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Non-destructive analysis of popular cigarette brands utilizing attenuated total reflectance (ATR) and diffuse reflectance infrared fourier transform spectroscopy (DRIFTS) and chemometrics

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BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

**NON-DESTRUCTIVE ANALYSIS OF POPULAR CIGARETTE BRANDS
UTILIZING ATTENUATED TOTAL REFLECTANCE (ATR) AND DIFFUSE
REFLECTANCE INFRARED FOURIER TRANSFORM SPECTROSCOPY
(DRIFTS) AND CHEMOMETRICS**

by

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B.S., California State Polytechnic University, Pomona, 2022

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requirements for the degree of
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ABSTRACT

Cigarettes have a long history in the United States, and remain popular to this day. Due to their prevalence in society, cigarettes are often discarded in public areas. This leads them to being found in many areas, including crime scenes. Since cigarettes are in proximity with biological fluid such as saliva, DNA analysis may be the first method used for forensic analysis. However, other methods of analysis, such as analysis of the various components that make up a cigarette, may also be useful.

Cigarette butts are comprised of a filter that is enclosed in an outer wrapping paper that is inserted into the mouth. Additionally, when the cigarette butt is discarded, a small amount of burnt tobacco may remain in the cigarette butt. Although these components are shared among many cigarettes available on the market, there may be differences among the various brands and manufacturers that might make brand differentiation possible.

Fourier Transform Infrared Spectroscopy (FTIR) is a traditionally nondestructive, common instrumental approach employed in forensic chemistry, and is often employed in forensic analysis of trace evidence. Due to its ability of analyzing different sample matrices with a variety of techniques, infrared spectroscopy is highly effective at identifying the chemical composition of unknown samples. Popular techniques include Diffuse

Reflectance Infrared Fourier Transform Infrared Spectroscopy (DRIFTS) and transmission FTIR. When combined with chemometrics, a mathematical approach to finding similarities and differences among samples, infrared analysis can be used to distinguish between samples and classify unknowns.

This research explores the possibility of utilizing various infrared analysis techniques and chemometric software in an effort to differentiate between cigarette brands. Ultimately, it was found that the methods used in this research could differentiate certain brands from each other, but could not differentiate between all the brands tested.

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LIST OF ABBREVIATIONS

2D	Two Dimensional
3D	Three Dimensional
AM	American Spirit
AM LB	American Spirit Light Blue
ATR	Attenuated Total Reflectance
B	Blue
CM	Camel
CM B	Camel Blue
DNA	Deoxyribonucleic acid
DRIFTS	Diffuse Reflectance Infrared Fourier Transform Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
G	Green
GC - MS	Gas Chromatography – Mass Spectrometry
HPLC	High Performance Liquid Chromatography
IR	Infrared
KBr	Potassium Bromide
LB	Light Blue
LC-MS	Liquid Chromatography – Mass Spectrometry
MB	Marlboro
MB R	Marlboro Red
mL	milliliters

mm	millimeters
NP	Newport
NP G	Newport Green
NIR	Near Infrared spectroscopy
NIPALS	Non-Linear Iterative Partial Least Squares
PR	Parliament
PR B	Parliament Blue
PCA	Principal Component Analysis
PG	Parental Guidance
R	Red
SVD	Singular Value Decomposition
µg	micrograms

1. INTRODUCTION

1.1 Cigarette Usage in the United States

Despite the health concerns surrounding smoking, cigarettes are among some of the most heavily advertised products in the United States, with over \$8 billion being spent on advertising and promotions, and the majority of the budget being spent on price discounts for wholesale (1,2). Although radio and television advertisements of cigarettes were banned in 1971, companies have found other ways to advertise cigarettes discreetly. Movies, especially those rated PG, or parental guidance 13 and up, have been influencing the younger audience into smoking cigarettes. A study in 2006 suggested that almost 90% of movies feature some aspect of cigarette imagery, and influenced approximately 390,000 teenagers to pick up the habit (3).

There are a large variety of cigarette brands available for purchase; a 2013 study suggests the most popular is Marlboro, which dominated the market, as nearly 40% of smokers (aged 12 or older) preferred this brand. Following closely behind Marlboro is Newport. The cigarette brand that experienced the greatest increase in usage between the years 2002 through 2013 was American Spirit, at 553.3% growth (2). Further research has found that brand preference differs among demographics, including race, age, and sexuality. For example, one study found that lesbian, gay and bisexual smokers were 3 times more likely to smoke American Spirit cigarettes compared to heterosexual smokers. African Americans, Hispanics and Latinos were more likely to smoke Newport cigarettes compared to the other brands in the study, such as Marlboro and Camel (4).

While Phillip Morris International, an international tobacco company, is popular for their Marlboro cigarettes, the company advertises its Parliament brand as the “premium price” compliment to the Marlboro cigarette. The company also advertises that Marlboro is the “world’s best-selling international cigarette”, as Marlboro cigarettes made up 37% of the total shipment volume in 2020 (5). One other major international tobacco company, R.J. Reynolds Tobacco Company, owns many popular cigarette brands, including Newport and Camel (6). Other cigarette brands are subsidiaries, such as American Spirit, which is owned by Santa Fe Natural Tobacco Company Inc., of the Reynolds American Tobacco Company umbrella (7,8).

Due to the prevalence of smokers of all demographics in the United States, remnants of cigarettes can often be found littered and not discarded properly. In particular, cigarette butts remained among the most recovered item on beaches during cleanup efforts worldwide in 2020 (9). Since discarded cigarette butts are a waste item, they may provide probative information if recovered at crime scenes. Cigarette butts are in direct contact with human saliva, which contain epithelial cells that contain deoxyribonucleic acid (DNA) (10). One study found that, with four DNA extraction methods, full or partial profiles were obtained from the trace DNA left on cigarette butts. One extraction method in particular was able to generate a full profile for 74.6% of samples, and a partial profile for 25.4% of samples (11). However, like all DNA-type evidence, cigarette butts are susceptible to factors that may degrade DNA, such as moisture, bacteria, sunlight and heat. The amount of saliva, and essentially, DNA containing epithelial cells, that may potentially be left

behind on the cigarette butts is also dependent on factors such as hereditary factors, hormonal factors, and general oral hygiene (12).

