defraud, a dwelling, public building, motor vehicle or aircraft, personal property of another” [26]. In 2011 alone, there were 52,333 arson cases reported throughout the country. From 2011 to 2012 crimes, involving arson

increased by 3.2%, and this was only for the first six months of 2012. Within those first six months, three of the countries' regions reported an increase in arson. The Midwest reported an 11% increase; the West reported a 6.4% increase; and the Northeast reported a 5.7% increase in arson cases [26]. Arson cases are continually rising. Because of this increase, it is important for forensic scientists to be able to determine the cause of these fires in an efficient manner. In the investigation of arson, it is extremely difficult to identify the accelerants used at the scene of the crime, partly due to the fact that they can evaporate over time [27, 28]. Studies show that gasoline is the most common accelerant used for arson [28,29,30]. Being able to link the gasoline type found at the scene of the crime to a container of gasoline found at a suspect's house could assist arson investigators in solving more arson cases. [31,32,33,34,35,36,37,38,39,40]

Gasoline was first distilled in the mid 1800's as a byproduct to kerosene production. At the time there was no use for gasoline, and it wasn't until the late 1800's and the invention of the automobile that gasoline found its usefulness. [41] As the popularity of the automobile increased and the demand for gasoline also increased, scientists began experimenting with the composition of gasoline.

The efficiency of gasoline is measured using an octane rating scale. The octane rating is a measure of a gasoline's compressibility and resistance to premature ignition. Gasoline octane ratings are determined by using isooctane and heptane as a reference mixture. Pure isooctane has an octane rating of 100 and mixtures of isooctane and heptane are analyzed to determine their ability to resist premature ignition. The properties of gasoline are measured in the same manner as the isooctane and heptane mixtures in order to determine their properties. Gasoline is assigned an octane rating based on the isooctane/heptane mixture that most closely matches its properties. A

gasoline with an octane rating of 90 would have the same properties of a mixture that contains 90% isooctane and 10% heptane. It is possible for gasoline to have an octane rating higher than 100, as some fuels are better able to resist premature ignition than pure isooctane, but the standard octane rating for gasoline is 83 in the United States. [42,43,44,45,]

In a typical engine, a gasoline/air mixture is heated due to compression, and is ignited by a spark from the spark plug causing the gasoline/air mixture to rapidly burn, creating carbon dioxide and hydrogen that expands and forces the cylinder to cycle creating mechanical force. If the composition of the gasoline is not correct it can cause the gasoline/air mixture to ignite prematurely or not at all, leading to damage to the engine. Car manufacturers design engines that work best with the standard gasoline octane ratings widely used in the intended country. At the same time gasoline manufacturers develop gasoline blends that burn more cleanly and efficiently to cause less damage and buildup on engines. [46,47,48,49]

Unprocessed gasoline from refineries mostly consists of hydrocarbons with four to twelve carbons per molecule. The structures of the hydrocarbons fall into three categories; alkanes, hydrocarbon molecules with only single bonds; cycloalkanes, hydrocarbon molecules that contain carbons bound in a ring with only single bonds; and alkenes, hydrocarbon molecules that contain at least one double bond. The ratios of the hydrocarbons differ based on the initial crude oil used, the refinery processing the crude oil, and the octane rating of the gasoline being produced. After refining the gasoline from crude oil it is further processed via a chemical transformation such as catalytic reforming, catalytic cracking, and alkylation among others. These chemical processes convert lower quality hydrocarbons to higher quality hydrocarbons needed to raise the gasoline octane rating. In addition to the chemical processes, gasoline refineries also add chemicals to



increase the octane rating, reduce the oxidation potential for gasoline, and even reduce carbon buildup on the inside of the engine. Each major brand of gasoline has different additives or different amounts of common additives added to the final gasoline product. These additives, along with the slight differences produced during refining cause gasoline samples from different gas stations to have slightly different gas chromatograms when analyzed. These differences can aid forensic scientists in determining the origin of the gasoline, or determining if two samples originated from the same source, gas station or refinery. [50,51,52]

### 2.2.1 GAS CHROMATOGRAPHY (GC)

Gas chromatography (GC) is a separation technique used to separate mixtures of volatile chemicals or compounds. The chemicals are separated using a dual phase method. These phases interact together to move the analytes at different speeds which creates separation. Chromatography contains a mobile phase and a stationary phase.

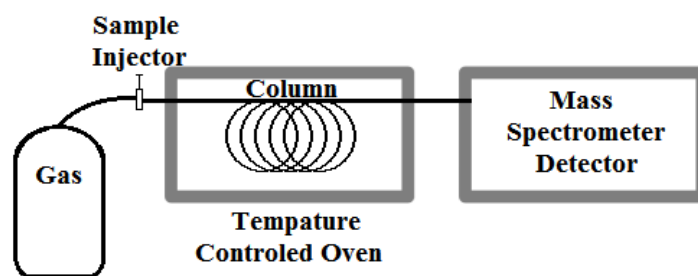


Figure 1. Gas Chromatograph schematic

In gas chromatography, the mobile phase is an inert gas that moves the analyte molecules through the column to the detector. The mobile phase gases are usually nitrogen, helium, or hydrogen, and do not interact significantly with the analyte molecules or the stationary phase/column. The column is the crucial component in the separation of mixtures. It interacts with

the various analytes in the mixture in different ways resulting in different retention times for the different components of the mixture. Typical GC columns are tens of meters long with a micrometer inner diameter and are called capillary columns. These columns are most useful for small sample volumes. Overloading a column can cause insufficient separation. The inside coating of these columns, consists of very high weight polymers, especially long chain substituted silicones. The majority of capillary columns contain a polar stationary phase that separates the analyte molecules based on their polarity. Analyte molecules with similar polarities are separated based on size differences. Heavier molecules take longer to travel through the column than lighter molecules. Molecules with similar polarities are also separated by boiling point differences. The column is heated in a controlled manner during analysis.

Gas chromatography can also determine the amounts of the individual components of the analyte molecules. Detectors on a GC not only record the time an analyte takes to transverse the column, but they also record the amount of the analyte that reaches the detector by monitoring the amount of time spent in the column and abundance of compound that passes the detector. When a standard is used, the concentration of the unknown analyte can be calculated from the collected data.

Gas chromatography is a useful separation technique used in analytical chemistry. When gas chromatography is combined with other techniques, it provides more information that can be used to accurately identify analytes. GC can be coupled with mass spectrometry, flame ionization, and other devices that allow different chemical information to be gathered depending on the sample to be analyzed. [53]

### 2.2.2 MASS SPECTROMETRY (MS)

Mass spectrometry (MS) is used to separate different positively charged ions based on their mass and charge. According to Jim Clarke, four stages occur in MS. The first stage is ionization. In this stage, the molecule is bombarded with electrons to form a positive ion. The second stage is acceleration, where the newly formed ions are accelerated with a magnetic or an electric field until all of the ions have the same kinetic energy. The third stage is deflection, where ions are deflected by a magnetic field based on their mass and charge. The lower the mass of the ion the more the ion will be deflected. The fourth and final stage is detection of the ions that are moving through the instrument which are electrically detected. When MS is coupled with gas chromatography, it has been widely used by many researchers for identification and quantification purposes [54].

Quadrupole mass spectrometry is a commonly used mass separator in mass spectrometry. It consists of four rods set up in a square configuration as seen in figure 2. Two sets of opposite rods oscillate between positive and negative voltages. The oscillation of the voltages causes the charged ions to travel in a spiral trajectory as they are alternatively attracted and repelled by the sets of charged metal rods. At a given voltage the path of the spiraling ions is only stable for a specific mass to charge ratio. Only ions with the appropriate mass to charge ratio will reach the detector. All other ions will collide with the rods or the walls of the quadrupole and be lost. The voltage applied to the quadrupole can be changed during analysis, allowing for a range of mass to charge ratios to be detected. [54]

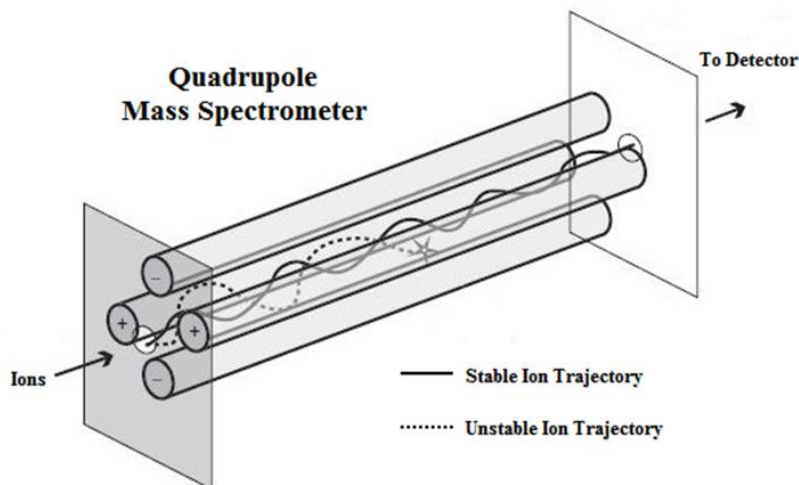


Figure 2. Schematic of a quadrupole mass spectrometer

### 2.2.3 INTERNAL STANDARD

Internal standards are used to help reduce the uncertainties and variabilities in results produced during sample preparation and sample loading. An internal standard is a compound that is similar in chemical characteristics to the target analyte that can be introduced to the sample during sample preparation. Internal standards are useful for samples like gasoline due to the complex makeup and the required dilution of the samples prior to analysis. An internal standard reduces the need for multiple external standards and adjusts for slight variations and human errors during sample preparation. Many of the studies that have been researched in the literature have not included an internal standard during sample preparation. These studies are intended to qualitatively study gasoline, while this study aims to compare peak ratios as the gasoline samples are evaporated [29,30,53,55,56,57]. The addition of an internal standard to samples at different levels increases the accuracy in determining the differences in peak abundances and quantifying the compounds in gasoline. An internal standard will also provide a reference to measure accurate retention times and changes in peaks heights. The use of internal standards provides both

qualitative and quantitative results that can assist in characterizing components of different gasoline samples.

## 2.3 MATERIALS AND METHODS

### 2.3.1 MATERIALS

Gasoline samples were obtained from a Texaco and Shell gas station in Oxford, MS during the summer and winter of 2013. Samples of three gasoline grades, (87, 89, and 93) were collected from both gas stations and were divided into two batches totaling twelve samples. The samples were all collected from the gas stations on the same day. Samples for the winter testing were collected from the same gas stations as those used in the summer testing. Along with the gasoline samples carbon disulfide (Fisher Scientific C184-500) and di-n-decyl sulfide (Fisher Scientific 50-700-8680) were used as the solvent and internal standard, respectively.

### 2.3.2 SAMPLE PREPARATION WITHOUT INTERNAL STANDARD

Gasoline samples were transferred to glass vials with air tight caps for storage between evaporation levels. The sample vials were marked every 25% based on volume in order to visibly determine evaporation levels during the experiment. Gas chromatograms and mass spectra were taken at 0, 25, 50, 75, and 99% evaporation levels for all samples. Samples obtained in the winter of 2013 were also evaluated at 83% and 91% due to the rapid decrease in volatile compounds between 75% and 99% evaporation levels.

The samples at all evaporation levels were prepared using the same preparation method. In

a clean beaker, 1 mL of carbon disulfide was mixed with 7 $\mu$ L of the gasoline sample. GC-MS sample injections were performed either, manually or automatically as subsequently described. After a successful gas chromatogram and mass spectra were obtained, the caps were left off the sample vials in a fume hood. The samples were all allowed to evaporate in an open-air environment at room temperature for 2 hours to a month. Once the desired evaporation level was reached, samples were capped to prevent over-evaporation until analysis could be performed. [36]

### 2.3.3 SAMPLE PREPARATION WITH INTERNAL STANDARD

Winter samples were also analyzed with an internal standard solution of 0.01M di-n-decyl Sulfide. The samples prepared with internal standards were also prepared by diluting 7 $\mu$ L of the gasoline samples with 1mL of carbon disulfide. 40 $\mu$ L of the internal standard solution was added to the gasoline and carbon disulfide sample. Di-n-decyl sulfide was used in this research for its ability to elute during gasoline analysis, its increased safety, and its ease of use when compared to deuterated analogs of compounds found in gasoline.

### 2.3.4 MANUAL INJECTION

For manual injection, a glass syringe was used to inject samples onto the GC-MS. The syringe was washed with 5 $\mu$ L of carbon disulfide, 3 to 4 times. To ensure that the previous samples did not contaminate the sample of interest, the syringe was washed with the sample mixture 3 to 4 times. After washing, 1 $\mu$ L of the mixture was carefully drawn into the syringe, making sure all air bubbles were removed. The sample was then injected into the GC-MS and the start button was pushed after a 3 second wait.

### 2.3.5 AUTO SAMPLER INJECTION

An auto sampler was used for a large portion of samples including most of the internal standard samples. The auto sampler was programmed to rinse the syringe three times with 5 $\mu$ L of carbon disulfide before washing the syringe three times with 2.5 $\mu$ L of the sample. To eliminate all air bubbles, 1 $\mu$ L of the sample was drawn up by the syringe rapidly for 10 strokes before a final volume of 1 $\mu$ L was obtained. The syringe was then plunged into the injection port on the GC. A programmed three-second delay was observed after the syringe's plunger was depressed and the instrument began its readings. The auto sampler parameters were set up to mimic the manual injection parameters as closely as possible.

### 2.3.6 INSTRUMENTAL PARAMETERS

The GC-MS analysis was conducted using a Thermo Scientific Trace GC Ultra and a Thermo Scientific ITQ1100 mass spectrometer. Separation was achieved on a 30m x 250 $\mu$ m x 0.25 $\mu$ m column. 1 $\mu$ L sample volumes were placed into auto sampler vials and each sample was injected into a splitless injector with 1 mL of constant H<sub>2</sub> as the carrier gas [5]. The inlet temperature was set at 230°C to vaporize the gasoline molecules. The mass spectrometer had a source temperature of 300°C and was set for full scan over an m/z range of 33-300 atomic mass units in electron ionization mode. The oven temperature was initially held at 37°C for 2 min, ramped at 5°C/min to 120°C, and then ramped at 12°C/min to 280°C, where it was held for a final 4 min. The first ramp elutes the compound of interest, and the second ramp cleans and prepares the column for the next sample. Sample preparation was conducted during this time. Before any

samples were run, a blank was run followed by carbon disulfide alone.

## 2.4 RESULTS AND DISCUSSION

### 2.4.1 WITHOUT INTERNAL STANDARD

The analysis of both the Shell and Texaco gasoline samples allowed for easy identification of each peak. The major peaks found in these samples, with retention times of 1) 3.34 minutes, 2) 4.92 minutes, 3) 7.64 minutes, and 4) 11.46 minutes were identified by the NIST library as 1) 2-methyl-1-nitropropane, 2) toluene, 3) xylene, and 4) tri-methyl benzene, respectively (Figure 3). These peaks are found in various concentrations in all brands and grades of gasoline.

When comparing the unevaporated chromatogram with the 99% evaporated chromatogram (Figure 4), most of the major peaks present in the unevaporated sample are no longer detectable in the 99% evaporated chromatogram. Tri-methyl benzene is the only original major peak that appeared at the 99% evaporated level. The slight difference in retention times (RT) in the chromatograms is the result of a shorter column following instrument maintenance.



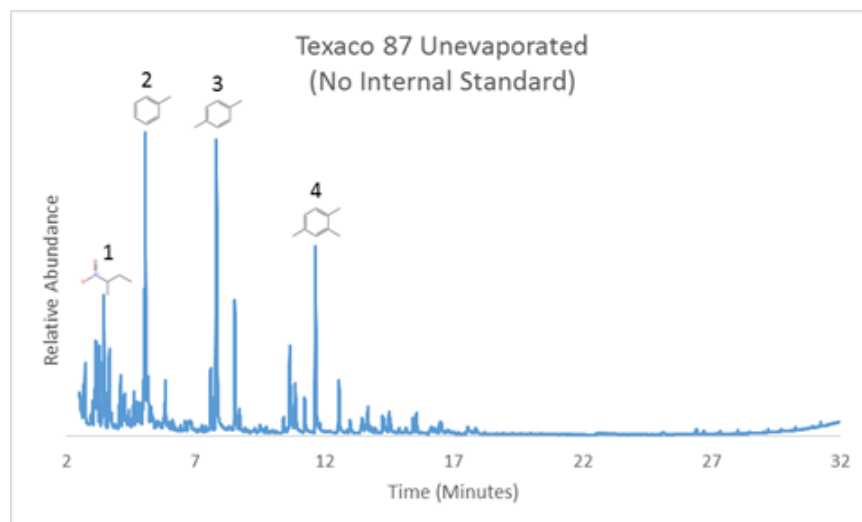


Figure 3. Chromatogram of Texaco grade 87 unevaporated gasoline without the internal standard. The NIST library was used to assign the most abundant peaks. (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene)

By observing evaporation patterns and attenuation rates of the samples, the volatility of the gasoline components can be determined. The peaks that attenuate and disappear the fastest represent compounds that have smaller mass to charge ratios and appear to be more volatile. The 2-methyl-1-nitropropane peak at 3.34 min in Figure 4 is the first major peak to evaporate fully throughout the evaporation process. It is the lightest peak examined in this study and is absent in the 75% evaporated sample. The evaporation levels of Texaco 93 are compared in Figure 5. 2-methyl-1-nitropropane is present in the 75% evaporated sample. The concentration of 2-methyl-1-nitropropane is greater in the Texaco 93 gasoline when compared to the Texaco 87 gasoline as evident by the amount of time needed to evaporate out the compound. When adding the internal standard (Figure 9) to the sample the 2-methyl-1-nitropropane peak in Texaco 93 is calculated to be six times the height of the 2-methyl-1-nitropropane in Texaco 87.

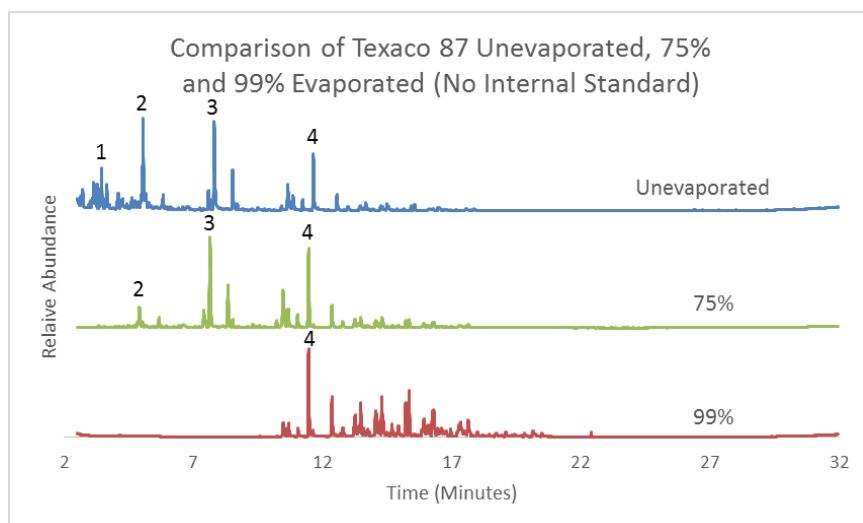


Figure 4. Chromatogram of Texaco grade 87 gasoline at 0%, 75% and 99% evaporation levels without internal standard. (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene)

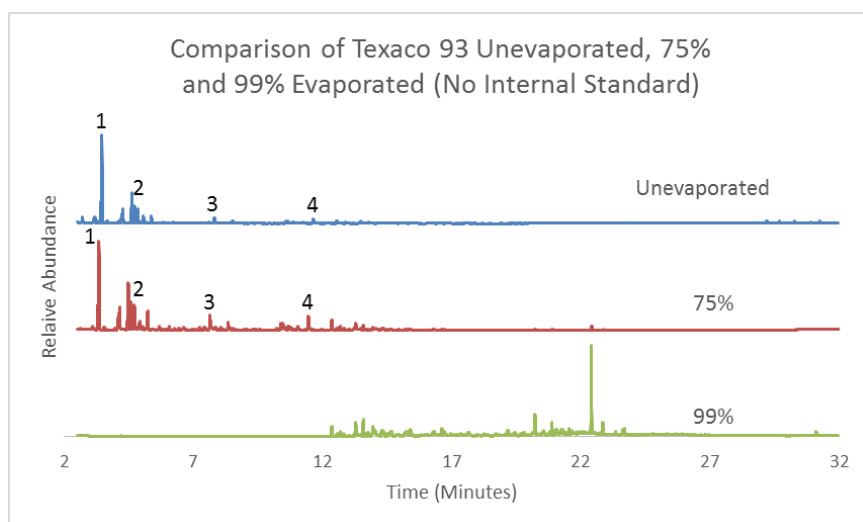


Figure 5. Chromatogram of Texaco grade 93 gasoline at 0%, 75% and 99% evaporation levels without internal standard. (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene)

The results in Figure 4 and Figure 5 show 2-methyl-1-nitropropane is the most volatile compound found in the Texaco grade 87 gasoline sample since it was the first component to completely evaporate out of the Texaco 87 gasoline samples. Another key observation of the gasoline sample minus internal standard results shows many new developing peaks between 11.5 and 22.5 minutes as the 99% evaporation level is achieved. As the evaporation level increases

without an internal standard, it appears that these peaks between 11.5 and 22.5 minutes are increasing in concentration and are becoming more abundant. Without an internal standard, it is difficult to determine the significance of the peaks between 11.5 and 22.5 minutes. Research done without an internal standard presents a problem in working with evaporated gasoline samples. The abundance indicated on the chromatogram only shows a frozen frame of the composition of the gasoline. To obtain the entire and accurate picture of the attenuation of old peaks and the generation of new peaks during gasoline evaporation studies, a reference should be used.

#### 2.4.2 WITH AN INTERNAL STANDARD

The internal standard chosen for this research was di-n-decyl sulfide. Di-n-decyl sulfide was researched to be a safer option than internal standards containing benzene and toluene, which are carcinogens. Compared to other internal standards used in the literature, di-n-decyl sulfide is relatively inexpensive and has a retention time of 30.46 minutes which is outside the target peak range of gasoline samples.  $4 \times 10^{-4}$  M was determined to be the optimal concentration of the internal standard used in the sample.

When examining the chromatograms without the internal standard in Figure 4, the concentration of the peaks between the retention times of 11.5 min and 22.5 min are increasing as the gasoline evaporates to 99%. The two chromatograms of Texaco 87 unevaporated and 99% evaporated with internal standard (Figure 6) also show that the peaks between 11.5 min and 22.5 min are increasing in concentration. As the gasoline samples evaporate the lighter compounds evaporate first. This causes the remaining compounds to become more concentrated in the remaining gasoline samples. This results in an increase in the heavier compounds before

decreasing as they eventually evaporate. Table 1 shows three heavier compounds that are relatively low in concentration when compared to the four compounds analyzed in this study. The compound at 14.31 minutes appears as if it is at the threshold of evaporating. It has just begun to evaporate out of the remaining sample, while the compounds at 14.70 minutes and 15.36 minutes have not yet begun to evaporate. This is observed by the 60-70% increase in concentration of the compounds at 14.70 minutes and 15.36 minutes, while the compound at 14.31 minutes decreased by about 15%.

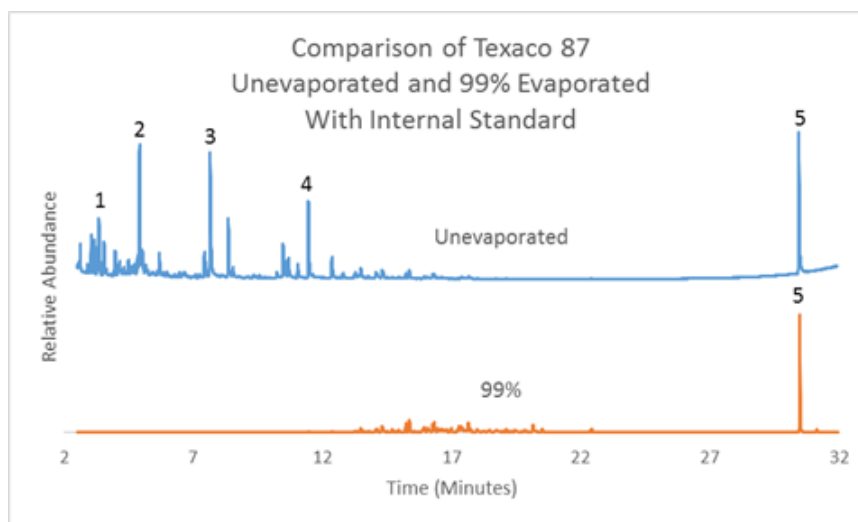


Figure 6. Chromatograms of Texaco grade 87 gasoline at 0% and 99% evaporation levels with internal standard. (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

Retention time (min)	0% evaporation level	99% evaporation level
14.31	0.067	0.057
14.70	0.022	0.031
15.36	0.065	0.107

Table 1. Peak ratios using area under the curve of Texaco 87 at 0% and 99% evaporation

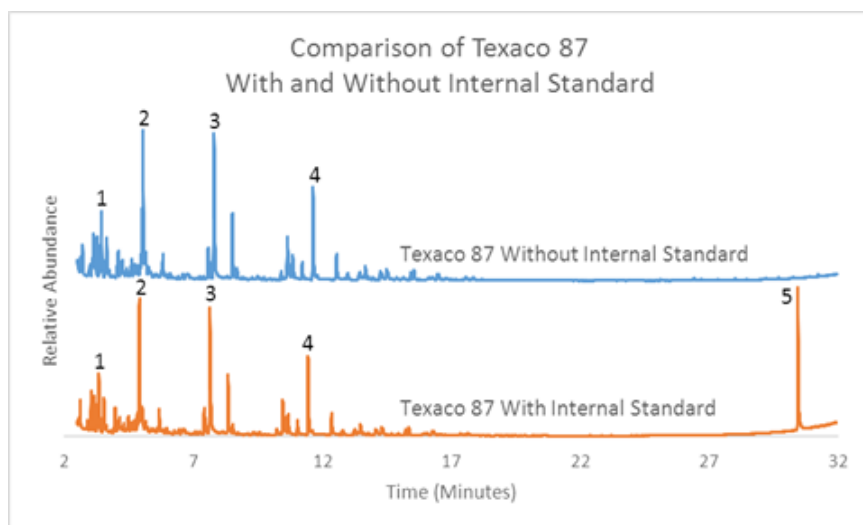


Figure 7. A comparison of unevaporated Texaco 87 gasoline with and without internal standard to show the internal standard does not chemically interact with the gasoline. (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

Figure 7 compares the chromatograms of Texaco 87 with and without the internal standard, di-n-decyl sulfide. The internal standard can be used to quantify the various peaks as the gasoline sample evaporates. Addition of the internal standard to the gasoline sample does not change the composition of the sample. This was an important factor in choosing di-n-decyl sulfide as the internal standard. It is easy to handle and makes the results easy to view and interpret.

In the chromatograms without the internal standard (Figure 8), the comparison of the Texaco 87 and Texaco 93 gasoline samples show that 2-methyl-1-nitropropane is the most abundant compound in the higher-octane gasoline, while it is the smallest analyzed compound in the lower-octane fuel. High 2-methyl-1-nitropropane propane levels are added to gasoline to increase the efficiency of the fuel. However, it is difficult to determine an accurate concentration of 2-methyl-1-nitropropane in the gasoline samples without the use of an internal standard.

With the internal standard added, as seen in Figure 9, a more elevated level of 2-methyl-1-nitropropane (1) is present in the higher-grade fuel compared to the lower grade fuel than initially

thought. The peak ratio between the internal standard and the 2-methyl-1-nitropropane peak for the lower octane fuel (Texaco 87) is 0.53 compared to 3.19 for the higher grade fuel (Texaco 93). The 2-methyl-1-nitropropane (1) in the 93 grade is so prominent that it makes accurate comparison of the concentration of the other compounds in both grades difficult to determine. When the 2-methyl-1-nitropropane (1) peak is subtracted and the chromatogram's y-axis is zoomed in, there are lower peak ratios of the heavier target peaks in the higher-grade fuel (Figure 10). Xylene has a peak ratio of 0.86 in Texaco 87 and 0.16 in Texaco 93, while tri-methyl benzene has a peak ratio of 0.54 in Texaco 87 and 0.11 in Texaco 93.

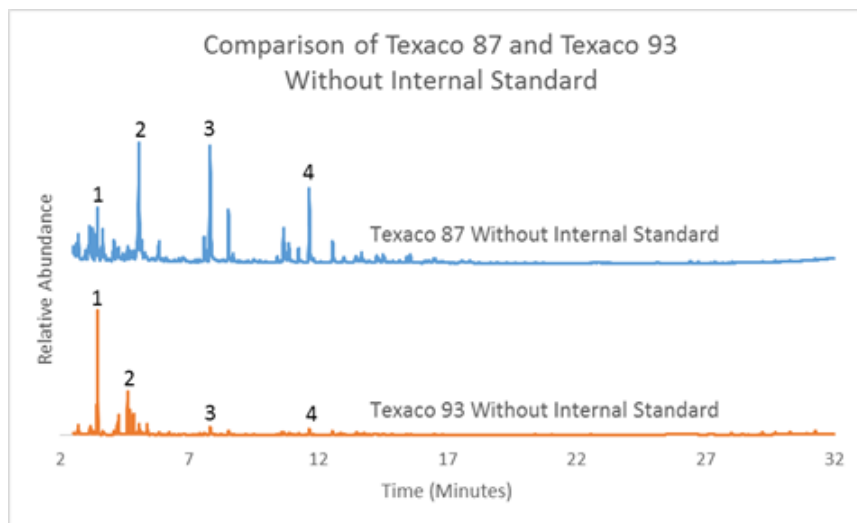


Figure 8. Chromatogram comparison of Texaco 93 and 87 gasoline that shows the relative abundance of 2-Methyl-1-Nitropropane in Texaco 93. (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

This data supports published reports that the higher-grade fuels use lighter hydrocarbon compounds to improve gas efficiency and more complete combustion of the fuel. The less expensive 87-grade fuel undergoes less refinement and is more concentrated with the heavier compounds making it less efficient.

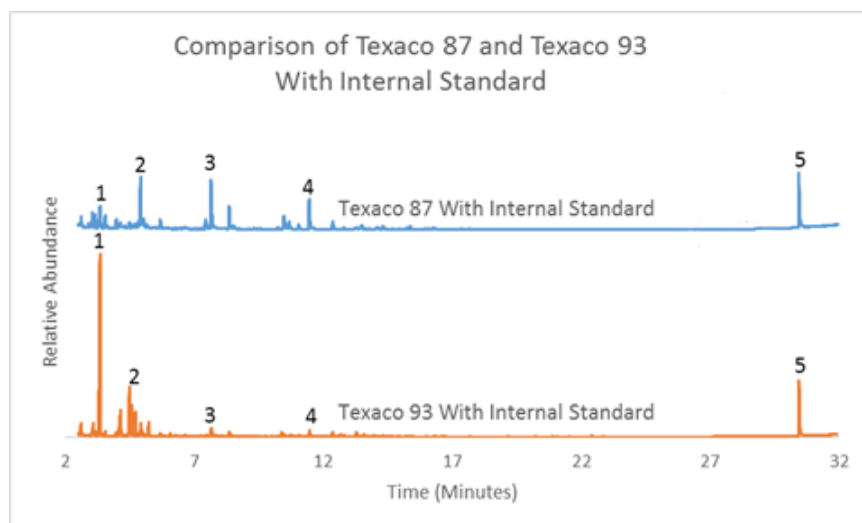


Figure 9. Chromatogram comparison of Texaco 87 and 93 gasoline shows a significant difference in the relative abundance of the peaks in the lower and higher grades of gasoline (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

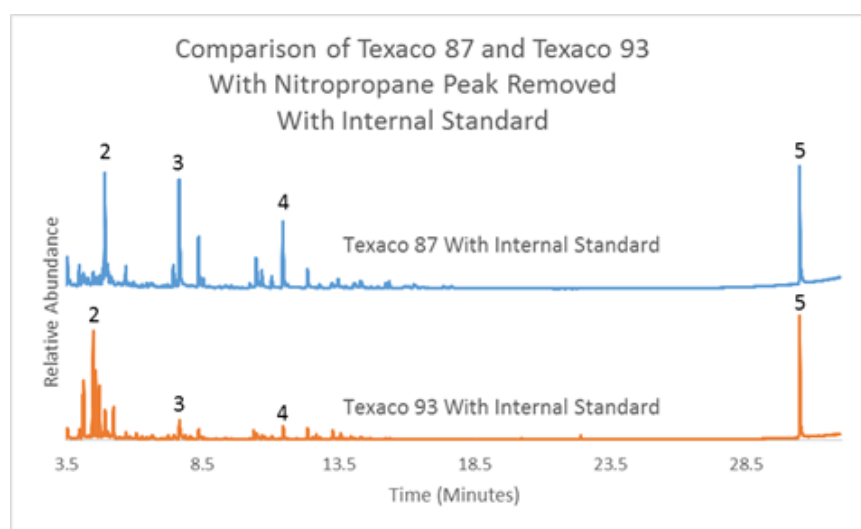


Figure 10. Chromatograms comparing Texaco 93 and 87 without the large propane peak to illustrate that the differences between the heavier compounds of the two grades (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

Shell gasoline is similar to Texaco gasoline as seen in Figure 11. Shell 93 has an increased concentration of 2-methyl-1-nitropropane (1), and a decreased concentration of heavier compounds when compared to Shell 87. This difference is easier to see in Shell gasoline due to the smaller concentration of 2-methyl-1-nitropropane (1) when compared to Texaco gasoline.

Unlike Texaco gasoline Shell's ratio of toluene (2), xylene (3) and tri-methyl benzene (4) is similar between the two grades.

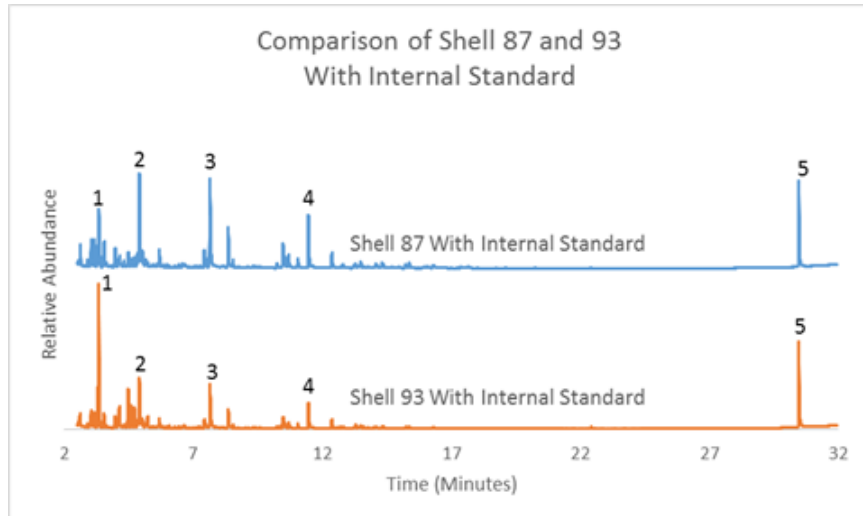


Figure 11. Chromatogram comparison of Shell 87 and 93 gasoline with internal standard that shows a significant difference in the relative abundance of the lower and higher grades of gasoline. (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

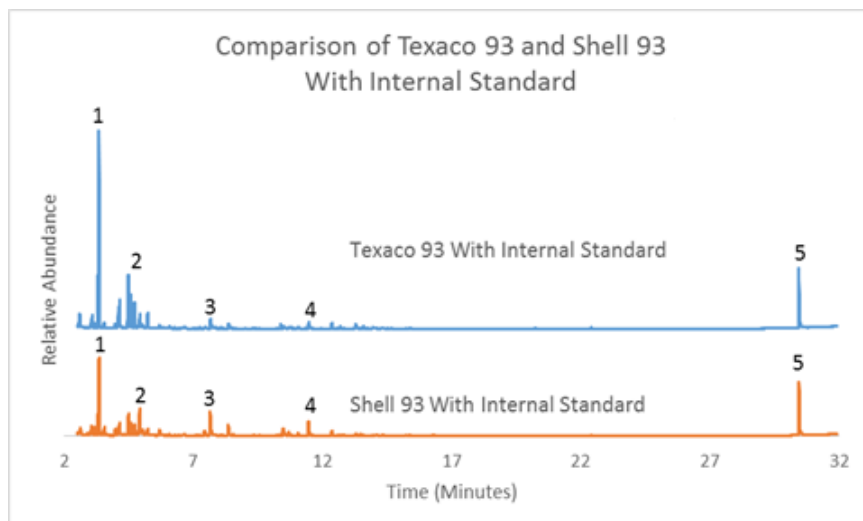


Figure 12. Chromatogram comparison of Texaco 93 and Shell 93 gasoline with internal standard that shows the difference in the composition of the two brands (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)



	Texaco 93	Shell 93
2-Methyl-1-Nitropropane	3.22	1.46
Toluene	0.88	0.51
Xylene	0.13	0.45
Tri-Methyl Benzene	0.11	0.26

Table 2. Peak ratios of 2-Methyl-1-Nitropropane, Toluene, Xylene, and Tri-Methyl Benzene in Texaco 93 and Shell 93 gasoline to show the differences in concentrations between the two gas stations

Figure 12 and Table 2 show the different composition ratios between Texaco 93 and Shell 93. The 2-methyl-1-nitropropane peak for Texaco 93 is more than twice the size of the same peak in Shell 93. The toluene peak in Texaco 93 is almost twice the size of its counterpart in Shell 93, but the xylene and tri-methyl benzene peaks are both smaller in size in the Texaco 93 gasoline compared to Shell 93. The 2-methyl-1-nitropropane ratio is a good indicator of gasoline grade and brand. In high grade gasoline, 2-methyl-1-nitropropane has a significantly larger ratio when compared to the toluene ratio. In the lower grade gasoline, the toluene ratio is larger than the 2-methyl-1-nitropropane ratio (Figure 13 and Table 3). The brand of gasoline can be determined by comparing the 2-methyl-1-nitropropane ratio with the toluene ratio. In Texaco 93 gasoline, the ratio between 2-methyl-1-nitropropane and toluene is 3.7 and the ratio in Shell 93 is 2.9. In Texaco 87 the ratio is 0.46 and in Shell 87 it is 0.64. These differences can be used to distinguish the two gasoline brands. While the 93 grade has obvious concentration differences between the two brands, the 87 grade has more subtle differences. Figure 13 and Table 3 shows the differences between Texaco 87 and Shell 87. It's here that the internal standard is most useful in distinguishing the brand of the gasoline. The ratios of the compounds and the internal standard are higher across the board for shell 87 gasoline. In addition the ratio of 2-methyl-1-nitropropane to toluene is 0.46 in Texaco 87 and 0.64 in Shell 87. The differences are subtle, but the internal standard makes

determining the origin of the gasoline sample easier.