1.2 Cigarette Construction

1.2.1 Cigarette Components

Cigarette construction is relatively simple, which contributes to their ability to be mass produced. When reduced to their basic components, cigarettes are simply a portion of tobacco blend, wrapped by paper. In truth, there are more factors that make up a cigarette, including adhesives to glue the paper together, and the filter that is included in the cigarette butt that is often discarded (13). Figure 1 depicts a standard cigarette, with labeled components.

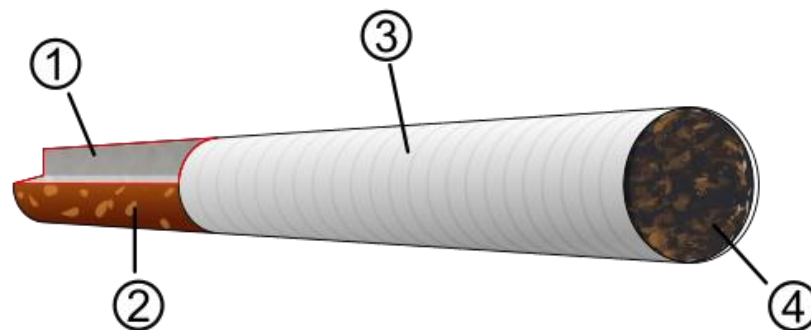


Figure 1. Diagram of Cigarette Components. A diagram of an unsmoked cigarette, labeled (1) the cigarette filter, (2) the outer wrapping paper, (3) the rolling paper, often with a brand identifier present at the base, near the wrapping paper, entirely filled with (4) the tobacco blend. Obtained from Wikimedia Commons (14).

The tobacco in commercial cigarettes often comes from the genus *Nicotiana*. Although the species remains the same among brands, the manufacturing process differs, which in turn, alters the tobacco's properties. The tobacco can either be air-cured, cured with artificial heat, or sun-cured (15). Tobacco that is dried with artificial heat, for example, contains more carbohydrates and a lower nitrogen level than those that are air-cured.

Commercial brands often have their own unique blend, which contains various amounts of flavorings and humectants. Humectants maintain the moisture content within the tobacco blend, in order to alter the pH, taste and nicotine content (15,16). Modern cigarettes often contain additives in order to enhance the taste and smell of cigarettes. As much as 10% of the cigarette's weight comes from additives, with monosaccharides and disaccharides being the most popular additive (15). Since all cigarette companies aim to sell a unique cigarette, a number of different additives may be added to create a signature taste. In the United States specifically, the tobacco industry has admitted to using 599 different cigarette additives (17,18). Additionally, even among the same brand, different batches and blends may differ due to the species used, the region harvested from, and the composition of the leaves during harvesting and processing (19).

1.2.2 History of the Cigarette Filter

While the main purpose of a cigarette filter is to prevent tobacco particulates from entering the smoker's lungs, the concept of a filter as a means to protect the lungs from smoke was invented prior to the invention of the cigarette (20,21). The earliest instance of a respirator with a filter was in 1849, when Lewis Haslett was awarded a patent for a "Lung Protector". Its purpose was to filter out "injurious substances" such as dust and prevent them from being inhaled (22). The first cigarette brand to sell a filtered cigarette was Parliament in 1931, which was constructed with a cardboard mouthpiece plugged with cotton. However, it was not until 1952 that Viceroy introduced the cellulose acetate filter, which replaced the paper filter (23,24). Filtered cigarettes were promoted in an effort to

increase sales among female smokers (21). By the 1960s, filtered cigarettes made up 51% of the consumer market, and by 2005 they represented 99% of the market (25).

1.2.3 Cellulose Acetate

Cellulose acetate is a polymer largely sourced from cellulose in wood, through an acetylation reaction. Due to the chemical modification that this polymer undergoes to become cellulose acetate, it is considered a semi-synthetic polymer, and is used in many industries besides the cigarette filter industry, including the textile and packaging industries (26). Although cellulose acetate is a resistant product that is capable of removing undesirable components from cigarette smoke such as nicotine, tar and phenols, the author of one of the patents granted by the United States for a cellulose acetate filter stated that it would not be “desirable [to remove these substances] due to considerations of taste and flavor” (27,28).

1.3 Infrared Spectroscopy and Chemometrics

1.3.1 Infrared Spectroscopy and its Forensic Applications

Infrared spectroscopy (IR) is a non-destructive, analytical method utilized in chemistry to determine the molecular structure of a sample. This is achieved due to the nature of the specific frequencies that the chemical bonds within the molecular structure begin to vibrate. The frequencies of vibration can be influenced by factors, such as proximity to other atoms, add more factors. By comparing a generated spectrum of an

unknown sample to documented spectra in literature, a chemist can qualitatively identify their unknown (29).

1.3.2 ATR - FTIR

Attenuated Total Reflectance – Fourier Transform Infrared spectroscopy (ATR-FTIR) is a technique used for infrared spectroscopy analysis. It is considered a non-destructive method of analysis that takes advantage of the fact that no sample preparation is required prior to analysis and the fact that only a small sample size is required for measurement (30). Combined with chemometrics, ATR-FTIR can be used in many forensic applications, including the estimation of the age of bloodstains, the differentiation of various body fluids, and identification of car bumper components for brand identification in hit and run crime scenes (31–33).