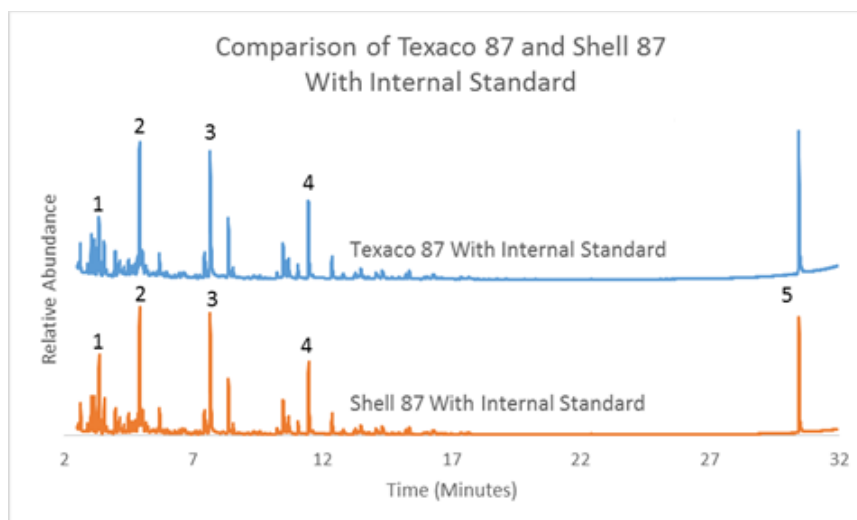


Figure 13. Chromatogram comparison of Texaco 87 and Shell 87 gasoline with internal standard that shows the difference in the composition of the two brands (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

	Texaco 87	Shell 87
2-Methyl-1-Nitropropane	0.42	0.68
Toluene	0.92	1.07
Xylene	0.86	1.03
Tri-Methyl Benzene	0.54	0.62

Table 3. Peak ratios of 2-Methyl-1-Nitropropane, Toluene, Xylene, and Tri-Methyl Benzene in Texaco 87 and Shell 87 gasoline to show the differences in concentrations between the two gas stations

Figure 14 compares all the evaporation levels for the Texaco 87 grade sample. Initially the peaks evaporate off slowly with the lighter compounds evaporating first. Table 3 provides additional insight into what is happening in Figure 14. The ratio of the peak compared to the internal standard was determined for the four major peaks at all evaporation levels. From unevaporated to 50% evaporated, the chromatograms retain their characteristic peaks that identify it as gasoline. After 50% evaporated, the samples slowly lose the lighter compounds until the samples are no longer identifiable as gasoline. Using an internal standard to build a library of gasoline chromatograms at different evaporation levels can alleviate this issue. The heavier

compounds with retention times greater than 10 minutes are detectable in the sample until the gasoline is completely evaporated and this technique can aid in the identification of different brands and grades of gasoline. Figure 15 shows that Shell 87 gasoline evaporate in a similar manner as Texaco 87. Both brands of gasoline lose 2-methyl-1-nitropropane peak around 50% evaporated. At the 91% evaporation level, tri-methyl benzene was the only remaining peak of those examined in this study.

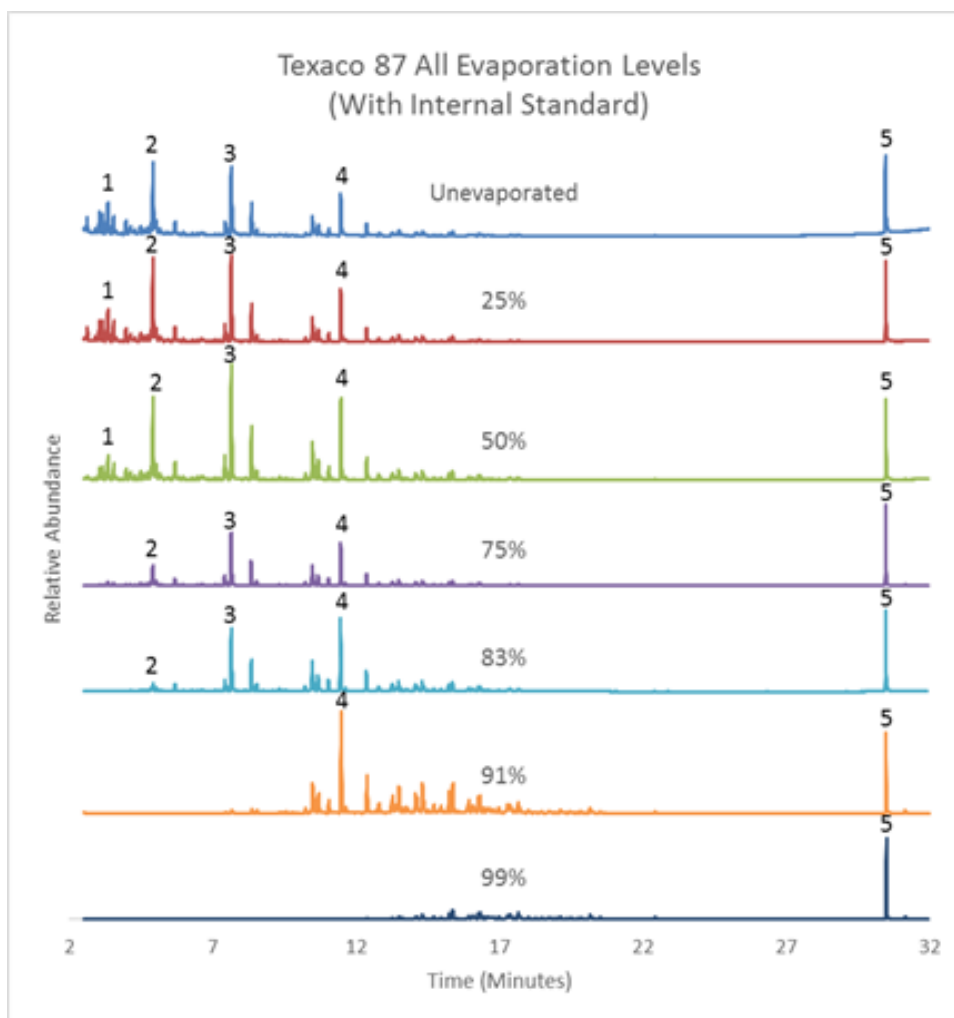


Figure 14. Comparison of all evaporation levels of Texaco 87 with internal standard. All chromatograms are measured in relative abundance (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-

n-Decyl Sulfide)

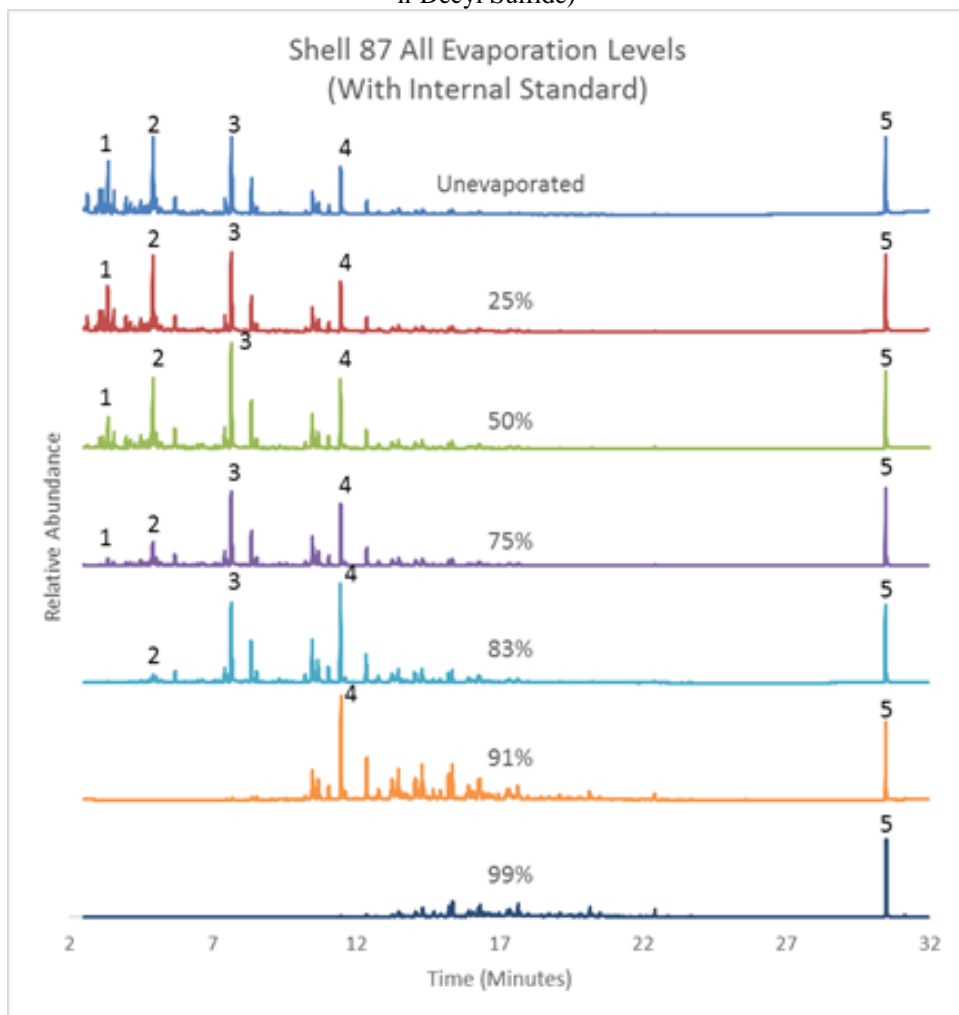


Figure 15. Comparison of all evaporation levels of Shell 87 with internal standard. All chromatograms are measured in relative abundance (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

Compared to the 87 octane gasoline, the 93 octane gasoline retains its characteristic chromatogram for longer than the 87 grade. It begins to lose its characteristic chromatogram at 75-83% evaporated as seen in Figure 16 and Figure 17. After 25% evaporated the 2-methyl-1-nitropropane (1) peak is so diminished that it is difficult to determine the origin of the gasoline. Evaporated gasoline is not suitable for origin determination. This is another benefit to developing a database of gasoline samples of all evaporation levels. The database can be used to determine if a gasoline sample is

suitable for origin determination.

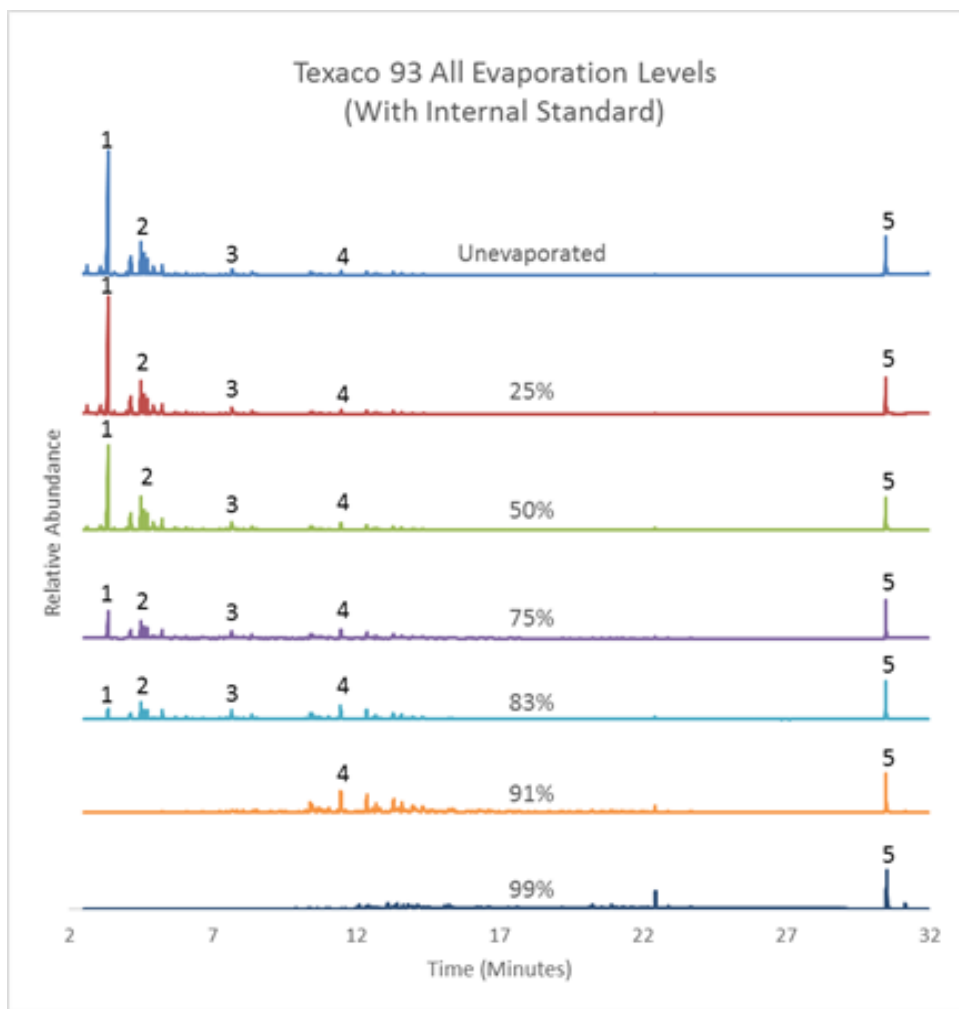


Figure 16. Comparison of all evaporation levels of Shell 87 with internal standard. All chromatograms are measured in relative abundance (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

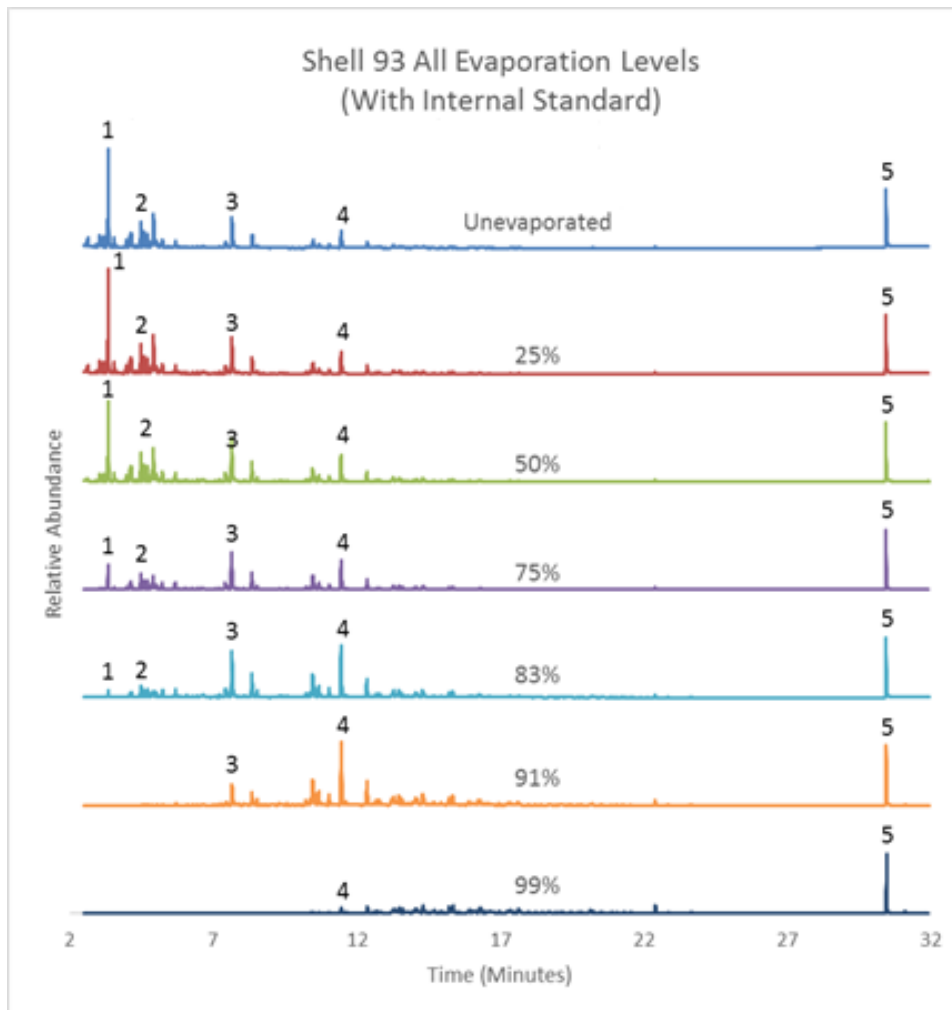


Figure 17. Comparison of all evaporation levels of Shell 93 with internal standard. All chromatograms are measured in relative abundance (1: 2-Methyl-1-Nitropropane, 2: Toluene, 3: Xylene, 4: Tri-Methyl Benzene, 5: Di-n-Decyl Sulfide)

Graphs of the ratios between the major peaks and the internal standard for Texaco 87 can be seen in Figures 18. This graph was created using JMP software from the data in Table 4. The JMP software plotted the ratios of 2-methyl-1-nitropropane, toluene, xylene, and tri-methyl benzene with the internal standard against the percent of evaporated gasoline. The software was also used to add a smoothed trend line that allowed the evaporation of the compounds of Texaco 87 to be visualized. The graph generated using the JMP software shows that as the gasoline

samples evaporated, the higher molecular weight compounds increased in concentration before eventually decreasing towards zero. This phenomenon is due to the complexity of gasoline and the limitations of gas chromatography. Gas chromatography analyzes only the volatile compounds in gasoline. Though the mixture has a large number of volatile compounds it is also composed of nonvolatile compounds that are not present in the chromatogram. As these nonvolatile, and lower mass compounds, evaporate the concentration of higher mass compounds appear to increase. The loss of the lower mass compounds results in the decrease in total sample volume during the evaporation process while the number of moles of higher mass compounds remains constant. If the number of moles of solute in a solution remains constant as the solution decreases, the molarity of the higher mass compounds would increase. Because of the inherent concentration issues during the gasoline evaporation process, an internal standard useful for the quantification of evaporated samples. However, the application of an internal standard to determine the small differences between gasoline brands could be a useful arson investigators tool.

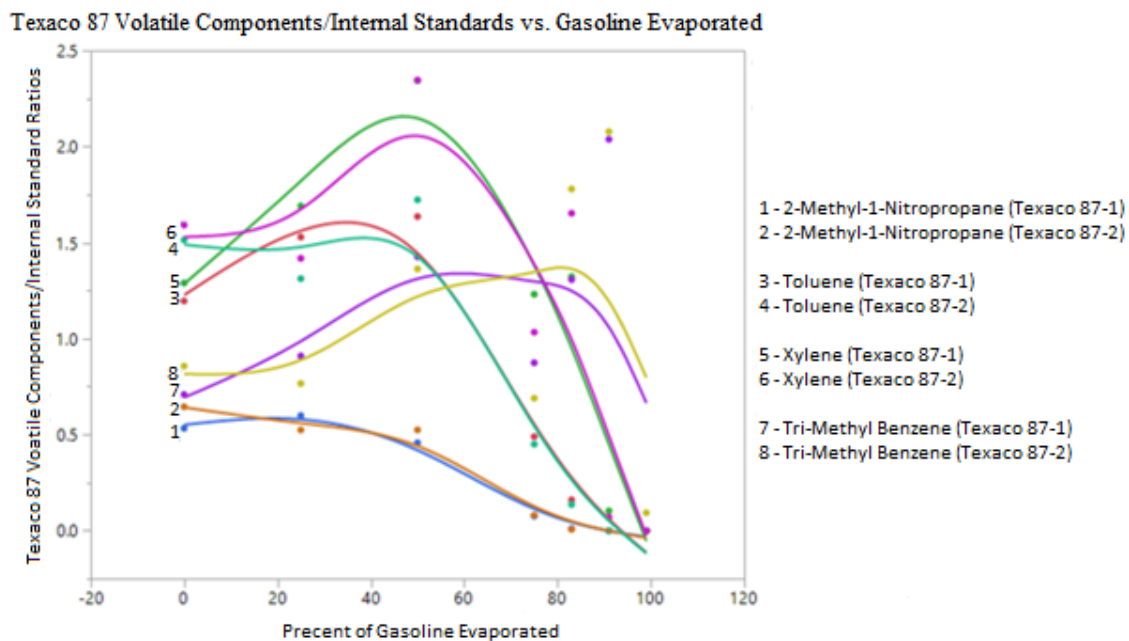


Figure 18. A graph of the major peaks in gasoline as the sample evaporated fully for Texaco 87-1 and Texaco 87-2

Retention Times (Minutes)	Texaco 87-1						
	0%	25%	50%	75%	83%	91%	99%
2-Methyl-1-Nitropropane	0.42	0.41	0.31	0.05	0.01	0	0
Toluene	0.92	1.04	1.03	0.25	0.11	0.01	0
Xylene	0.86	1.06	1.42	0.65	0.78	0.05	0
Tri-Methyl Benzene	0.53	0.65	1.01	0.54	0.9	1.25	0
	Texaco 87-2						
	0%	25%	50%	75%	83%	91%	99%
2-Methyl-1-Nitropropane	0.46	0.37	0.36	0.06	0.01	0	0
Toluene	1.08	0.95	1.1	0.25	0.09	0	0
Xylene	1.01	0.93	1.4	0.58	0.96	0.02	0
Tri-Methyl Benzene	0.62	0.57	0.95	0.43	1.21	1.26	0.06

Table 4. Peak ratios of major peaks in Texaco 87-1 and Texaco 87-2 when compared to the internal standard.

## 2.5 CONCLUSION

This study aimed to 1) establish a preliminary database of gasoline chromatograms with an internal standard, 2) identify an internal standard to use in the analysis of the gasoline samples,



and 3) assist arson investigators by reducing problems associated with analyzing accelerants at arsons. Using two brands of gasoline a preliminary database was established. Texaco and Shell gasoline samples were evaporated with and without internal standards. The internal standard used in this study, di-n-decyl sulfide, was ultimately used due to its safety and low cost when compared to deuterated compounds. After numerous tests an internal standard concentration of  $4 \times 10^{-4}$  M was found that would allow for its use in all grades of gasoline despite the concentration differences. The peak ratios calculated with the internal standard allowed for the concentration of the different components of gasoline to be compared. It was found that different brands of gasoline have differing peak ratios and these differences can be used to help determine the origin of gasoline samples. The internal standard also allows for the standardization of gasoline chromatograms, allowing for easier comparison between different samples. This data can be used by arson investigators to assist in the identification of gasoline used as an accelerant at arson scenes, and possibly help them identify the origin of the gasoline.

## CHAPTER 3

### METHOD DEVELOPMENT FOR THE DETERMINATION OF CAFFEINE IN PRESSED FINGERPRINTS USING MATRIX ASSISTED LASER DESORPTION IONIZATION

#### 3.1 ABSTRACT

Fingerprints play a major role in identifying suspects in criminal cases. Due to the variability of ridge formation and the minute details used in fingerprint comparison the odds of two people having the same fingerprints as someone else is very improbable. For decades fingerprints have been limited to a comparative identification source in crime laboratories. Recent studies have shown that fingerprints contain a wealth of chemical information that could give investigators valuable information ranging from recent drug use to traces of explosives. The purpose of this study was to show proof of concept that drug metabolites can be detected from the fingerprints of a suspect. Fingerprints along with sweat and saliva were collected from volunteers and analyzed using matrix assisted laser desorption ionization (MALDI) to test for the presence of caffeine. Samples were taken both during the normal course of the day and timed periods after consumption of caffeine to determine if caffeine could be qualitatively determined using MALDI. 3 matrix application methods were investigated with the best method yielding a calibration curve with an  $R^2$  value of 0.97. Initial results show that MALDI was able to detect caffeine in fingerprints for 2-5 hours after consumption. These results show that MALDI is capable of

detecting drugs that have been consumed and excreted in the sweat that forms fingerprints. Work can now begin on optimizing these methods to facilitate illicit drug detection and quantification in fingerprints.

## 3.2 INTRODUCTION

### 3.2.1 FINGERPRINTS IN FORENSICS

Fingerprint examination is one of the oldest divisions of forensic investigation. Fingerprints have been used as a means of identification for thousands of years. The first recorded use of fingerprints for identification was discovered in Chinese records from around 200 BCE, where fingerprints were used for identification in a robbery case. [59] As the world's understanding of science, and fingerprints, increased so did their use in solving crime. Fingerprints are one of the most common types of evidence collected at crime scenes today. [59]

The ability to detect, collect, and analyze fingerprints has increased since ancient times. Forensic scientists no longer have to rely on collecting fingerprints left in blood, paint, or malleable materials that could be seen with the naked eye. Fingerprints not visible to the naked eye can be visualized with powders, chemicals, and other techniques that allow forensic scientists to use these fingerprints for identification. [60] A large amount of fingerprints collected by forensic scientists turns out to be unusable for identification purposes due to insufficient ridge detail for comparison. The prints that cannot be used for identification have no further use in current forensic laboratories because fingerprints are only used for identification. There is a wealth of chemical information that could be gleaned from these unusable fingerprints.

### 3.2.2 BIOLOGY OF FINGERPRINTS

Fingerprints are composed of small friction ridges that have evolved to enhance humans' ability to grasp and pick up objects. [61] Friction ridges are developed in the womb and babies are born with the fingerprints that they will have for their entire life barring accidents. It is generally accepted, though not thoroughly understood, that friction ridges are caused as the basal layer of skin grows at an accelerated rate when compared to the upper layers of the epidermis and the layers of the dermis. Since the basal layer is the innermost layer of the epidermis, the layer right before the dermis begins, the excess growth is believed to buckle and fold causing ridges to form on the outermost layer of skin. The skin on the hand and feet, despite having friction ridges not present on the rest of the skin, contains a multitude of sweat glands essential for forming the fingerprints that forensic scientists collect and process. [62]

### 3.2.3 SWEAT AND SWEAT SOLUBLE DRUGS

Sweat glands are biological features found on the surface of the skin that secrete a mostly water discharge primarily for temperature regulation. The cells in the human body require a narrow optimal temperature range to function and the human body has developed a complex system comprised of sweat glands to maintain this optimal temperature. If the body begins to overheat sweat glands will excrete a watery discharge that when it evaporates off the skin it will absorb energy and cause the skin to cool [63]

Sweat is a complex secretion that is composed almost entirely of water and waste materials. The composition of sweat varies from person to person and day to day. Sweat contains waste materials that the body wants to get rid of including salts, minerals, lactic acid, and various organic compounds. [64,65] Many drugs of abuse are water soluble and can partition into sweat as they

travel through the blood stream. It is possible to detect trace amounts of these drugs by analyzing sweat samples. [66,67]

Research has been conducted into the viability and sensitivity of detecting drugs in sweat compared to more traditional testing of urine and blood. Urine analysis can detect drugs as they are metabolized and excreted as waste after ingestion with most drugs being excreted within 48 hours. [68] Drug analysis of blood is useful for determining current level of intoxication as it measures the amount of illicit drugs and metabolites present in the blood at the time of collection.

Sweat analysis is gaining momentum as an alternative to urine or blood analysis. Collection of sweat for analysis is preformed using a patch that subjects wear over a period of time that absorbs any sweat produced. The patches are more tamper resistant than urine analysis and less invasive than both urine and blood analysis. Some of the largest issues with sweat analysis is the low level of illicit drugs and their metabolites excreted in sweat and the potential of removal during the collection phase. Drugs of abuse including cocaine, opiates, methamphetamines, cannabinoids, and others have been studied using sweat patches and sweat wipes have been analyzed and shown to be present in sufficient quantities for identification using GC-MS and LC-MS. [69,70,71,72,73]

### 3.2.4 CAFFEINE

Caffeine is a commonly consumed chemical found in a wide variety of food and drinks. It is a central nervous system stimulant and primarily acts as an antagonist of the adenosine receptors in the brain which blocks drowsiness brought on by adenosine. Caffeine is absorbed in the stomach and intestines and reaches peak concentration levels 15-45 minutes after ingestion. Caffeine travels through the body via the circulatory system and has a half-life of approximately 6 hours. [74]

The metabolism of caffeine follows a couple different pathways in the body. Caffeine can

undergo N-3 demethylation to paraxanthine, which occurs 70-80% of the time. Caffeine can also undergo reactions that converts it to theobromine and theophylline, which occurs about 12-14% and 4-5% of the time respectively. Caffeine is metabolized in the liver and can partition into all of the tissues of the body, before it is excreted by the kidneys. [74] Caffeine was used in this study because it is not a controlled substance, it is a lower molecular weight molecule similar to common controlled substances, and it is widely available.

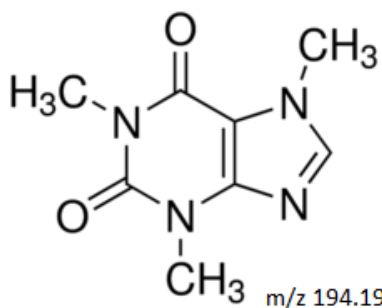


Figure 19. Molecular structure of caffeine.

### 3.2.5 MATRIX ASSISTED LASER DESORPTION IONIZATION (MALDI)

Matrix assisted laser desorption ionization (MALDI) is a soft ionization technique that allows for the analysis of molecules without fragmentation and is usually combined with time-of-flight (TOF) mass spectrometry. Samples analyzed using MALDI are coated with a matrix that is energized during ionization. MALDI ionization works by pulsing a laser at the matrix coated analyte on a metal plate. The energy from the laser energizes the matrix/analyte sample and aerosolizes small droplets. The matrix/analyte droplets are charged, and as the matrix evaporates, the charge is retained by the analyte. This charge allows the analyte to be accelerated towards the mass analyzer. All analytes have the same acceleration when entering the time of flight mass spectrometer. The differing mass to charge ratios of these charged species result in separation of the charged fragments in the flight tube. The different charged fragments are measured as the

charged fragments with the smaller mass to charge ratios hit the detector first. [75,76]

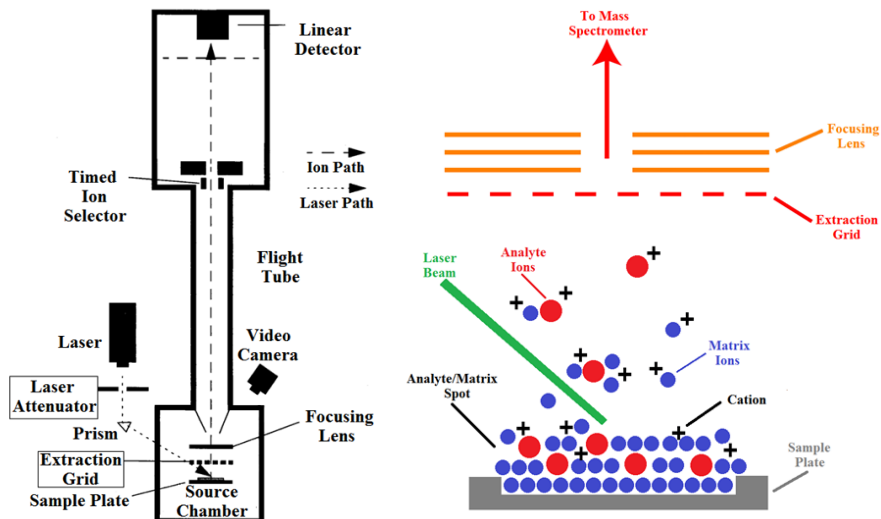


Figure 20. A schematic of the Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) instruments, with a close up depicting the ionization of the sample/matrix.

### 3.2.6 CURRENT RESEARCH INTO DRUG DETECTION IN FINGERPRINTS

Fingerprints have played a large role in forensics since 1892 when Sir Francis Galton wrote the first book on fingerprints and how to use them for identification, but only recently has scientists started analyzing the composition of fingerprints for more information into the lifestyle of the owner of the fingerprint. Fingerprints are primarily composed of sweat and contain water-soluble metabolic waste with a makeup based on the individual's consumption. Techniques and experiments are being developed to determine the extent of data that can be gathered from the chemical composition of fingerprints. [77]

In 2007, Richard Leggett et al of the United Kingdom began looking into fingerprints and their analysis with gold nanoparticles. Leggett group was interested in determining drug usage of an individual based on metabolites found in fingerprints. The research team developed a gold nanoparticle ligand system that binds to metabolites of drugs and can be detected by fluorescence spectroscopy. Use of the gold nano particle allowed for more antibodies to bind to the fingerprint allowing for lower detection rates. The use of this technique allowed for the preservation of

fingerprint ridge detail. [78]

Also in 2009, Benton et al in Singapore researched the detection of nicotine from contact with smokers and secondhand smoke. Moving slightly away from looking at secretions of metabolites from fingerprints, this research was focused on determining if the concentration of external contamination from person to person contact, contact of contaminated surfaces, or second hand smoke was present in significant concentrations to render a non-smokers fingerprint indistinguishable from a smokers fingerprint. It was found that passive smoking caused significant contamination only from less than 0.1 meter away. Handshakes between a smoker and a non-smoker indicated a small transfer of nicotine, but it was not found to be significantly different from the baseline levels found in non-smokers fingerprints. [79]

In 2010 Hazarika et al of the United Kingdom researched an immunoassay based technique to detect metabolites of heroin and cocaine with brightfield and fluorescence microscopy. Unlike the Leggett research group, Hazarika's research group focused on using antibodies bound to magnetic particles for detection of illicit drug metabolites. The binding of the antibody/magnetic particle system produced a visible color reaction from a grey fingerprint to a yellow-brown fingerprint upon its binding to the metabolites. The presence of the metabolites was confirmed using brightfield and fluorescence microscopy. The research indicated that metabolites of heroin and cocaine could be detected separately and simultaneously while leaving the fingerprint with enough detail for comparison purposes. [80]

In 2013 Kuwayama et al of Japan conducted research on the measurement of caffeine and its metabolites in fingerprints as a function of time after coffee ingestion. Fingerprints from subjects were taken after 1, 3, 5, and 7 hours. The fingerprints were studied using LC-MS. This research was conducted as a precursor to analysis of illicit drugs and their metabolites in



fingerprints. The researchers were able to detect caffeine and its metabolites over the seven hour period and were able to see a significant change in the amount of caffeine before and after the intake of coffee. Unlike previous methods the fingerprint is destroyed and cannot be used for further comparison identification. [81] The Kuwayama's research group also studied the detection of commercially available cold medicines and their metabolites in fingerprints. They were able to detect ibuprofen, dihydrocodeine, chlorpheniramine, methylephedrine, and their metabolites in fingerprints up to 7 days after ingestion. Analysis of fingerprints was faster and less invasive than blood analysis without loss of detectability. [82]

### 3.3 METHOD

#### 3.3.1 SELECTION OF MALDI MATRIX

Detecting drugs in fingerprints using MALDI-TOF is an exacting process. MALDI analysis is typically performed on molecules with higher masses such as proteins. The illicit drugs of interest and the caffeine used in the method development all have smaller masses that make them more difficult to detect. Most of the common matrices used in MALDI analysis produce peaks in the mass range of the analytes of interest. Two matrices were tested in the laboratory to determine the viability in detecting caffeine in fingerprints. 2,5-dihydroxybenzoic acid (DHB) and  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) were both tested due to their use in literature to detect low to mid-range weight molecules. After preliminary experiments and analysis were performed on DHB and CHCA only CHCA yielded peaks from caffeine and was subsequently chosen for all further testing. Although not an issue in this study, CHCA can affect other drug detection protocols by MALDI in that the matrix mass peaks fall within the mass range of the analytes examined.

### 3.3.2 DETERMINATION OF IMAGE RESOLUTION

The MALDI instrument used in this study was equipped with imaging capabilities that were used in this research. The imaging software uses camera-guided positions to overlay the data onto a photo of the target plate. Figure 21 demonstrates how the imaging software creates a grid for analysis. The laser pulses 100 times in the squares formed by the grid in a randomized pattern before averaging the results. After the data is collected, it is overlaid on top of the image used to create the grid where mass to charge ratios can be selected to create intensity maps like the one shown in Figure 21. The images can detail the concentration intensity for a chosen target peak for the entire plate. This allows comparisons to be made visually before analyzing the data. Four resolution settings for the imaging of caffeine were tested to determine the optimal resolution and data collection time required for future images. The optimal resolution was determined to be 200x200.

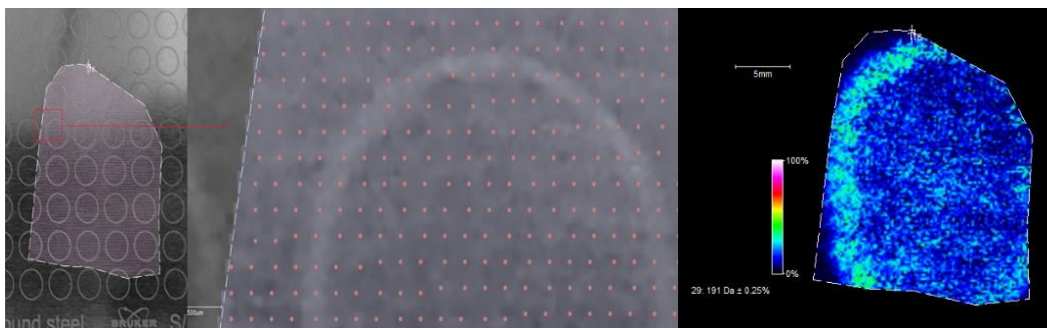


Figure 21. Visual representation of how the MALDI grid is laid out for imaging analysis

### 3.3.3 DETERMINATION OF MATRIX APPLICATION AND CALIBRATION CURVE

The main compound studied in this research was caffeine. Caffeine is a common ingredient in many foods and drinks, and is already a part of a large portion of the population diets. A caffeine standard was used to create a calibration curve to attempt to quantify the data obtained using MALDI imaging, and a caffeine standard was used during each analysis for confirmation that the

peaks analyzed in fingerprints and biological fluids were caffeine. The resultant mass spectra generated from the Thermo Scientific ITQ1100 mass spectrometer, Figure 23, was confirmed using NIST library reference spectra.

Caffeine standard was purchased from Sigma Aldrich (CAS Number: 58-08-2). Standard solutions of caffeine were dissolved in methanol and spotted onto a metal MALDI target plate and allowed to dry. To determine the best method to quantitatively measure caffeine using MALDI three matrix application techniques were investigated. The first method involved dissolving the matrix and caffeine standard together in methanol. The matrix/caffeine standard solution was spotted on the target plate and allowed to crystalize. The second method investigated involved dissolving caffeine standard in methanol and spotting it on the target plate. The matrix was applied via spray coating after the methanol had evaporated from the target plate. This method proved troublesome as the methanol had a tendency to disperse over a large area. After thorough literature research the third matrix application method was investigated. This method involved spotting droplets of water onto the target plate and then spotting the caffeine standard, which was dissolved in methanol, directly in the water droplets. The matrix was once again applied via spray coating after the solvents had evaporated off the target plate. The addition of the water droplet allowed the caffeine standard to remain in the desired location and not spread to the other samples causing the target plate to be redone. Calibration curves were formed using the three types of matrix applications. Each calibration curve contained a blank and test spots with the concentrations of 0.001 M, 0.01 M, 0.1 M, and 1 M.

### 3.3.4 CALCULATION OF CALIBRATION CURVE

Data was obtained for each of the three methods investigated. A total of 8,000 spectra were generated for the 15 spots. Each spectra was checked for caffeine peaks. A portion of the spectra, especially those in the blanks, contained no caffeine data. Those that did contain caffeine data were recorded and condensed into a single table for each spot. The spots were then analyzed using JMP software. The data from the four concentrations, 0.001 M, 0.01 M, 0.1 M, and 1 M, were graphed to look at the distribution and spread of intensities. The JMP software was also used to calculate the mean, standard deviation, standard error mean, and the mean of the top and bottom 95% of the data. The software also determined and displayed the outlier points for each spot.

### 3.3.5 SAMPLE COLLECTION AND PREPARATION

Three volunteers were selected for this study. Volunteer 1 consumed a small 12 ounce cup of coffee and a small sip of diet coke, 2 hours and 10 minutes before sampling, respectively. Volunteer 2 consumed a 20 ounce bottle of Mello Yellow 2 hours prior to sampling. Volunteer 3 consumed a 16 ounce Monster Energy drink at the time of sampling and consumed no other caffeine 224 hours prior to sampling. Biological samples including saliva, sweat, fingerprint residue, and pressed fingerprints were taken from Volunteer 1 and 2 during a typical day. The saliva and pressed fingerprints were applied directly to the target plate. The sweat residue was collected by rubbing a cotton swab along parts of the body including the neck and arms. The fingerprint residue was collected by rubbing a cotton swab along the palms and fingers. The residues were collected over a 5 minute period before soaking in methanol for 30 minutes. After soaking, the residues were spotted on the target plate alongside the saliva, pressed fingerprints,

and caffeine standard.

### 3.3.6 MALDI DATA COLLECTION

Fingerprints were placed on the plate with a minimum of fifteen seconds of contact, and biological fluids were spotted on the target plate and allowed to dry. After all solvents had evaporated the matrix,  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA), was sprayed onto the plate in thin layers until covered. After the plate was fully dry, it was placed into the Bruker Autoflex II MALDI-TOF instrument for analysis. Sections of the target plate with analyte were selected for analysis using the instrumental software. The areas were measured in 200x200 squares, where 100 spectra were collected and averaged. During analysis on the MALDI instrument the ion source 1, the ion source 2, and the lens was set at 20.00 kV, 18.45 kV, and 8.30 kV respectively. The laser strength was set between 75% and 78% strength. Masses were detected in the range of 100 m/z and 1000 m/z. Once all spectra were obtained the software was able to overlay the obtained data to form the imaging results that showed the concentrations of the samples.