1.3.3 DRIFTS

Diffuse Reflectance Infrared Fourier transform spectroscopy (DRIFTS) is another analytical infrared spectroscopy method that can be used for the analysis of forensic samples. DRIFTS is a traditionally non-destructive method of analysis, as the powdered sample is recovered at the end of analysis. Compared to transmission infrared spectroscopy, DRIFTS is highly sensitive and able to analyze samples with varying concentrations. DRIFTS is even capable of analyzing “neat” powders, without the need to

dilute a sample with KBr, or potassium bromide (34). Additionally, a very small sample size is needed in order to generate usable spectra; as small as 100 micrograms (μg) (35).

1.3.4 Chemometrics

Since the 1970s, chemometrics has been used in many scientific disciplines for pattern recognition. One of the primary uses of chemometrics was analytical data analysis of near infrared spectroscopy, (NIR) (36). However, chemometrics is also capable of analyzing data received from Mid IR and visible IR ranges, as well as, other analytical chemistry methods such as gas chromatography - mass spectrometry (GC-MS), and liquid chromatography - mass spectrometry (LC-MS) (37). Chemometric software is primarily utilized for Principal Component Analysis (PCA) (36). Principal Component Analysis data is generated through various algorithms, including, Non-Linear Iterative Partial Least Squares (NIPALS), or through a matrix algorithm, such as Singular Value Decomposition (SVD). These algorithms are used in order to interpret large amounts of data and find a common pattern among the dataset and create groups based on the components of the data (37).

PCA has many utilities, such as clustering, discrimination or differentiation, classification or identification, and discriminative power estimation (38). Clustering involves the grouping of samples based on their properties, such as in the grouping of illicit narcotics based on their elemental composition (38,39). Discrimination is the process of using chemometrics to separate samples, such as in the differentiation between menstrual blood and non-menstrual blood (38,40). Identification is primarily used to classify an unknown sample after training the software with known samples. Estimation of

discriminative power is particularly utilized in forensics in order to provide a numerical value in the instrumental technique's ability to differentiate a known and unknown sample that came from different sources (38).

1.4 Previous Research and Research Objectives

It has been established that infrared spectroscopy is a valued instrumental approach in forensics for the analysis of a wide range of sample types. When cigarettes are found at crime scenes, they may only be considered for the possibility of extracting a DNA profile. However, it may be possible, through the use of infrared spectroscopy and chemometrics to differentiate between various cigarette brands. One study aimed to differentiate between cigarette filters of various brands through analysis in the mid-IR range in an effort to create a database of cigarettes for the purpose of brand identification (41). This study aims to analyze various components of the cigarette, including the filter, the tobacco and the outer wrapping paper surrounding the filter in an effort to determine brand identification of cigarettes.

2. MATERIALS AND METHODS

2.1 Cigarette Brands

Various cigarette brands were utilized during the course of the experiments. Brands included American Spirit, Camel, Marlboro, Newport, and Parliament. Table 1 displays the cigarette brands, along with the flavor or type of cigarette, and the designation given during data analysis. Each cigarette brand was analyzed as an unsmoked cigarette and as a smoked cigarette. Figure 2 depicts the cigarette brands chosen for this research.

Table 1. Cigarette Brands. Information about samples chosen, including cigarette brand, flavor or type, parent company, and designation.

Cigarette Brand	Flavor/Type	Parent Company	Designation
American Spirit (AM)	Light Blue (LB)	Reynolds American	AM LB
Camel (CM)	Blue (B)	R.J. Reynolds Tobacco Company	CM B
Marlboro (MB)	Red (R)	Phillip Morris International	MB R
Newport (NP)	Green (non-menthol) (G)	R.J. Reynolds Tobacco Company	NP G
Parliament (PR)	Blue (B)	Phillip Morris International	PR B



Figure 2. Cigarette Brands. An image of the cigarette brands, with their boxes and respective cigarettes below, chosen for this research. From left to right, American Spirit Light Blue, Camel Blue, Marlboro Red, Newport Non-menthol, Parliament Blue.

A digital caliper (General Tools and Instruments, Secaucus, N.J) was used to take measurements of the unsmoked cigarette filter length and outer diameter to gather information about class characteristics and note if any similarities or differences among brands was noticed. Three cigarettes from each pack were chosen at random and measured. Table 1 and 2 in the results and discussion section displays the measurements. Parliament cigarettes contain a paper wrapping around the cigarette filter. The paper was left on during these measurements, as cutting and removing the paper would result in the filter unraveling and affected the measurements.

To simulate the action of smoking a cigarette, the cigarettes were inserted into 2 milliliter (mL), disposable syringes. The handmade apparatus was then taken to the fume

hood, where the end of the cigarette was lit with a match. While the match was lit, the plunger was pulled outward, to light the cigarette. The plunger was then pushed in and out repeatedly, to create suction. The cigarette was “smoked” by the apparatus until it had been consumed down to the butt of the cigarette, which was designated as the area where the cigarette paper color changed from white to brown. In the case of Parliament cigarettes, since the entirety of the cigarette is white, the cigarette was smoked down to where the paper appeared to change between the slightly transparent wrapping paper, and the thicker paper that was wrapped around the cigarette filter.

The cigarette was then extinguished and brought over to the sample prep benchtop for analysis. While all other cigarettes were able to be smoked with no modifications to the cigarette, the filter in the Parliament cigarettes was recessed, so approximately 3 millimeters (mm), was cut from the butt of the cigarette to ensure that the filter was flush with the plunger. This allowed for proper airflow, and without this step, the Parliament cigarettes did not properly light with the apparatus. The smoking apparatus is depicted in Figure 3.



Figure 3. Image of Handheld Smoking Apparatus. (Left) A Marlboro Red cigarette, unsmoked, inserted into the handheld smoking apparatus; and (Right) the same cigarette, now consumed down to the butt, still inserted into the handheld smoking apparatus.