## 3.4 RESULTS AND DISCUSSION

### 3.4.1 DETERMINATION OF IMAGE RESOLUTION

An aspect of the imaging software explored is the resolution capabilities. The software allows the user to specify the resolution used during analysis. Lower resolution decreases both the data collection speed time and the image quality by a significant factor. Figure 22 shows the results of the resolution optimization tests. Box 1, Box 2, Box 3, and Box 4 represent a sample analyzed at 1000x1000, 600x600, 200x200, and 100x100 resolutions, respectively. The resolutions detail

the dimensions of the grid overlaid on the sample by the software. 100 spectra are gathered in each square and averaged to form the final spectra. Box 4 offered the best resolution of the settings examined and took nearly 19 hours to complete the analysis. Conversely, Box 1 offered the worst resolution and took less than a half hour to complete. The resolution settings of 200x200, Box 3, was chosen for this study since it yielded satisfactory images that took only 4 hours to complete compared to the 19 hours needed to analyze images with 100x100 resolution.

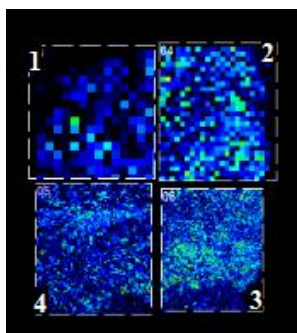


Figure 22. Caffeine resolution test for MALDI imaging with (1) 1000x1000, (2) 600x600, (3) 200x200, and (4) 100x100 resolution

### 3.4.2 MATRIX APPLICATION METHOD

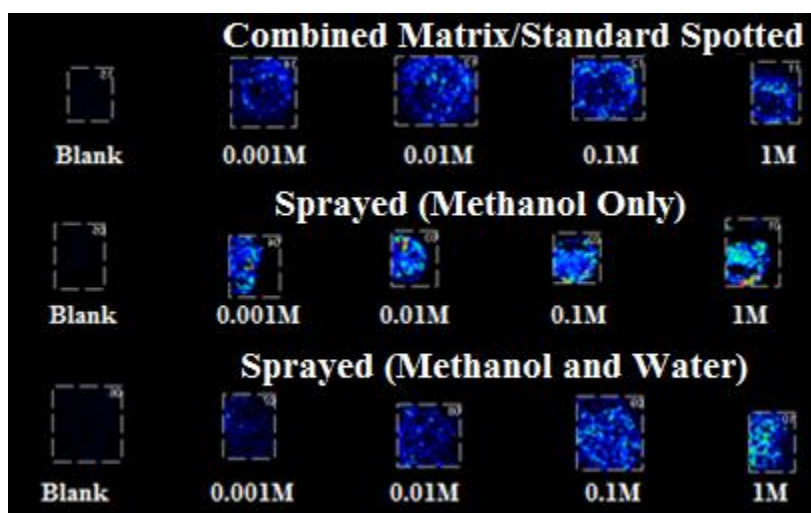


Figure 23. MALDI imaging results of matrix application techniques and calibration curves

Three matrix application methods were examined to help select the most appropriate quantitative analysis method for this study (Figure 23). The first application method, the matrix  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) dissolved in methanol, was added to the caffeine standard

and spotted on the target plate. Once the solvent evaporated, the caffeine crystallized on the target plate in an irregular and unreproducible manner. In the second and third method, the CHCA was sprayed onto the target plate creating an even coating layer. The second method involved carefully spotting and re-spotting caffeine standard solution dissolved in methanol until usable spots were achieved. When various concentrations of caffeine standard solutions dissolved in methanol were spotted on the target plate, the solutions spread uncontrollably across the target plate. The irregular distribution of sample on the target plate made accurate and precise measurements difficult. The third application method tested was similar to the second application method tested. The third application method involved the addition of water. The water was spotted on the target plate and the caffeine standard solution was then spotted directly into the water droplet. The cohesive properties of the water molecules helped to contain the standard solution to a small area on the target plate. The confinement of the caffeine standard by the water allowed the caffeine to dry in a uniform layer. The even distribution of sample on the MALDI target plate made the idea of generating a calibration curve plausible. The caffeine standard solution was confirmed using Thermo Scientific Trace GC Ultra and Thermo Scientific ITQ1100 mass spectrometer (Figure 24).

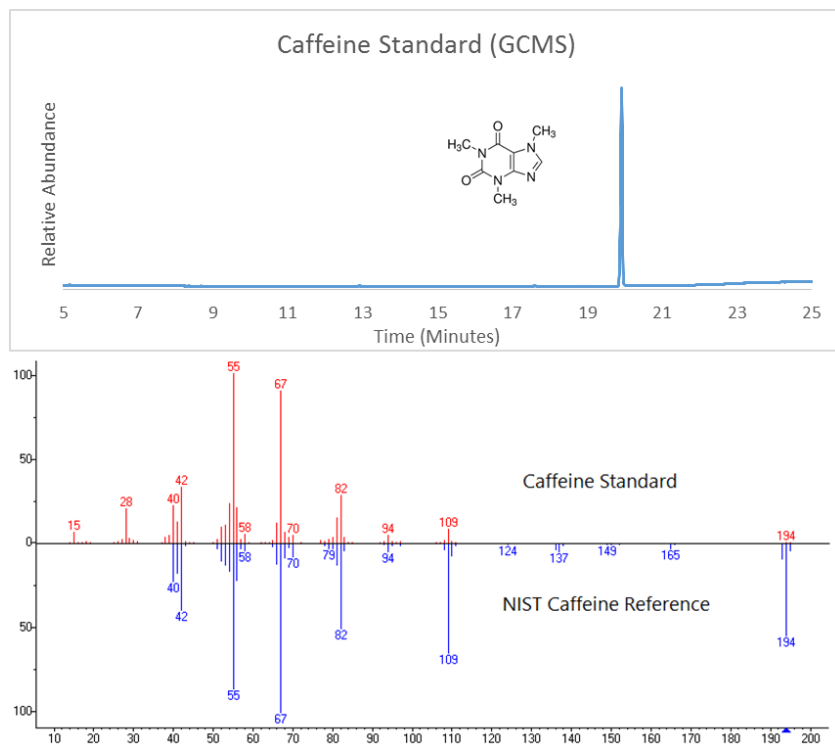


Figure 24. GC/MS Results of caffeine used in this study

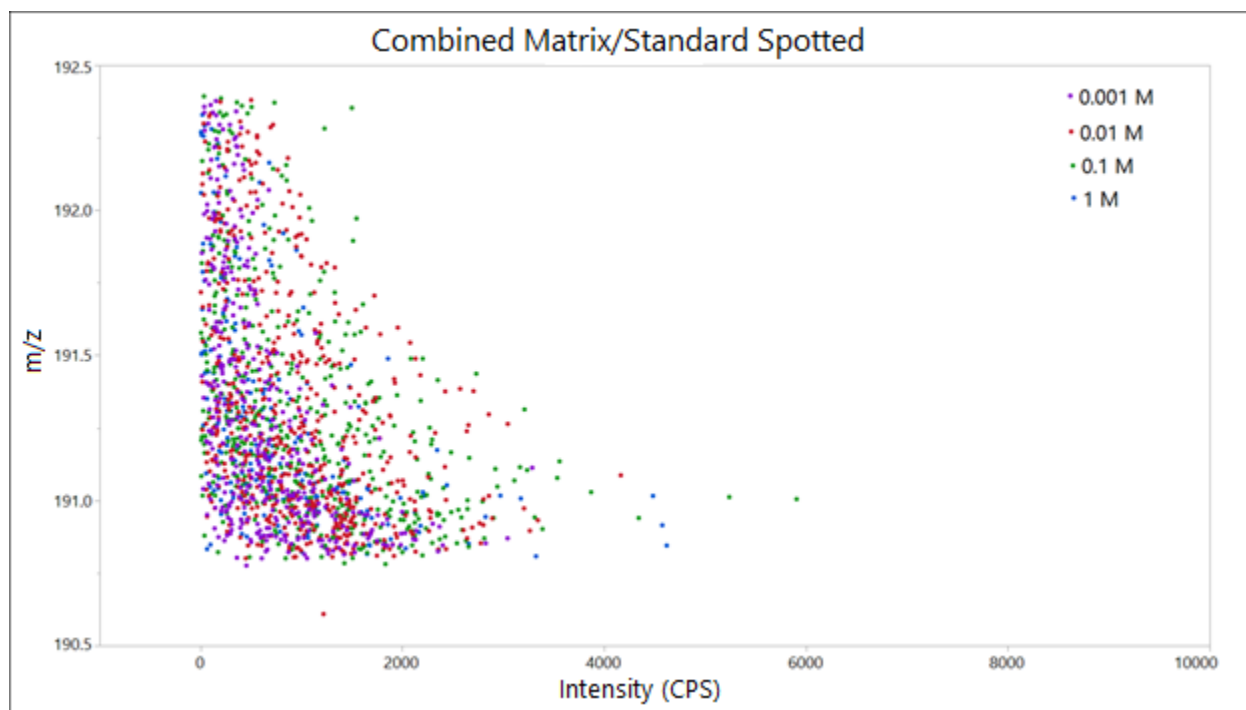


Figure 25. Distribution analysis of 0.001M, 0.01M, 0.1M, and 1M caffeine for the calibration spots where the matrix was dissolved with the caffeine standard and spotted on the plate.



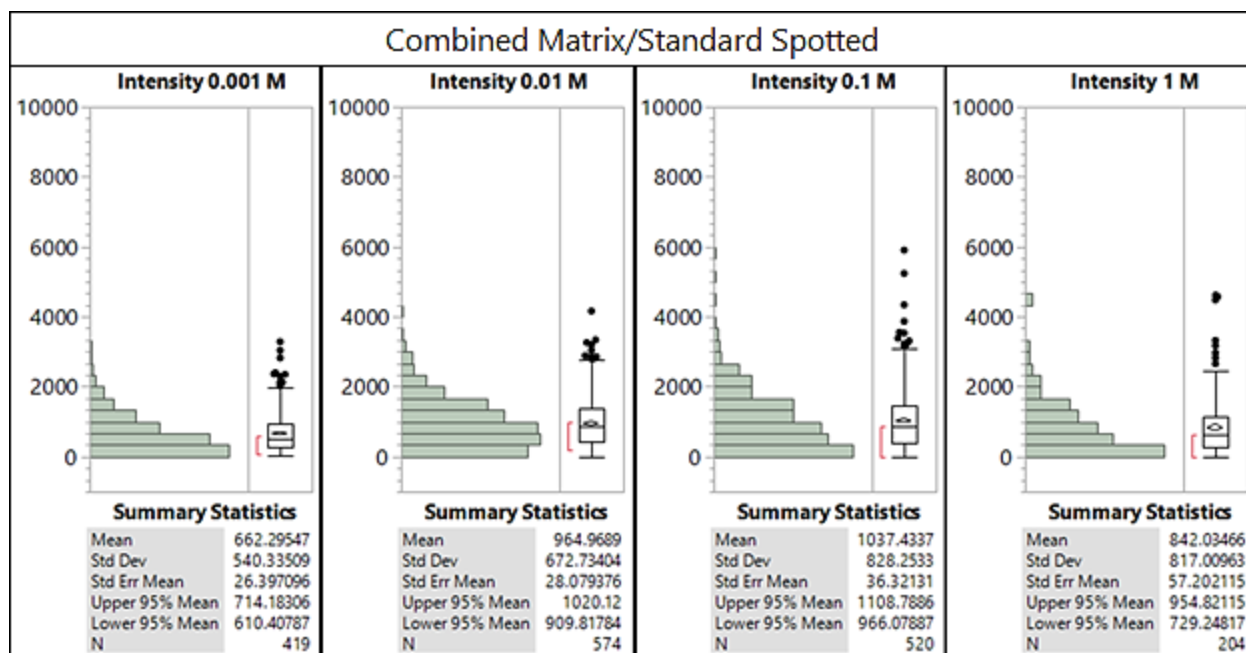


Figure 26. Distribution summaries of .001M, 0.01M, 0.1M, and 1M caffeine for the calibration spots where the matrix was dissolved with the caffeine standard and spotted on the plate.

Figures 25, 27, and 29 illustrate the scope of the data generated during analysis using MALDI imaging. Each figure is broken down into 200x200 nm squares, and each square generates a spectra. That spectra was then analyzed to obtain the intensities of the caffeine peaks. After analyzing each spectra for the spots using the first application method, where the matrix, CHCA, was dissolved with the caffeine standard and spotted on the plate, the resulting intensities can be seen in Figure 25. Figure 25 plots the intensities obtained from all concentrations versus the mass to charge ratio of the detected species. The figure shows the large amount of overlap each concentration has, with a large concentration of intensities between 0 and 2000. Figure 26 breaks the data in Figure 25 down into each individual concentration. It displays the distribution of intensities along with average intensity and standard deviation. Examining at the averages for the concentrations reveals why dissolving the matrix with the standard and spotting them together is not the best method to use for creating a calibration curve. The averages were 662, 965, 1037, and 842 for the concentrations 0.001 M, 0.01 M, 0.1 M, and 1 M respectively. The method proves to

be useful for qualitative analysis, but not quantitative analysis.

The data for the matrix application method where the caffeine standard was spotted and a sprayed matrix coat was applied, as seen in Figure 27, show several differences compared to the data from the first method. The data have more outliers in the higher intensities. The data is also more distributed over the intensity range, but the different concentrations have little separation and significant overlap. This can be seen in their average intensities, found in Figure 28, which are 1245, 1507, 1638, and 1743 for the concentrations 0.001 M, 0.01 M, 0.1 M, and 1 M respectively. The data create a linear calibration curve, Figure 31, with an  $R^2$  value of 0.95. The method produces usable results for a calibration, but applying the standards to the target plate make this method less desirable than the third method, where water was spotted on the target plate first.

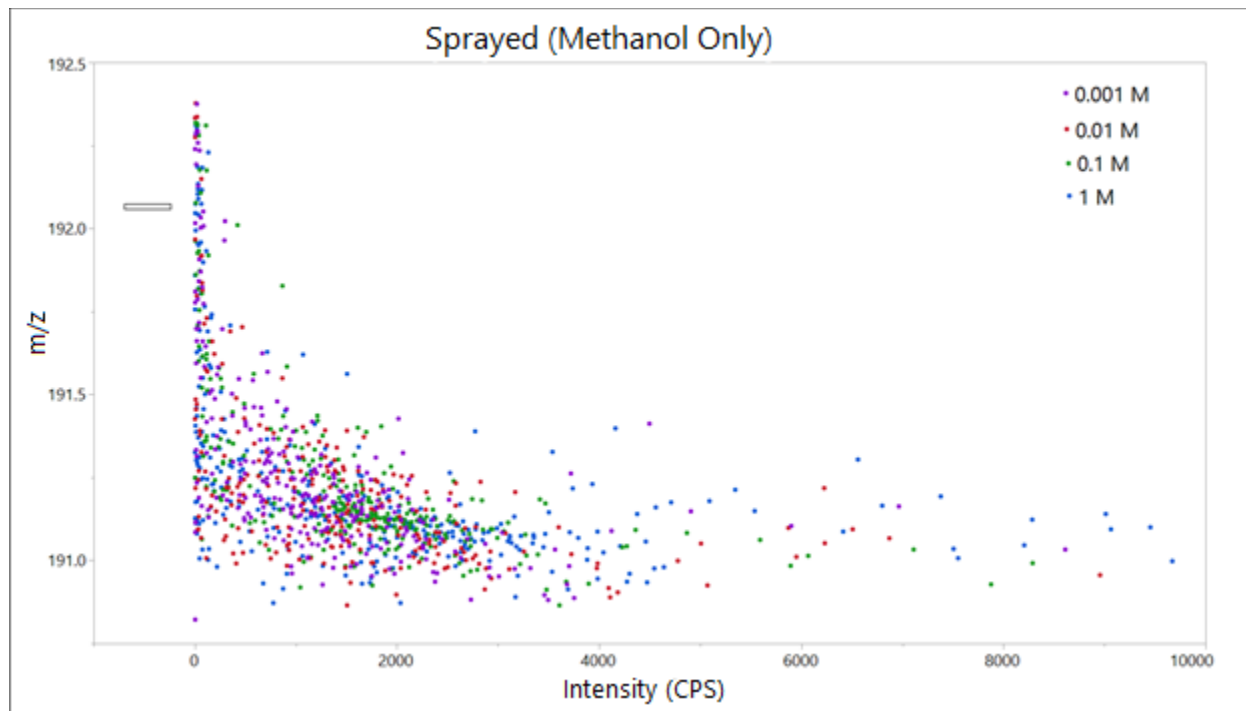


Figure 27. Distribution analysis of .001M, 0.01M, 0.1M, and 1M caffeine for the calibration spots where the caffeine standard was spotted and a sprayed matrix coat was applied.

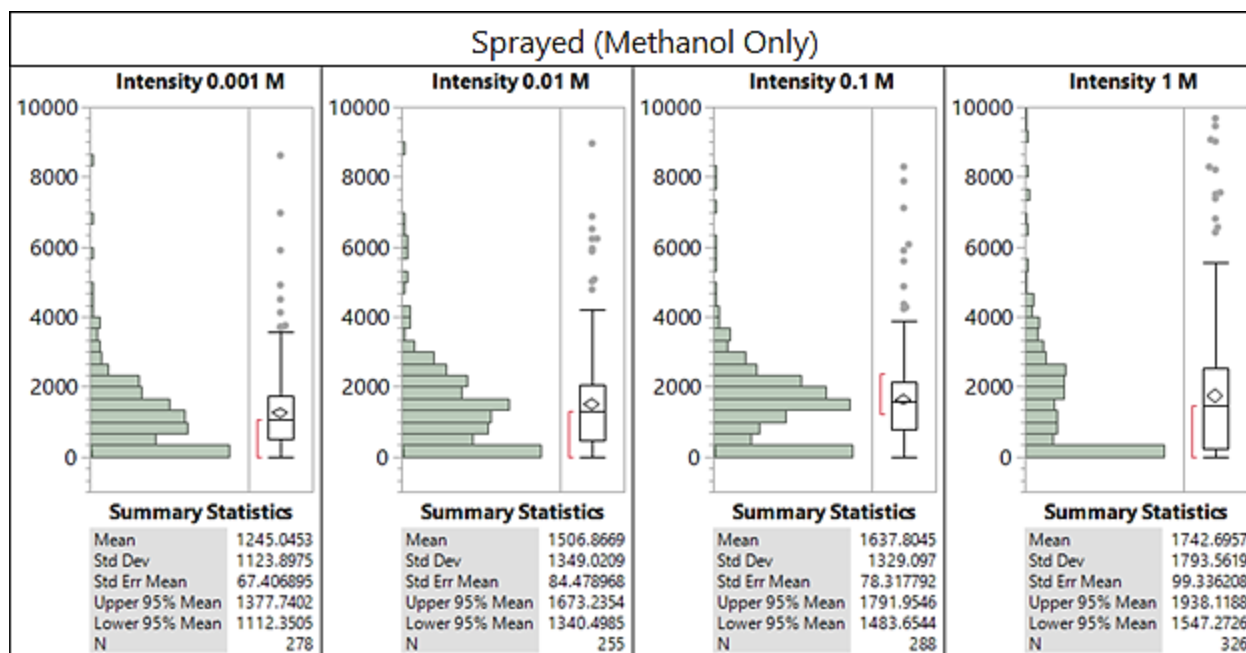


Figure 28. Distribution summaries of .001M, 0.01M, 0.1M, and 1M caffeine for the calibration where the caffeine standard was spotted and a sprayed matrix coat was applied.

The third matrix application method, where first water was spotted and then the caffeine standard was spotted and a sprayed matrix coat was applied, produced the best data out of the three application methods. While there is some overlap, in Figure 29 individual concentrations (as represented by the colored dots) appear in distinguishable bands. The addition of the water to the target plate before spotting the caffeine standard allowed the standard to spread evenly over the target plate and form a more uniform spot for analysis. These results can be seen in Figure 22 and are also shown in Figure 30. The distribution plots for the different concentrations show the positive correlation between increasing intensities with higher caffeine concentrations. The averages are 490, 673, 1206, and 1501 for the concentrations 0.001 M, 0.01 M, 0.1 M, and 1 M respectively. The calibration curve generated from these averages had an  $R^2$  value of 0.97. These results produce a reproducible and useable calibration curve that can be used to determine the concentration of unknown caffeine samples. The ease of sample preparation along with the more uniform distribution of the standard over the spot method led to the spray method being chosen as

the best method for forming a calibration curve using MALDI imaging.