2.2 ATR Infrared Analysis

A Nicolet 6700 Fourier Transform Infrared Spectrometer (Thermo Electron Corporation, Waltham, MA) with OMNIC™ software was utilized, with each sample being analyzed with 64 scans. An Attenuated Total Reflectance attachment, a transmission attachment, and a DRIFTS attachment were utilized with the IR instrument. No spectral correction in the experimental setup was added to any of the experiments.

The cigarette filters along with the cigarette paper that is located at the cigarette butt were deposited directly onto the ATR attachment. The ATR diamond cell and probe were cleaned with sterile alcohol prep pads (Fisher Scientific, Waltham, MA) prior to each sample being analyzed.

Sterilized scalpels and tweezers were used to gather five samples from each smoked and unsmoked cigarette. The cigarette paper contained two sides, designated side A, the

outside, and side B, the inside. In most cigarettes, side A is the brown side, while side B is the white part that is in direct contact with the cigarette filter. However, it should be noted both sides of the Parliament cigarette paper are white. Additionally, Parliament cigarettes have areas along the paper of the cigarette butt of varying thickness and construction, that resulted in inconsistent results and significant noise. In order to prevent this, an area was designated on the Parliament paper to collect ATR – FTIR data. Due to the thickness of the paper itself, sterilized scissors were used to cut these samples, rather than a sterilized scalpel. Figure 4 displays the area chosen for sampling.

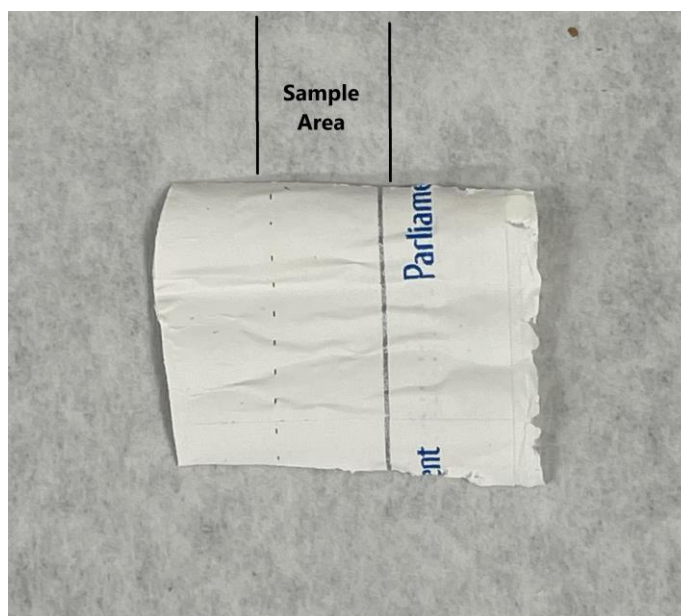


Figure 4. Image of Parliament Sampling Area. Parliament cigarette paper, cut and removed from the cigarette, with markings to designate the sampling area.

2.3 Nicotine Extraction Procedure

2.3.1 Transmission Infrared Analysis

This procedure involved the extraction of nicotine, an alkaloid found in tobacco leaves, from the smoked cigarette filters. Since nicotine is soluble in many solvents, including alcohols, ether and kerosene, this extraction procedure involved the use of methanol (42).

The cigarette was smoked as normal with the handheld apparatus. Once it had been smoked down to the cigarette butt, the filter was detached from the surrounding paper and placed into a 4 mL black top, disposable, glass vial. Two mL of high-performance liquid chromatography (HPLC) grade methanol (Fisher Scientific Waltham, MA) was pipetted into the vial with the filter. The vial was then placed onto a Speci-mix rocker (Barnstead Thermolyne, Ramsey, MN) for 20 minutes. After the elapsed time, the vial was removed.

Polyethylene IR sample cards (International Crystal Laboratories, Garfield, NJ) with two sample areas, A and B, were used with the transmission attachment. Fifty μL of methanol were pipetted onto side A and allowed to dry, to be scanned for the background. Fifty μL of the sample extract were then pipetted onto side B and allowed to dry. This amount of liquid initially caused the sample to spread farther than the polyethylene sample area, so the amount was reduced to 30 μL .

Ultimately, this sampling method proved unviable for detection, and this portion of the experiment was discarded and replaced with DRIFTS analysis due to sensitivity concerns. While the instrument was capable of detecting methanol during the background scan, the instrument was not able to detect any of the sample extracted.

2.3.2 DRIFTS Analysis – Filter Extraction

The cigarettes were smoked in the same fashion as all previous trials. The filter was removed and placed into a black top, disposable, glass vial. It was important at this step to ensure that the filter was not damaged in any way, as this would cause it to absorb all the added liquid. To ensure that the filter was not damaged, sterilized scissors were used rather than a scalpel to remove the filter paper. The vial was filled with 1.25 mL of HPLC grade methanol and placed on the rocker for 20 minutes. For the Newport cigarettes only, 1.5 mL of methanol was added to the vial. This was because the Newport filters tended to unravel when removing the outer paper, and all of the initial 1.25 mL of methanol was soaked up. To ensure that the cigarette filter did not soak up all available liquid, an additional 250 μ L of methanol was added.

One hundred and fifty microliters of methanol were added to a crucible (Fisher Scientific, Waltham, MA), along with a pea sized amount of potassium bromide, or KBr (BDH Chemicals, Radnor, PA). The sample was mixed with the bottom of a disposable culture test tube (DWK Life Sciences, Millville, NJ) until the sample had homogenized into a fine powder. This powder served as the sample for the background scan. One micro DRIFTS cup was created to serve as the background for the five replicates.

One hundred and fifty μ L of the sample extract were added to separate crucibles, along with a pea sized amount of KBr, and mixed with the bottom of a disposable culture test tube. A portion of the powder was added to a clean micro DRIFTS cup with the aid of a funnel, and placed into the DRIFTS apparatus to be analyzed.

2.3.3 DRIFTS Analysis – Tobacco Extraction

The cigarettes were all smoked as previously described utilizing the plunger syringes. To ensure there was enough tobacco for analysis, a visual target area was designated for all cigarettes. The cigarette butt was to be approximately 30 mm in length. Figure 5 indicates the visual target area for all smoked cigarettes.



Figure 5. Image of Target Area for Smoked Cigarettes. A visual identifier of where each cigarette should be smoked to. Before smoking, each cigarette was compared to the area written on the bench paper.

Once the cigarette had been smoked in the smoking apparatus down to the measured amount, the cigarette butt was cut open. The remaining tobacco contents were measured on a weighing balance (Denver Instrument, Bohemia, NY) in a weigh boat. After the remaining tobacco was measured, approximately 20 mg from each cigarette was placed

into a 4 mL vial. Table 2 shows how much tobacco was remaining after smoking, and how much was used.

Table 2. Tobacco Measurements used in Extraction. Recording of how much tobacco was remaining in each cigarette after smoking, and how much was used in the extraction. Note: Acronyms used in column 1 are defined within Table 1.

Cigarette	Tobacco Left Over (mg)	Tobacco Used in Extraction (mg)
American Spirit Light Blue (AM LB)	96.2	19.5
Camel Blue (CM B)	50.8	19.6
Marlboro Red (MB R)	94.9	20.1
Newport Green (NP G)	119.6	20.4
Parliament Blue (PR B)	55.2	20.3

Five hundred μL of methanol were pipetted into each of the vials containing samples. Each sample was put on a rocker for 20 minutes. Once the 20 minutes elapsed, the samples were removed from the rocker, and taken to the benchtop for DRIFTS analysis. Due to the presence of solid plant matter that had settled on the bottom of the vial, liquid was carefully collected from the top to ensure no particulates were collected with the sample. One hundred and fifty μL of the extract was pipetted into a crucible and a pea sized amount of KBr powder was deposited into the crucible. The mixture was macerated with the bottom of a culture tube until the sample had become a homogeneous powder. A small amount of sample was removed from the crucible and deposited into the DRIFTS

sample cup for analysis. Background scans were collected with a sample created from 150 μL of the same methanol used in the extraction and KBr powder.

2.4 Chemometrics

In order to analyze the spectral data, Mass Mountaineer™ software, version 7.01.5.0, developed by Diablo Analytical, Inc., (© R.B. Cody, 2022) was utilized. While Mass Mountaineer™ is primarily utilized for mass spectral data analysis, the software was only used in this case to generate two dimensional, (2D) and three dimensional, (3D) Principal Component Analysis plots. Plots were generated for comparison between the smoked cigarette brand components as well as comparison between the unsmoked cigarette brand components. PCA plots were also generated for comparison among the smoked cigarette filter extracts and smoked cigarette tobacco extracts.

3. RESULTS AND DISCUSSION

3.1 Cigarette Class Characteristics

As discussed in the materials and methods section, measurements of the filter length and filter outer diameter were taken. The values are recorded in Tables 3 and 4.

Table 3. Cigarette Filter Lengths in Millimeters. The lengths of three cigarettes selected from the box, including the average length. Note: Acronyms used in column 1 are defined within Table 1.

Filter Length (mm)	1	2	3	Average Length
AM LB	22.93	22.81	22.84	22.86
CM B	25.87	25.88	25.93	25.89
MB R	18.42	18.57	18.46	18.48
NP G	20.66	19.94	19.96	20.18
PR B	19.68	19.71	19.67	19.68

Table 4. Cigarette Filter Outer Diameters in Millimeters. The outer diameters of three cigarettes randomly selected from the box, including the average outer diameter. Note: Acronyms used in column 1 are defined within Table 1.

Outer Diameter (mm)	1	2	3	Average Length
AM LB	6.62	6.88	6.70	6.73
CM B	6.50	6.51	6.51	6.50
MB R	6.64	6.65	6.65	6.64
NP G	6.43	6.35	6.44	6.40
PR B	6.20	6.20	6.20	6.20

The American Spirit Light Blue filters tended to be the thickest by a small margin, while the Parliament Blue filters tended to be the thinnest. The results for the Parliament

filters were also the most consistent among the cigarette filters, which may have to do with the thin filter paper that covers the filter and prevents unraveling when the outer paper layer is removed. Contrary to this, Newport filters tended to unravel the most when the outer paper layer was cut off, which made taking reproducible measurements difficult, due to the soft nature of the filter, and concern of the filter itself being compressed to read an inaccurate result.

While the filters among brands varied little in outer diameter, there was significantly more variation among the lengths of the filters. Camel Blue filters were the longest and thickest amongst the cigarette brands. This was evident during testing as well, as the filter would burn if the cigarette was smoked down to what was considered the cigarette butt for this portion of the experiment. Marlboro Red filters tended to be the shortest filter, and when the cigarette was smoked down to the butt, there would be a significant amount of ash as well as partially burnt and unburnt tobacco left over within the wrapper, compared to other brands such as Parliament Blue and Camel Blue, as seen in Table 2 in the materials and methods section.

3.2 Smoked and Unsmoked Cigarette Comparison

3.2.1 Unsmoked Cigarettes

Once all of the replicates were taken, the spectra were analyzed visually, to observe if there were any visual differences among the different brands. Figure 6 depicts a representative spectrum of each of the brands.