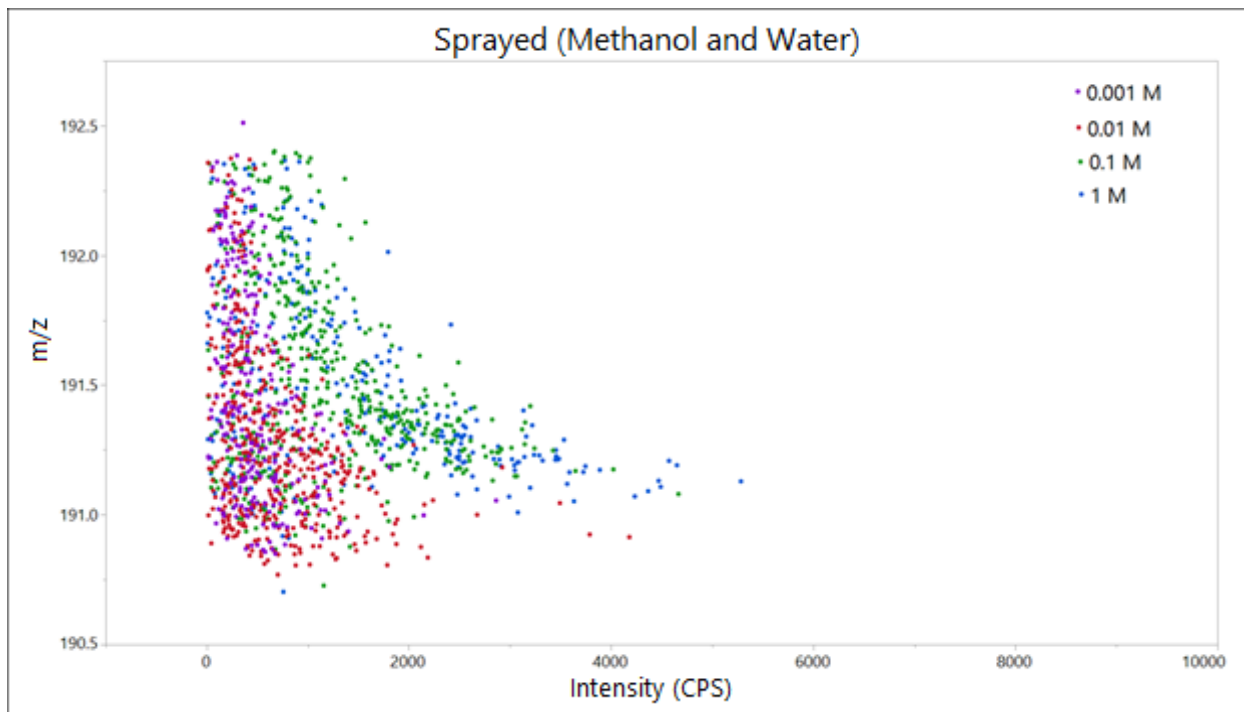


Figure 29. Distribution analysis of .001M, 0.01M, 0.1M, and 1M caffeine for the calibration where first water was spotted and then the caffeine standard was spotted and a sprayed matrix coat was applied.

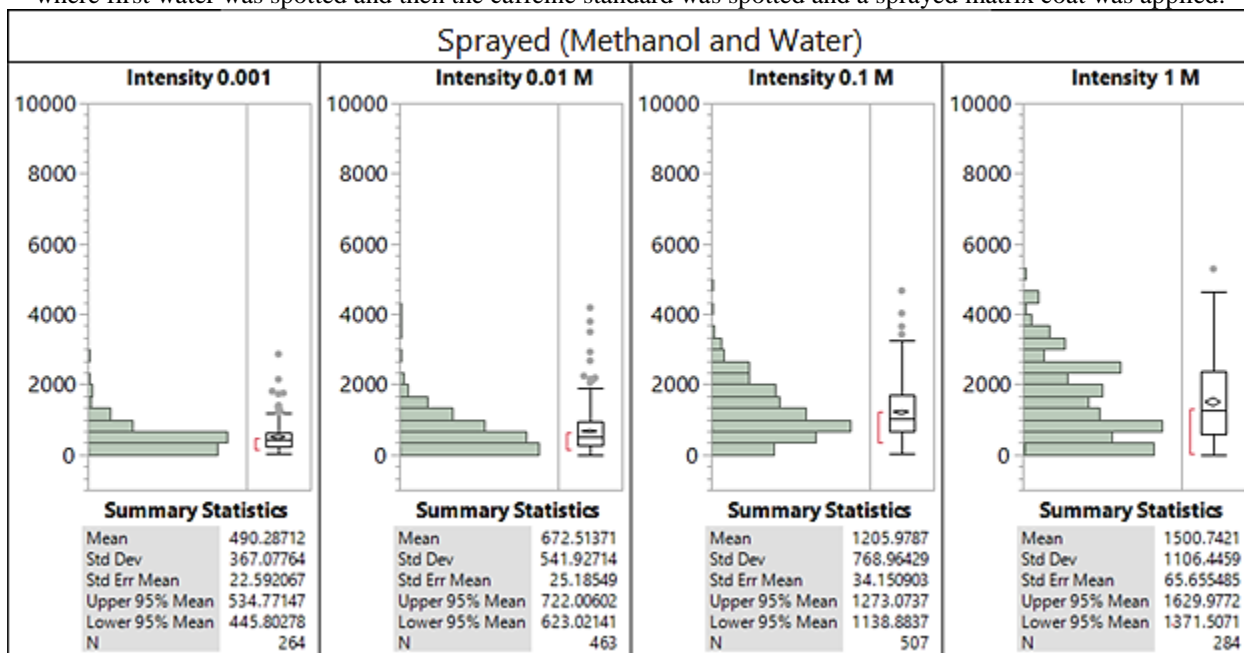


Figure 30. Distribution summaries of .001M, 0.01M, 0.1M, and 1M caffeine for the calibration where first water was spotted and then the caffeine standard was spotted and a sprayed matrix coat was applied.

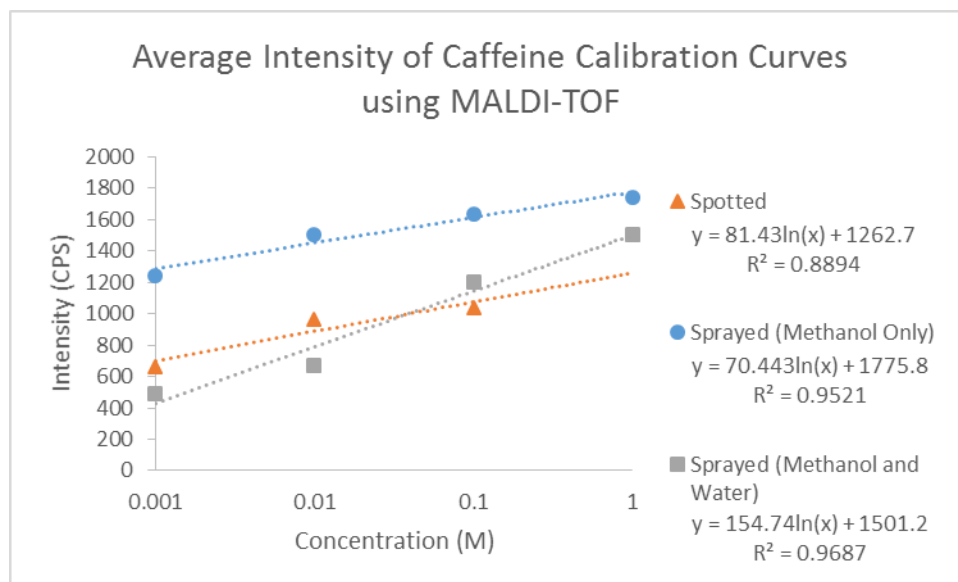


Figure 31. Calibration curves of the MALDI data obtained from the different matrix application methods.

### 3.4.3 SAMPLE COLLECTION

After the intricacies of the method were determined, samples were collected to determine if caffeine could be detected in biological fluids and fingerprints. Three volunteers were selected for this study. Volunteer 1 consumed a small 12 ounce cup of coffee and a small sip of diet coke, 2 to 3 hours and 10 minutes prior to sampling, respectively. Volunteer 2 consumed a 20 ounce bottle of Mello Yellow 2 hours prior to sampling. Volunteer 3 consumed a 16 ounce Monster Energy drink at the time of sampling after refraining from caffeine for 24 hours. Samples from Volunteer 1 and 2 were collected to represent the caffeine concentrations for a typical day in a person's life. Samples from Volunteer 3 were collected to show the changes in concentration levels detected by MALDI after caffeine consumption.

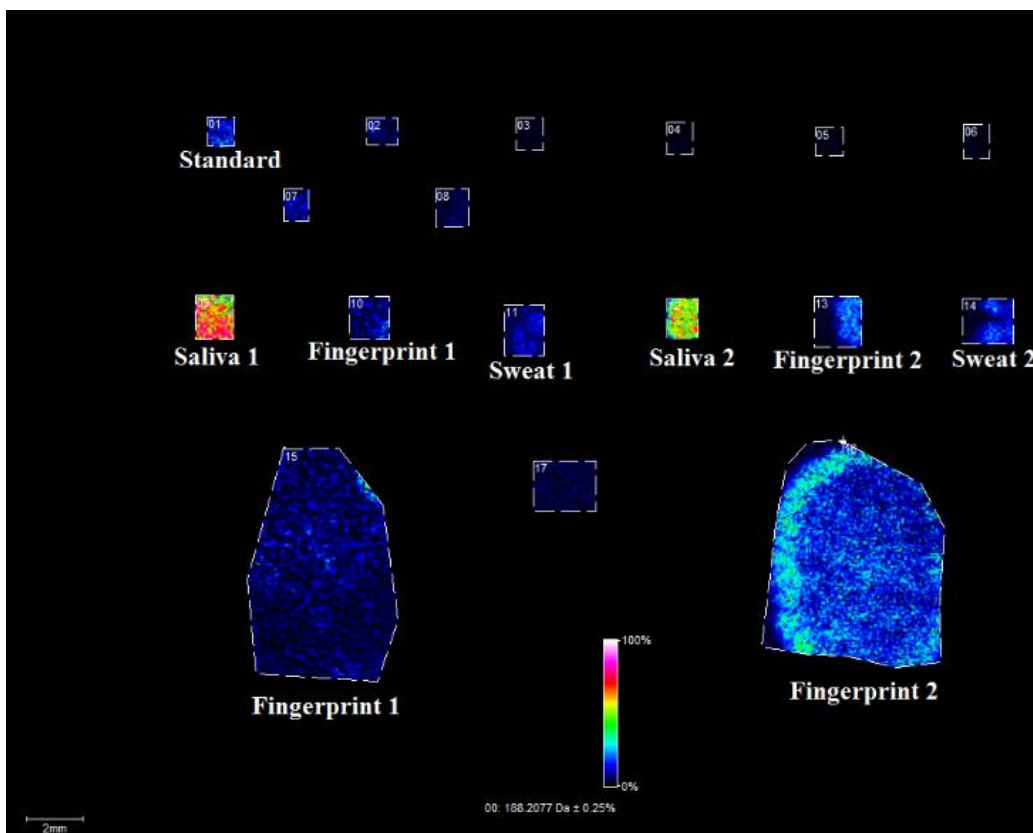


Figure 32. Analysis of saliva, fingerprints, and sweat from Volunteer 1 and 2 collected using a cotton swab, and pressed fingerprints.

In Figure 32, the pressed fingerprint 1, fingerprint residue 1, and sweat residue 1 showed very little caffeine present while the saliva 1 sample revealed, as expected, a high concentration of caffeine. This is due to the rate that caffeine travels throughout the body. Volunteer 1 did not have enough time between caffeine consumption and sample collection for the body to process the caffeine and for the caffeine to travel throughout the body to the sweat glands.

In contrast, Volunteer 2 consumed caffeine 2 hours prior to sample collection. Which allowed sufficient time for the body to metabolize some of the caffeine and for the caffeine to travel throughout the body to the sweat glands. This conclusion is supported by the higher concentrations of caffeine present in the pressed fingerprint 2, fingerprint residue 2, and sweat residue 2. The decreased amount of caffeine present in the saliva of Volunteer 2 when compared to Volunteer 1 is also attributed to the different ingestion times of the caffeinated beverages.

Figure 33 shows the change in concentration levels of caffeine as the body metabolizes caffeine. The caffeine levels in the fingerprints can be seen to increase after only 5 minutes. The caffeine levels peak around 30 minutes and begin to return to original levels over the next 5 hours. Volunteer 3 did not consume any sources of caffeine, food or drink, during the sampling period. The increase in concentration of caffeine is due to the consumed Monster Energy drink only. These results are consistent with the metabolism of caffeine that has been reported in the literature [83]

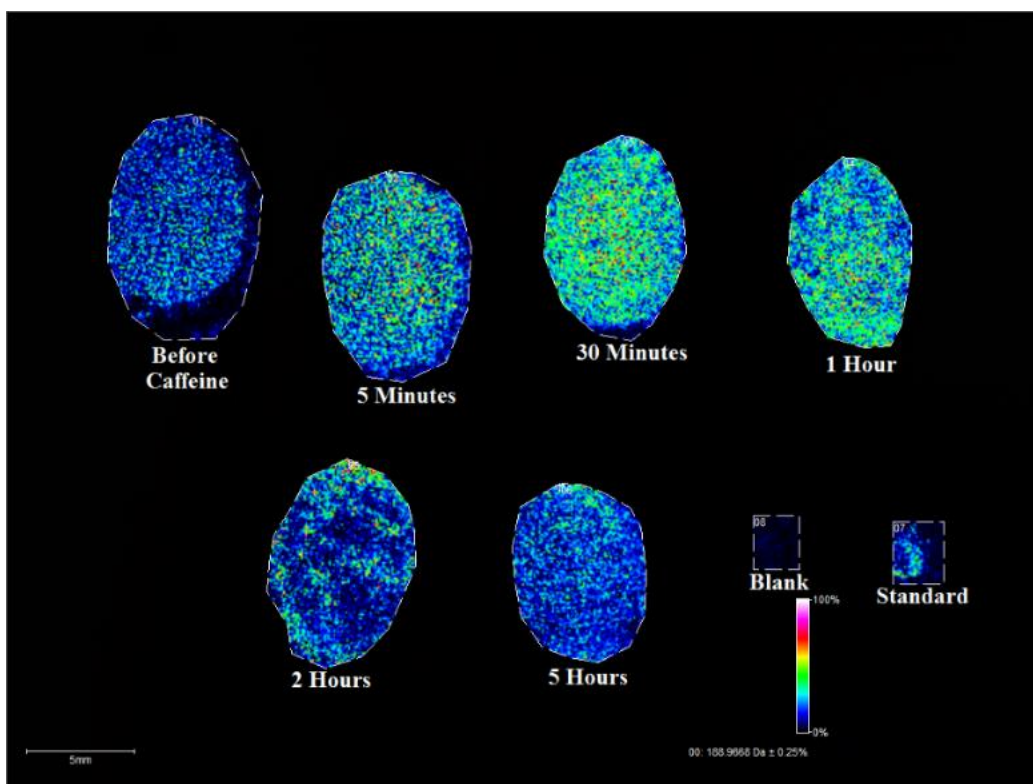


Figure 33. Analysis over a period of 5 hours of Volunteer 3's fingerprints after the ingestion of a 16 ounce Monster® energy drink.

### 3.5 CONCLUSION

MALDI analysis is a powerful tool that allows for sensitive analysis of samples with minimal sample preparation. The results observed in this research show that trace amounts of caffeine could be found in fingerprints and other biological matrices. This research is the first step in developing methods to detect sweat soluble drugs of abuse in fingerprints found at crime scenes. Additional work will be performed in order to detect and quantify metabolites of illicit drugs in

fingerprints, sweat, and saliva. Once these new noninvasive methods are fully developed, they can be used to assist the criminal justice system in determining abuse and intoxication levels of individuals using marijuana or other illegal or prescription drugs.



## CHAPTER 4

### METHOD IMPROVEMENT FOR DETECTION OF $\Delta^9$ -TETRAHYDROCANNABINOL IN HAIR

#### 4.1 ABSTRACT

Marijuana one of the most commonly used drugs in the United States and is one of the most analyzed illicit drug in forensic laboratories [83]. Despite the increasing number of states that have legalized marijuana it is still illegal at the federal level. Current drug testing methods for the detection of marijuana usage can detect marijuana metabolites in blood and urine samples of suspected marijuana users. Current analytical methods are useful in determining recent use of marijuana but have a limited window for detection in blood and urine samples of 2 days and 2 months respectively. Hair analysis for marijuana is an option for analysts that need to test for prior marijuana usage of suspects or personnel that ingested the drug months before drug samples could be taken for blood or urine analysis. The difficulty with current hair analysis techniques is the lengthy time needed for sample preparation which include washing, drying, pulverization or other hair size reduction techniques, and digestion. The purpose of this study is to reduce the preparation time needed for marijuana analysis from 2 days to 2-5 hours making hair analysis a more practical option for the backlogged forensic laboratories. The methods in this research aimed to reduce the amount of time needed for drying and to determine the best method of hair preparation for

extraction. Results from this study suggest that the total hair sample preparation time can be reduced by nine hours or more without sacrificing detection limits. The different methods had an average concentration of 120 pg/mg  $\pm$  10 pg/mg with 50pg/mg being the cutoff recommended by the Society of Hair Testing [84]. The more than 1 day in time savings and unchanged detection limits offered by these methods could result in an increase in hair analysis in forensic laboratories across the United States.

## 4.2 INTRODUCTION

### 4.2.1 WHAT IS CANNABIS?

Marijuana, or cannabis, is one of the most commonly used street drugs in the United States of America [83]. According to the National Institute on Drug Abuse, roughly 50% of adults have used marijuana at one point during their lifetime [85]. Under the Controlled Substance Act of 1970, marijuana was declared a ‘Schedule I’ drug and illegal to possess or ingest in the United States. A ‘Schedule I’ drug is a drug that has a high potential for abuse and no medical use [86].

Marijuana has not always been an illegal drug. Its use has been recorded for thousands of years. Early use of the plant was recorded dating back to the end of the Stone Age. People living in Central Asia used one species of the plant to make rope and clothing as well as food. They also discovered another species of the plant that had psychoactive properties. The names given to these two plants eventually morphed into the modern day names of hemp and cannabis. Written accounts of cannabis use as a fiber, food source, and psychoactive drug, can be found throughout the writings of many different cultures around the world. The Greeks mostly used cannabis for its practical uses and had little contact with it as a drug. There are accounts of the plant’s use in the

Middle East, Africa, Asia, and Europe indicating that cannabis is a plant that can be found in many differing cultures around the world. [87]

In 1973, a Swedish botanist formally studied cannabis creating a new genus, *Cannabis*, in which there are multiple subspecies due to human cultivation. The three main subspecies are *Cannabis Sativa*, *Cannabis Indica*, and *Cannabis Ruderalis*. As scientists began to study the plants of the *Cannabis* genus, they were able to isolate compounds that distinguished the plants from others with similar familiar characteristics, such as varieties of hops, mulberries, or hackberries. These compounds are collectively known as cannabinoids. There are over 100 naturally occurring cannabinoids divided into 8 main classes, as shown in Table 5, determined by the way the base structure of the compound is cyclized. [87,88]

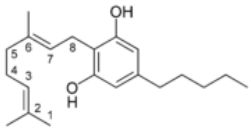
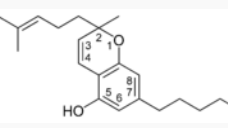
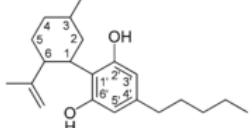
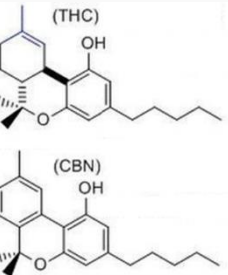
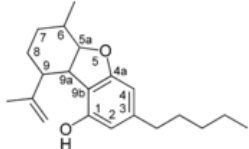
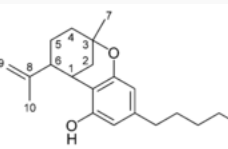
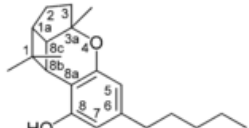
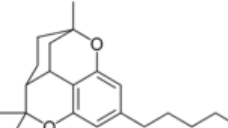
Cannabigerol <b>CBG</b>		Cannabichromene <b>CBC</b>	
Cannabidiol <b>CBD</b>		Tetrahydrocannabinol and Cannabinol <b>THC, CBN</b>	
Cannabielsoin <b>CBE</b>		<i>iso</i> - Tetrahydrocannabinol <b><i>iso</i>-THC</b>	
Cannabicyclol <b>CBL</b>		Cannabicitran <b>CBT</b>	

Table 5. The eight main classifications of cannabinoids and their base structure.