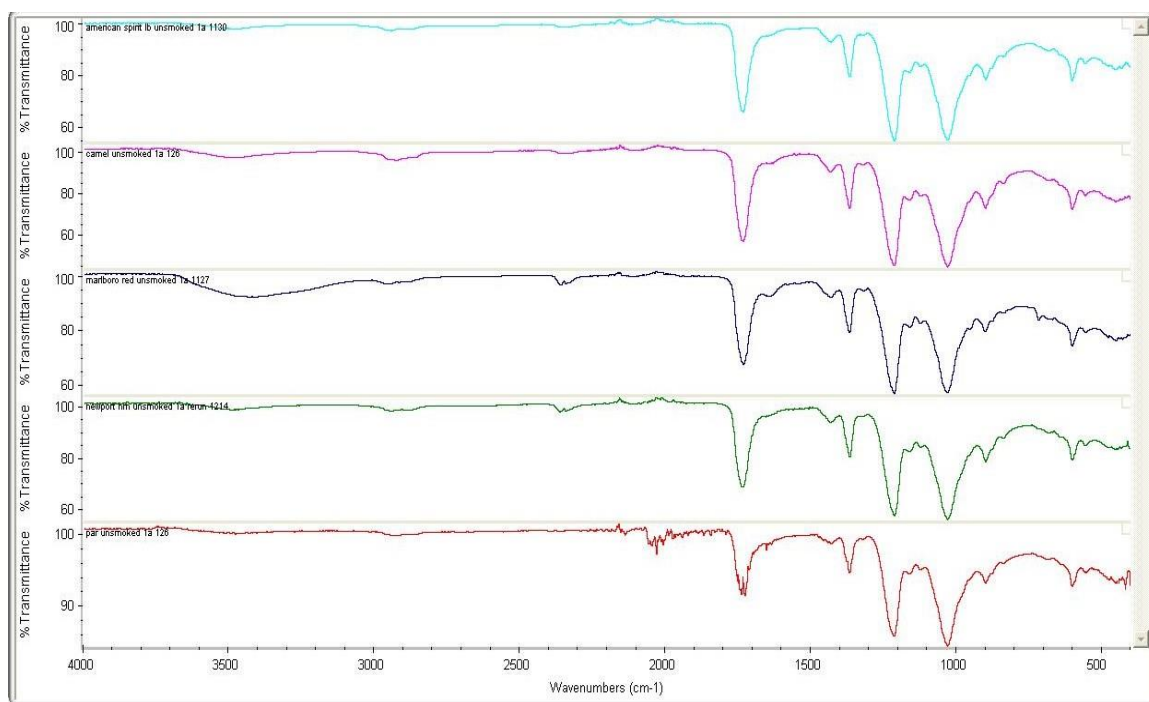


Figure 6. Infrared Spectra of the Unsmoked Cigarette Filters. Generated with OMNIC™ software. From top to bottom; American Spirit Light Blue, Camel Blue, Marlboro Red, Newport Green, and Parliament Blue. The Y axis of each spectra represents the % Transmittance, while the X axis depicts the wavenumbers in cm^{-1} .

Visually, there were no differences amongst the cigarette brands, apart from some peaks between 2200 and 1600 cm^{-1} in the Parliament Blue spectra, which may be attributed to noise from the instrument. There was a slight difference among Marlboro Red as well, as an additional peak was slightly visible, around 1600 cm^{-1} . Since the spectra looked

visually similar between all cigarette brands, Mass Mountaineer™ software was used in order to mathematically analyze the data.

Principal Component Analysis 3D and 2D plots were generated with the Mass Mountaineer™ software to evaluate any correlations among the data. Figures 7 and 8 depict the PCA 3D and 2D Class plots of the unsmoked cigarette filters, respectively.

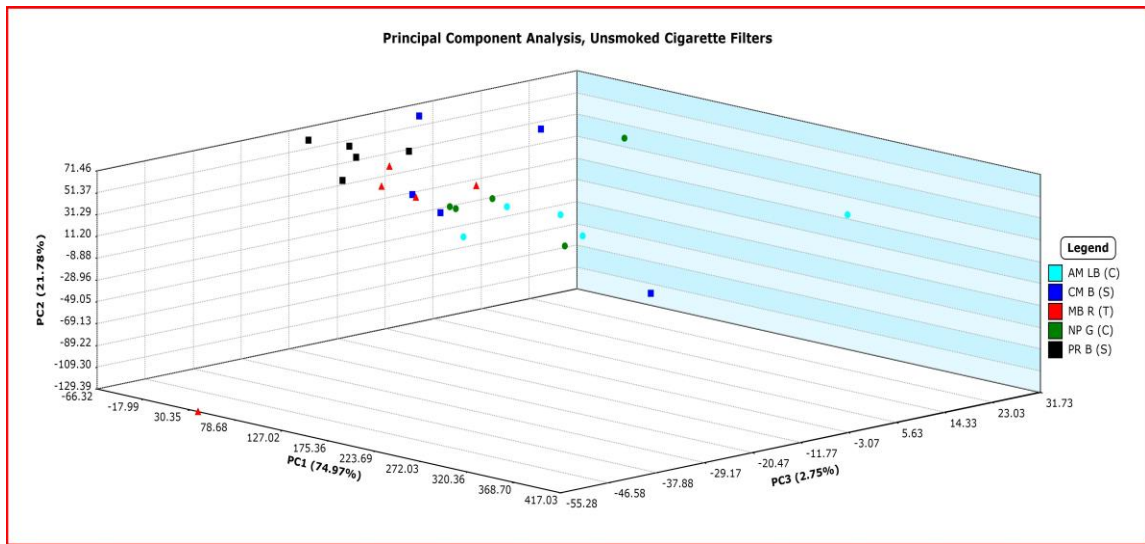


Figure 7. 3D Principal Component Analysis, Unsmoked Cigarette Filters. Generated with Mass Mountaineer Software™ version 7.01.5.0.

There appeared to be a large spread of the data among some brands as seen in figure 7, including the Camel Blue, and the American Spirit Light Blue. However, other brands such as Parliament Blue and Marlboro Red clustered more closely together. Newport Green cigarettes, had some data points that are relatively close together, with some outliers

present. Overall, figure 7 depicted the trend that a majority of the datapoints from the unsmoked cigarette filters tended to cluster together in the 3D space.

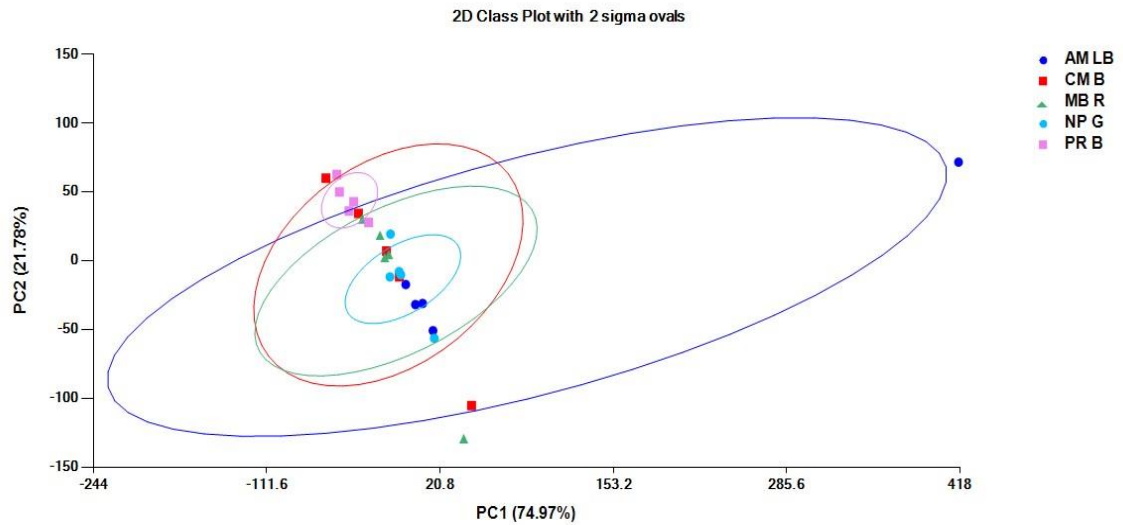


Figure 8. 2D Principal Component Analysis, Unsmoked Cigarette Filters. Generated with Mass Mountaineer Software™ version 7.01.5.0.

The similarities amongst the brands seen in figure 7 were evident from the 2D PCA plot generated from the data, represented in figure 8. The datasets generally overlapped with each other, which can be expected as most cigarette filters are all made of cellulose acetate. However, certain brands have a smaller range of variation, such as Parliament Blue and Newport Green, where they appeared to be clustered closer together, compared to other brands such as American Spirit Light Blue and Camel Blue.

Although the data represented in figure 8 cannot be definitively used for brand differentiation, the tightness of some of the ovals generated could potentially aid in discrimination. Parliament Blue and Newport Green ovals did not overlap, and there may be potential for brand differentiation between these two cigarettes. Newport and Parliament cigarettes are both owned by different companies, so there may be some difference in the

manufacturing of the filter between companies that would change the infrared spectra generated. There was some slight overlap between Marlboro Red and Parliament Blue, which could lead to the potential for differentiation between these two cigarettes. With more replicates, it may be possible to completely isolate the two ovals.

Mass Mountaineer™ software allows users to select a certain range of data to generate 3D and 2D PCA plots. To see if it was possible to distinguish between certain brands further, the data was restricted to the “fingerprint region” of IR, and Mass Mountaineer™ only included data points that fell within 600 and 1400 cm⁻¹. These plots are represented in figures 9 and 10.

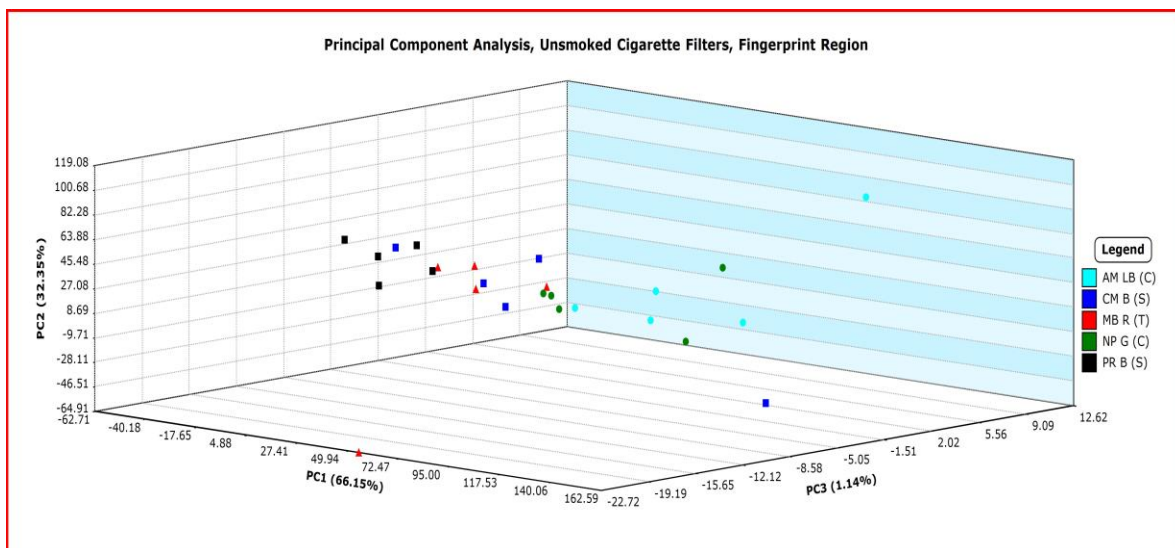


Figure 9. 3D Principal Component Analysis, Unsmoked Cigarette Filters, Fingerprint Region. Generated with Mass Mountaineer™ Software version 7.01.5.0.

Compared to figure 7 where the full range of data was included, the data in figure 9 appeared to be clustered more closely together, with very few outliers present. Brands such as Parliament Blue and Marlboro Red were similarly clustered closely together, while brands such as Camel Blue and American Spirit Light Blue were spread further apart.