Cannabinoids are comprised of psychoactive and non-psychoactive compounds. The most well-known cannabinoid is  $\Delta^9$ -tetrahydrocannabinol or  $\Delta^9$ -THC. The cannabinoid is the primary

chemical found in marijuana that is consumed recreationally throughout the United States. The metabolites of  $\Delta 9$ -THC are the main chemicals targeted for drug screenings for marijuana use. Selective cultivation of *Cannabis* plants been undertaken to increase and decrease the amount of  $\Delta 9$ -THC found in the plant. Plants with higher  $\Delta 9$ -THC concentrations produce stronger psychoactive symptoms, while plants with lower  $\Delta 9$ -THC concentrations are typically used for medicinal purposes.

*Cannabis* plants have a long history of use as medicine. Greek physicians prescribed *Cannabis* for a variety of medical ailments including stomach issues. The majority of Greek uses of *Cannabis* did not employ the psychoactive properties of the plant. *Cannabis*'s psychoactive properties are activated by high temperature, such as baking or smoking. Writings detailing the Greek use of the plant did not include heat in its preparation. Arabic speaking scholars wrote of their medicinal practices, which were based on Greek physicians. Little knowledge or use of the psychoactive properties of *Cannabis* appeared in text until the twelfth century. After the twelfth century, there was an increase of recorded accounts of the psychoactive properties of *Cannabis*, but the main use of the plant remained as food and textile products. [87]

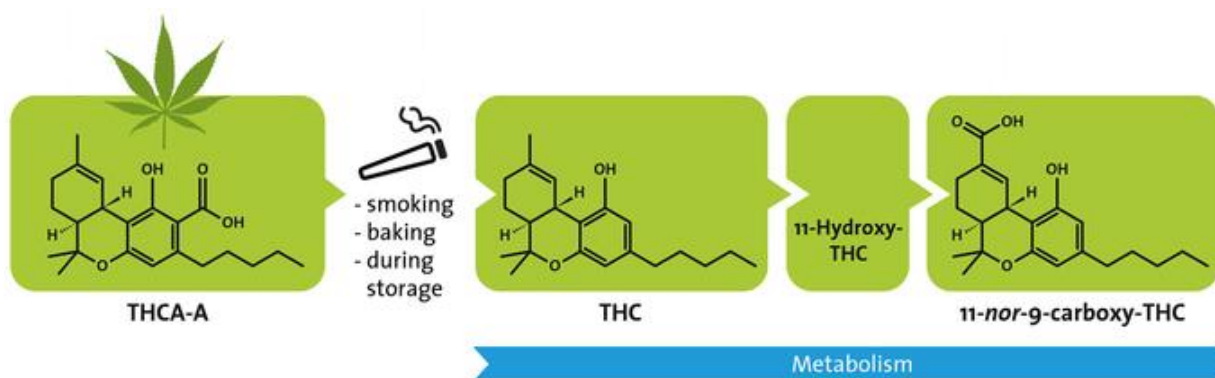


Figure 34: Major pathway of tetrahydrocannabinol (THC) metabolism. Tetrahydrocannabinolic acid (THCA), found in marijuana plants, is decarboxylated to THC through heat before being metabolized further in the liver via a cytochrome p450 gene to the psychoactive metabolite 11-nor-9-carboxy-THC [89]

Tetrahydrocannabinolic acid, THCA, is a non-psychoactive cannabinoid found in live *Cannabis* plants. As *Cannabis* plants are harvested THCA is converted to tetrahydrocannabinol, THC, through decarboxylation. The application of heat, through smoking or baking, can accelerate the decarboxylation process. THC is the main psychoactive component of *Cannabis* and is metabolized to 11-hydroxy-THC in the liver by the cytochrome p450 enzymes, CYP2C9, CYP2C19, and CYP3A4. 11-hydroxy-THC is further oxidized to 11-nor-9-carboxy-THC, the main metabolite detected in drug panels designed to screen for *Cannabis* use. THC and its metabolites travel throughout the body via the circulatory system and interact with cannabinoid receptors, CB receptors, in the central and peripheral nervous systems. [90,91,92,93,94]

#### 4.2.2 HISTORY OF AMERICAN LAWS REGARDING CANNABIS

*Cannabis* traveled to the Americas with the Spaniards and made its way into what is now the United States. It has been used in textiles such as rope, fabric, and paper since the 1600s. [95] Early American physicians even included *Cannabis* in their medicines that they sold to the public at large. Before 1910, *Cannabis* was referred to by its Latin name in America. As Mexican immigrants moved into the United States, the commonly used name of *Cannabis* began to shift slowly towards marijuana. Along with a name change, Mexican immigrants brought with them the practice of smoking marijuana. Prior to this, most Americans only ingested *Cannabis* in liquids or foods.

In the early 1900s, states began to take notice of marijuana use and enacted laws to regulate the possession, use, and sale of the plant. After the 18<sup>th</sup> amendment was repealed and prohibition ended the federal government turned their attention to marijuana with the 1937 Marijuana Tax. The increased government oversight and restriction on marijuana came at the start of the Great Depression in the 1930s. The government blamed marijuana smoking for corrupting the minds and

bodies of those who smoked it, unfairly targeting Blacks and Mexicans who were its largest consumers.

Henry Anslinger, the first director of the Federal Bureau of Narcotics, led the campaign against marijuana. Anslinger used the newly emerging silent movies, radio interviews, and newspaper articles to spread misinformation and fear against the plant, despite *Cannabis* already being accepted by the majority of Americans. Due to the rising tensions caused by the Great Depression, recent Mexican immigration, and continued racism of White Americans, Anslinger was able to turn a country against a plant by continually referring to it by its Mexican name and creating an association between the plant and the recent immigrants. By the time, White Americans realized the drug they were making illegal was *Cannabis* it was too late. By 1936, every state had laws regarding the plant, and in the 70's and 80's the federal government made marijuana illegal. [95,96]

In 1970, the government passed the Comprehensive Drug Abuse Prevention and Control Act of 1970, and in 1973, the Drug Enforcement Administration (DEA) was created. The Act of 1970 classified marijuana as a 'Schedule I' drug. Drugs in this schedule have a high potential for abuse and no medical use. It is illegal to possess or use drugs in this schedule. [95]

As the federal government began to increase the penalties for having or using marijuana, several states started relaxing the penalties for possessing small amounts of the drug. States also began to acknowledge once again the use of marijuana for medicinal purposes. This has caused a rift between state laws and federal law, especially in recent years as states have started legalizing recreational use of the drug. Since marijuana is still illegal on the federal level and in several states, research is ongoing to improve and increase marijuana analytical detection methods.

#### 4.2.3 DETECTION OF THC IN BIOLOGICAL FLUIDS

Testing for marijuana drug use is typically preformed using urine samples. Urine requires a noninvasive collection method and is generally easier to obtain. THC and its metabolites can take several hours to appear in urine after ingestion. Positive results can typically be found for up to a week after a single use. Chronic users of marijuana can have metabolites present in their urine for a couple of months after they discontinue using the drug. The main metabolite found in Urine is the non-psychoactive metabolite 11-nor-9-carboxy-THC. Due to the long retention time of marijuana metabolites in urine, it is impossible to measure impairment based on urine analysis alone. [97]

Blood is an alternative biological matrix that can be tested for marijuana use. It has several advantages over urine testing. Blood will contain traces of  $\Delta^9$ -THC, the psychoactive cannabinoid in marijuana, which can be used to indicate recent usage of the drug and can be used to measure a person's level of intoxication.  $\Delta^9$ -THC travels quickly through the blood and will be metabolized within 12-24 hours. After 24 hours, it is unlikely that any  $\Delta^9$ -THC will be found in a blood sample. Chronic users of the drug can build up plasma concentrations that could interfere in the determination of the timing of the most recent dose. Because obtaining a blood sample is also an invasive procedure that requires a medical professional, urine samples are used more frequently than blood samples in crime laboratories to test for marijuana usage. [98]

Another less commonly used biological matrix used in drug testing is hair. Hair is less commonly tested when compared to urine and blood samples because it details long-term use of the drug instead of immediate or recent use. THC and its metabolites can deposit into hair follicles as the hair is growing. Once incorporated into hair, the drug doesn't change form and will remain with the individual until their next haircut or the hair falls out. Since the average growth of hair is

known, hair can be used to determine patterns of usage. Hair testing can also be used to determine usage of marijuana that occurred outside of the window of detection for the other methods. [97]

#### 4.2.4 DETECTION OF DRUGS IN HAIR

Hair is a unique biological matrix that covers the majority of the human body in varying forms of thickness. The largest concentration of hair follicles are located on the head. Hair is comprised of keratin proteins, a fibrous structure protein. Hair, along with nails, are a unique part of the body because after growing past the skin the cells will remain unchanged. Unless changed by external chemical or physical means a shaft of hair will retain the chemical make-up it had at formation. [98,99]

Hair growth occurs in three distinct phases. The anagen phase is the first and the longest phase of hair growth. For 2-6 years, hair actively grows and has a steady flow of blood to nourish and promote growth. The catagen phase is a 2-4 week phase where the hair is beginning to die and lose contact with its blood supply. The final phase is the telogen phase where the hair is dead and a new hair begins to grow in the same follicle pushing the old hair out. During growth, hair follicles are nourished and supplied by the blood stream. As the blood flows to the hair and nourishes, waste material can be deposited in the growing hair shaft.

Current methods for testing hair samples for drug use are prohibitive to most non-specialist laboratories, especially in the forensic laboratory sector. The process for prepping the hair for extraction and the extraction procedure can be time consuming and take multiple days. Hair needs to be washed to remove all external contaminants and dried before extraction under current methods. After washing and drying, the keratin structure must be broken down to extract the drugs



trapped in the hair shaft. Work is ongoing to make drug analysis of hair a more efficient and reasonable method for use in crime labs. [100,101,102,103]

#### 4.2.5 LIQUID-LIQUID EXTRACTION

Liquid-liquid extraction is a technique that separates compounds based on their solubility in two immiscible liquids. In hair drug analysis, liquid-liquid extraction is used to extract the  $\Delta^9$ -THC from the keratin proteins that are broken down during digestion. [104]

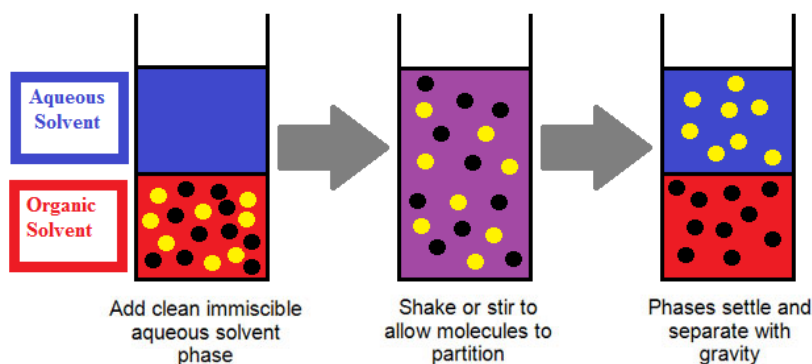


Figure 35. A visual representation of liquid-liquid extraction

#### 4.2.6 LIQUID CHROMATOGRAPHY (LC)

Liquid chromatography (LC) is a separation technique used to separate mixtures of chemicals or compounds. The chemicals are separated using a dual phase method. These phases interact together to move the analytes at different rates through a column, which creates separation. The two phases in chromatography are a mobile phase and a stationary phase.

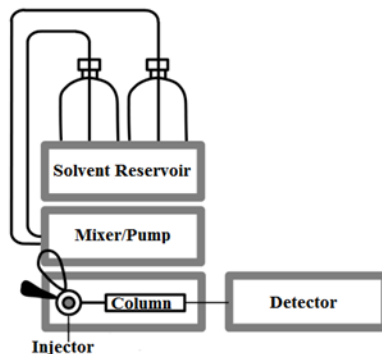


Figure 36. Schematic of a liquid chromatograph

In liquid chromatography, the mobile phase is a solvent or solvent gradient, while the stationary phase is a column. The mobile phase consists of solvents that can either remain the same concentration (isocratic) during the analysis, or change concentration (gradient) during analysis. Solvent manipulations allow for simultaneous analysis of compounds with dissimilar polarities. The solvents are chosen based on the polarity and potential interactions with the sample. The purpose of the mobile phase is to move the analyte molecules through the column to the detector.

The column interacts with the analytes causing the molecules to travel through the length of the column at different speeds where they reach the detector at different times. The solvents help move the analytes through the column. A gradient solvent flow can be used when a sample consists of analytes of different polarities requiring more than one solvent to carry all constituents to the detector.

This technique can also determine the amounts of the individual components of the analyte molecules. Detectors on a LC will not only record the time an analyte takes to reach the column, but it will also record the relative intensity of the analyte that reaches the detector. When a standard is used, the concentration of the unknown analyte can be determined.

Liquid chromatography is a technique that is becoming more commonly used in chemistry. While it is useful on its own, when it is combined with other techniques, it provides more

information that can be used to accurately identify analytes. LC is commonly coupled with mass spectrometry to provide separation of a sample before mass data is gathered.

As the instrumentation continues to improve, liquid chromatography moves to the foreground of separation techniques. With the ability to couple LC separation techniques to all manners of other instrumentation, the analytical benefit of the technique is limitless. [105]

#### 4.2.7 TANDEM MASS SPECTROMETRY (MS/MS/MS)

Mass spectrometry is a common analytical technique that measures the mass to charge ratio of molecules. In a typical mass spectrometer, a molecule is ionized and then separated using one of several mass separation components. Depending on the type of ionization the entire molecule is either ionized or is fragmented and then ionized during the ionization process. The whole or fragmented ions are sorted based on the ratio of their mass to their ionization charge. There are several types of mass separators including one of the most common, quadrupole mass separator.

Quadrupole mass separators consist of two pairs of rods that are parallel and form a diamond shape. Of the four rods there are two pairs formed as seen in Figure 37. The quadrupole works by applying a radio frequency voltage (RF) and a direct current (DC) voltage to the pairs of rods. The pairs will alternate between the RF voltage and the DC voltage with the pairs never having the same voltage type at the same time. The alternating voltages cause the ions to form a spiraling trajectory as they travel towards the detector. The strength of the voltage is used to separator ions. Only specific ions, based on their mass-to-charge ratio, will have a stable trajectory and reach the detector. All other ions will have unstable trajectories and collide with the rods or walls of the chamber before reaching the detector. The strength of the voltages applied to the rods is adjusted to allow a variety of mass-to-charge ratios to reach the detector one mass-to-charge

ratio at a time. Quadrupoles typically cycle through voltage strengths quickly, detecting hundreds of mass-to-charge ratios in milliseconds. Alternatively, the quadrupole can maintain one voltage strength only measuring one mass-to-charge ratio, which is known as single ion monitoring.

Tandem mass spectrometry was used in this research and consists of three sequential quadrupoles run in sequence. The first quadrupole (Q1) typically uses a soft ionization source that does not fragment the molecule. Q1 separates out a specific set of molecules with a specific mass-to-charge. These molecules are detected and then accelerated to the second quadrupole (q2). In q2, the molecules collide with an inert gas and are fragmented before being accelerated to the third quadrupole (Q3). Q3 is set up similarly to the first quadrupole. It separates and detects the most common fragments of the parent molecules. Multiple-reaction-monitoring (MRM) is a programmable mass selection method that allows the researcher to predetermine a set of mass-to-charge ratios to be analyzed. MRM is similar to the traditional scanning method and single ion monitoring method. MRM allows for multiple ions to be analyzed one after the other in a repeating cycle as does the traditional scanning method. Additionally MRM allows for the ions to be specified as in single ion monitoring and will only monitor those specific mass-to-charge ratios. In triple tandem mass spectrometry quadrupoles, 1 and 2 typically use MRM methods. Quadrupole 1 is set up to analyze and separate parent ions while quadrupole 3 is set up to analyze known fragments of the parent ion. This type of method allows for fast and sensitive analysis as only the ions of interest are detected. A drawback of the method is that all mass-to-charge ratios of interest need to be known before analysis. [106]

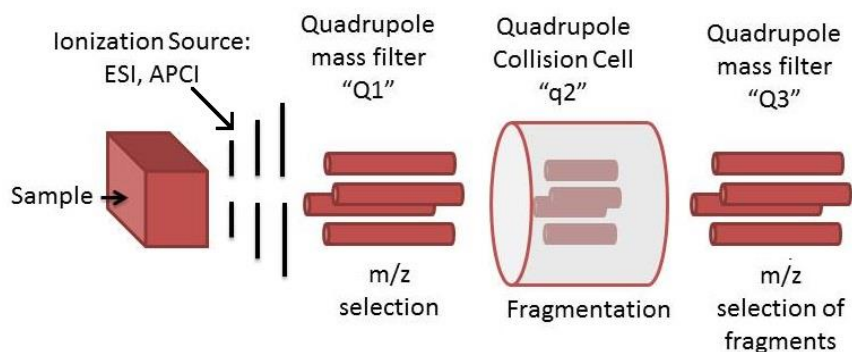


Figure 37. Schematic of a tandem quadrupole mass separator. After the sample is ionized, it is filtered in the first quadrupole, fragmented in the second quadrupole, and the fragments filtered in the third quadrupole. The ions are detected as they exit the first and third quadrupole.

#### 4.3 METHOD

Detection of THC in hair is an interest to forensic chemists and scientists who study the use of marijuana. The lengthy analysis time and difficulties involved with extracting THC and its metabolites from hair samples serve as barriers preventing hair analysis in crime laboratories. This study attempts to reduce the overall analysis time of THC in hair samples while improving or maintaining established detection limits.

Published hair preparation methods typically require a gentle wash and an overnight drying of the hair before the cutting and or pulverization of the hair and the final extraction process. Preparation techniques were chosen for this research to determine the impact washing and drying had on the detection of THC metabolite. The viability of using liquid nitrogen to pulverize the hair samples to allow for a more homogeneous sampling of hairs from different sources is proposed and investigated in this study.

##### 4.3.1 WASHING

Approximately 0.5 g of hair obtained after a hair cut from a self-reported marijuana smoker was used for each sample tested. Each batch of hair was washed with dilute soap for 10 minutes.

After 10 minutes of washing with diluted soap, the process was repeated three additional times with ddH<sub>2</sub>O for 10 additional minutes per wash. A final rinse for 5 minutes included an addition of 5ml of dichloromethane. Once the washing process was finished, the samples were dried in an oven at 30°C for 2 hours until the majority of the visible moisture was gone. To determine the necessity of a long drying process, a set of samples underwent the entire washing process, but did not undergo the drying steps. An additional set of samples were not processed using the washing or drying step to examine the effect that washing and drying has on metabolite levels in the hair sample.

#### 4.3.2 EXTRACTION

During extraction, 50mg of hair was added to 0.5 mL of a 0.5M sodium hydroxide solution. The resulting solution was heated to 95-100°C for 10 minutes, resulting in a liquefied digested hair solution. The 3 hair preparation techniques examined for this method included 1) the Godfrey/Glenn liquid nitrogen or G<sub>2</sub>LN<sub>2</sub> pulverized hair method (hair pulverized using liquid nitrogen and a mortar and pestle), 2) the uncut hair method (8-10 cm pieces of hair), and 3) the cut hair method (approximately 1cm pieces of hair). After the solution was allowed to cool, a 9/1 hexane/ethyl acetate solution was added to extract the Δ<sup>9</sup>-THC. The samples were vortexed for 10 minutes, and the organic phase was removed. The organic phase was evaporated under a gentle stream of nitrogen, and the sample was reconstituted in 100μL of acetonitrile for analysis on LCMS.

### 4.3.3 LC/MS/MS

Liquid chromatography separation was performed using a Shimadzu Nexera duel pump LC and a Phenomenex Synergi Hydro – RP 2.5 mm 100Å 50x4.6mm column. The instrument included an auto-sampler that injected 5µL of the sample into a gradient flow pumped at 0.8ml/min with a column temperature of 30°C. The solvents used in this analysis were solvent A, water w/ 0.1% formic acid, and solvent B, acetonitrile w/ 0.1% formic acid which were pumped through the instrument following the gradient program outlined in Table 6. The total runtime for the method was 10 minutes.

Time Program		
Time (Min)	A%	B%
0.0	60%	40%
3.0	60%	40%
3.2	10%	90%
5.2	10%	90%
6.0	05%	95%
7.0	60%	40%
7.5	60%	40%
10.0	Stop	Stop

Table 6: The parameters for the gradient LC program during analysis of the hair samples.  
Solvent A: water w/ 0.1% formic acid. Solvent B: acetonitrile w/ 0.1% formic acid.

Tandem mass spectrometry detection was performed using ABSciex QTrap 4500 ESI-QqQ MS. For this analysis, the instrument was operated in positive ionization mode (PIM). The source temperature was set at 650°C, the ion spray voltage was 5500V, and the curtain gas had a flow of 40 liters per minute (L/min) while source gases 1 and 2 had flows of 60 and 70 L/min, respectively. Multiple-reaction-monitoring (MRM) was used to detect and separate tetrahydrocannabinol (THC) and 11-nor-9-carboxy-THC (THC-COOH) in the first quadrupole. The second quadrupole fragmented the two molecules of interest and the two most common fragments were detected in the third quadrupole as shown in Table 7. Quadrupole 1 (Q1) allowed only 315m/z and 345m/z to

pass to quadrupole 2 (q2). Quadrupole 3 (Q3) detected the 193.1m/z and 122.9m/z fragments from the parent ion of THC, and the 327m/z and 76.9m/z fragments from the parent ion of THC-COOH. The declustering potential (DP) was set to 76 eV. The entrance potential was set to 10 eV. The collision cell exit potential was set at 8, 16, or 6 depending on the size of the fragment in Q3. Analysis was performed using Analyst v.1.6.2 and v.1.6.3 software.

MRMs					
	Q1	Q3	DP	EP	CXP
Tetrahydrocannabinol (THC) 1	315	193.1	76	10	8
THC 2	315	122.9	76	10	8
11-nor-9-carboxy-THC (THC-COOH) 1	345	327	76	10	16
THC-COOH 2	345	76.9	76	10	6

Table 7: Summary of the parameters for tandem MS.

#### 4.4 RESULTS AND DISCUSSION

The comparison of various hair preparation methods yielded some promising results. Four of the six samples types used in this study were allowed to dry in an oven at 30°C for 2 hours until all of the visible water was evaporated. One of the samples used was washed but not dried while the last set was neither washed nor dried. Table 8 contains the time needed for each method used in this research compared with the time taken for current literature methods.

Method	Wash	Dry	Cut	Pulverized	Total Time
Literature Pulverized	2h+	12h+	-----	20min	14h+
Godfrey/Glenn (G <sub>2</sub> LN <sub>2</sub> ) Pulverized	30min	4h	-----	30min	5h
Washed and Dried [Uncut]	30min	2h	0min	-----	2.5h
Washed and Dried [Cut]	30min	2h	5min	-----	2.5h
Washed and Not Dried [Cut]	30min	0min	5min	-----	35min



Not Washed or Dried [Cut]	0min	0min	5min	-----	5min
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Table 8. Comparison of the time required for the various hair preparation methods.

Three different hair sample preparation methods were examined in this study to maximize the liberation of  $\Delta^9$ -THC from hair samples using a sodium hydroxide solution 1) pulverized hair, hair samples were pulverized using liquid nitrogen and a mortar and pestle 2) uncut hair, hair left uncut at approximately 8-10cm lengths and 3) cut hair, hair cut into approximately 1cm strips using a scalpel blade as suggested in the literature. The cutting of the hair samples took only 5 minutes and did not offer a significant amount of time saving when compared to the uncut hair. In addition, the uncut hair had to be tapped against the lab bench multiple time throughout the digestion process in order liberate all of the hair for digestion. The cut hair did not require this step. The pulverized hair took a significantly longer time than the other preparation methods. Pulverizing the hair, using the Godfrey/Glenn liquid nitrogen pulverization method ( $G_2LN_2$ ) took approximately 45 minutes per sample including cleaning the mortar and pestle in-between samples. Due to the high air humidity, the liquid nitrogen caused the samples to become water logged requiring additional drying. The additional drying increased the preparation time for the pulverized samples by 2 hours.

While the  $G_2LN_2$  method required twice the preparation time as the uncut and cut hair methods, all of which were washed and dried, the  $G_2LN_2$  method is a 9+ hours improvement over the pulverization method used in the literature. The particles of hair produced were millimeters long and would lead to a better homogenization of hair samples. After slight modification, the  $G_2LN_2$  method has great potential for future use in forensic laboratories.

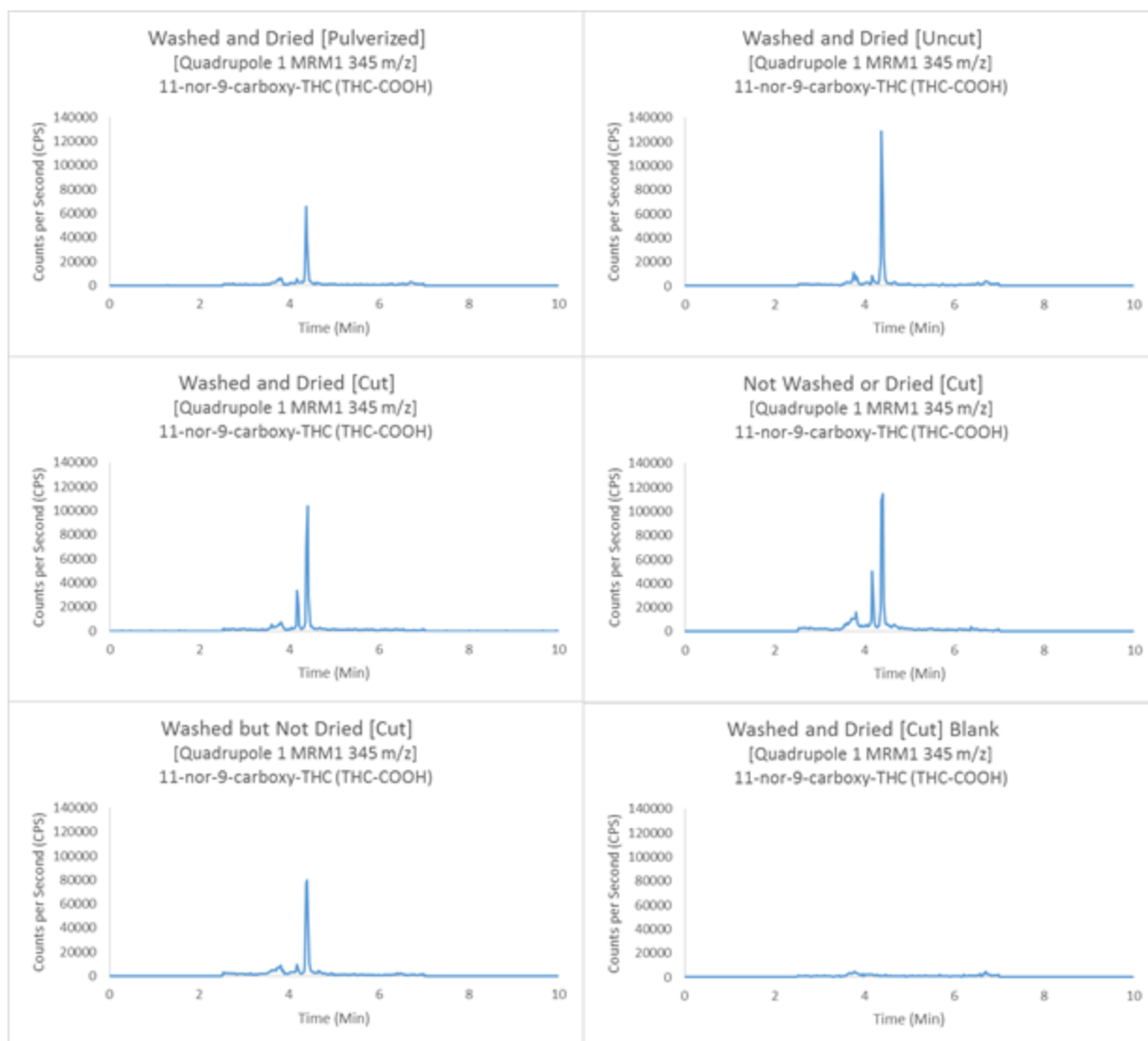


Figure 38. The mass spectra from quadrupole 1 of THC-COOH for the different hair preparations.

As seen in the Figures 38 each sample preparation method had detectable THC-COOH, the main metabolite of THC. An additional peak can be seen in the samples, with the highest concentration in the samples that were washed and dried and not washed or dried. This peak is believed to be an isomer of 11-nor-9-carboxy THC. Figure 39 shows the chemical structure of  $\Delta^9$ -THC and  $\Delta^8$ -THC, two cannabinoids found in marijuana. The only difference between the two compounds is the double bond between the 9/10 carbons and 8/9 carbons in  $\Delta^9$ -THC and  $\Delta^8$ -THC, respectively. The metabolite studied in this research is a metabolite of  $\Delta^9$ -THC, the most common

cannabinoid in marijuana. It is thought that the additional peak in the results come from the metabolite of  $\Delta 8$ -THC, but further analysis would be needed.

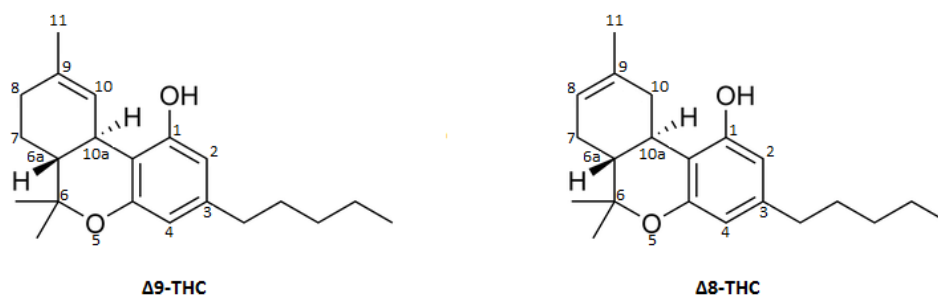


Figure 39. Comparison of  $\Delta 9$ -THC and  $\Delta 8$ -THC

The concentrations of each hair preparation method can be found in Table 9. They were calculated using the peak heights and the calibration curve found in Figure 35. The samples had an average concentration of  $120 \text{ pg/mg} \pm 10 \text{ pg/mg}$ . The concentrations of the metabolite in each sample are close in value as expected, because the hair was harvested from a single donor. Of the six methods tested, two of the methods produced results that were below  $120 \text{ pg/mg}$ . The method where the hair was pulverized and the method where the hair was washed but not dried had concentrations of  $105 \text{ pg/mg}$  and  $111 \text{ pg/mg}$  respectively. Out of the six methods these two were exposed to more water, or the water was not removed completely before extraction. The pulverized hair sample was pulverized with liquid nitrogen, and the humidity of the room caused the sample to become waterlogged. A 30-minute drying process was performed to remove most of the water, but it was not as long as the drying session after washing. The hair sample that was washed but not dried had water remaining from its washing. The initial step of extraction involved an aqueous  $0.5 \text{ M}$  sodium hydroxide solution. The water remaining in the two hair samples would have diluted the sodium hydroxide solution causing to become less efficient at extracting the 11-nor-9-carboxy THC. This loss in efficiency led to a lower concentration for the samples. The three samples without additional moisture have an average concentration of  $127 \text{ pg/mg} \pm 4 \text{ pg/mg}$  which is

nearly 20 pg/mg larger than the 108 pg/mg  $\pm$  3pg/mg of the two samples that had additional moisture during preparation.

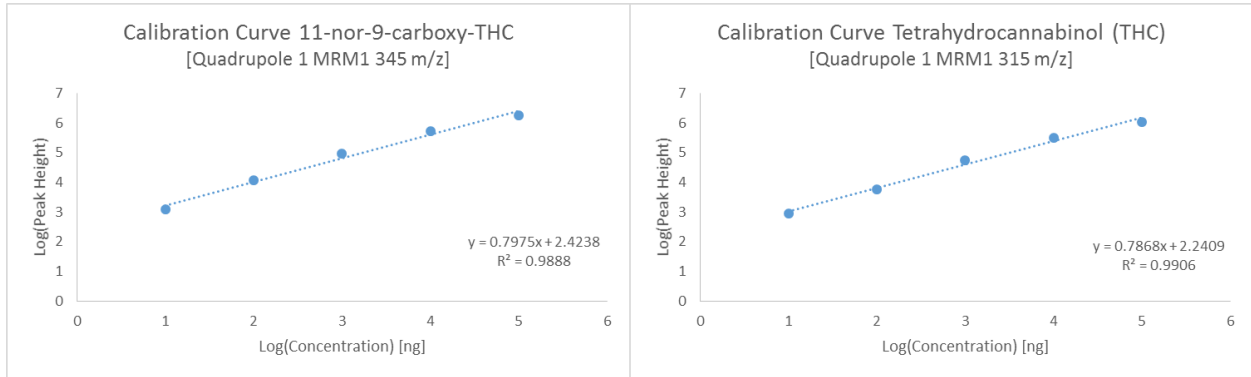


Figure 40. Calibration curve of Standard 11-nor-9-carboxy-THC that produced data for 345m/z (11-nor-9-carboxy THC) and 315m/z (THC) molecules

	11-nor-9-carboxy THC	Tetrahydrocannabinol
Washed and Dried [Pulverized]	105 pg/mg	25 pg/mg
Washed and Dried [Not Cut]	133 pg/mg	33 pg/mg
Washed and Dried [Cut]	123 pg/mg	25 pg/mg
Washed and Not Dried [Cut]	125 pg/mg	68 pg/mg
Not Washed or Dried [Cut]	111 pg/mg	77 pg/mg
Washed and Dried [Blank]	0 pg/mg	0 pg/mg

Table 9. The concentration of the 11-nor-9-carboxy THC and Tetrahydrocannabinol detected.

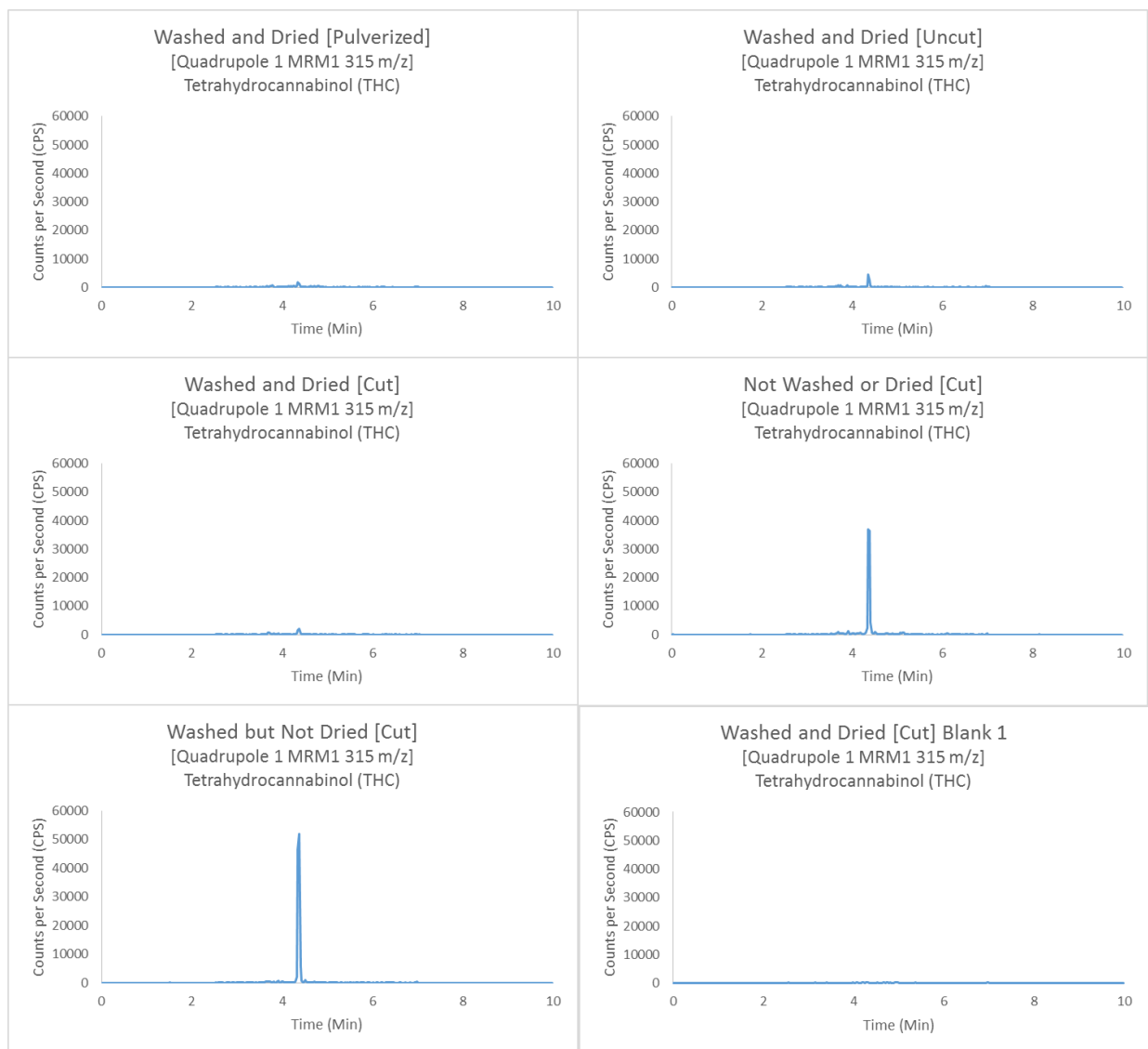


Figure 41. The mass spectra from quadrupole 1 of THC for the different of hair preparations.

Analysis of the samples and standards was performed using an LC/MS/MS method established at ElSohly Laboratories to analyze THC, its metabolites, and synthetic cannabinoids. The 11-nor-9-carboxy-THC metabolite was the compound of interest in the extraction and analysis of the hair samples. Due to the method used at ElSohly laboratories, it was possible to look at the results for the parent THC molecule. The presence of THC in the samples initially lead to the belief that there was THC present in the hair samples. Upon analyzing the standard 11-nor-9-carboxy THC it was found that THC was also present in the results. This indicates that during analysis 11-

nor-9-carboxy THC is becoming decarboxylated producing the parent THC molecule. The THC data gathered from the 11-nor-9-carboxy THC produces a linear calibration curve similar to calibration curve of 11-nor-9-carboxy THC. The 11-nor-9-carboxy THC's calibration curve has a slope of 0.7975 while the THC's slope is 0.7868. Both calibration curves have an  $R^2$  value of 0.99. This data shows a correlation between the 11-nor-9-carboxy THC data and the THC data. This correlation is not seen in the sample data. The samples of hair that received no drying (washed but not dried and neither washed or dried) had a higher concentration of THC than the samples that underwent the drying step. It is possible that the natural THC found in the hair could have been broken down by the heat of the drying step, and the remaining THC is the product of the decarboxylation of 11-nor-9-carboxy THC. This would account for the increased concentration of THC found in the samples that did not undergo the drying step. However, more research is needed to determine the exact nature of the THC data.

#### 4.5 CONCLUSION

Current literature details a lengthy method for analyzing the metabolite 11-nor-9-carboxy-THC in hair. The aim of this study was to reduce the amount of time needed for hair analysis, eventually making it a more practical option for use in forensic laboratories and for use in this laboratory. Examination of current literature identifies the drying of the hair sample after washing adds an additional 12+ hours to the hair preparation step. The different sample preparation methods yielded 11-nor-9-carboxy-THC concentration of  $120 \text{ pg/mg} \pm 10 \text{ pg/mg}$  that are comparable to the published results. The results of this research suggest that the length of the drying time does not have a significant impact on the quantity 11-nor-9-carboxy-THC of detected. The time saving measures and comparable detection limits of the Godfrey/Glenn Liquid Nitrogen Pulverization

method can make the lengthy hair analysis used in crime laboratories a routine and reliable service just as urine and blood.

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VITA

Bachelor of Forensic Science, 2012, University of Mississippi