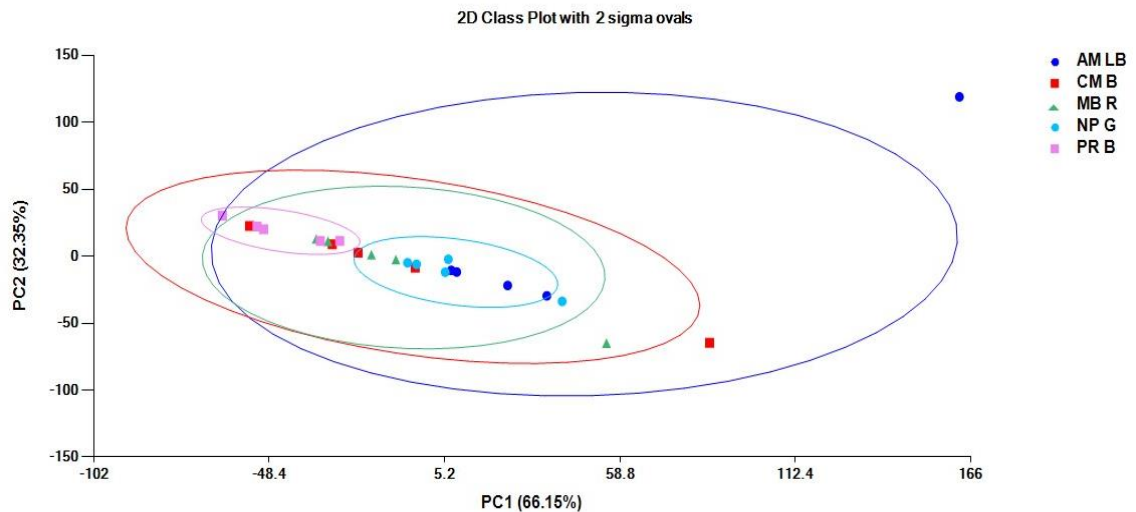


Figure 10. 2D Principal Component Analysis, Unsmoked Cigarette Filters, Fingerprint Region. Generated with Mass Mountaineer™ Software version 7.01.5.0.

The findings noted above, in figure 10, were represented in the ovals generated with the 2D PCA plot. Camel Blue and American Spirit Light Blue had the largest distribution of data, resulting in their ovals fully encompassing all other brands. This is comparable to figure 8, where the ovals for Camel Blue and American Spirit Light Blue encompassed all

other brands as well. However, as seen in the PCA plot generated in figure 10, American Spirit Light Blue had the largest distribution of data among all other brands.

Although the data was restricted to include only the fingerprint region, in an effort to differentiate brands, it had only reinforced the previously established notion that only Parliament Blue and Newport Green are capable of differentiation from each other.

Similar 3D and 2D Principal Component Analysis plots were generated for the unsmoked cigarette papers, sides A and B. Figures 11 and 12 depict the 3D and 2D PCA plots of side A of the unsmoked cigarette papers, respectively.

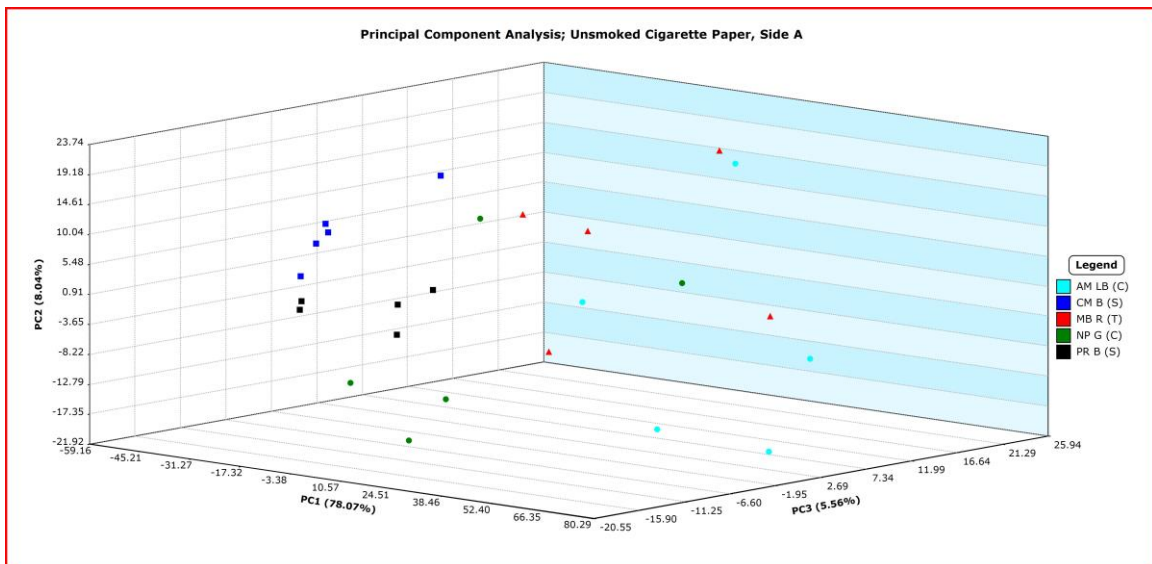


Figure 11. 3D Principal Component Analysis, Unsmoked Cigarette Paper, Side A. Generated with Mass Mountaineer™ Software version 7.01.5.0.

Similar to the data collected from the unsmoked cigarette filters, in figure 7, certain brands tended to cluster together, as seen in figure 11. As previously seen among the unsmoked cigarette filters, the Parliament Blue cigarette paper data points were relatively close together, while other brands were further apart. However, unlike the previous dataset in figure 7, Camel Blue cigarettes lacked a distinct outlier. Additionally, brands such as

Newport Green, Marlboro Red and American Spirit datapoints cover a large range in figure 11.

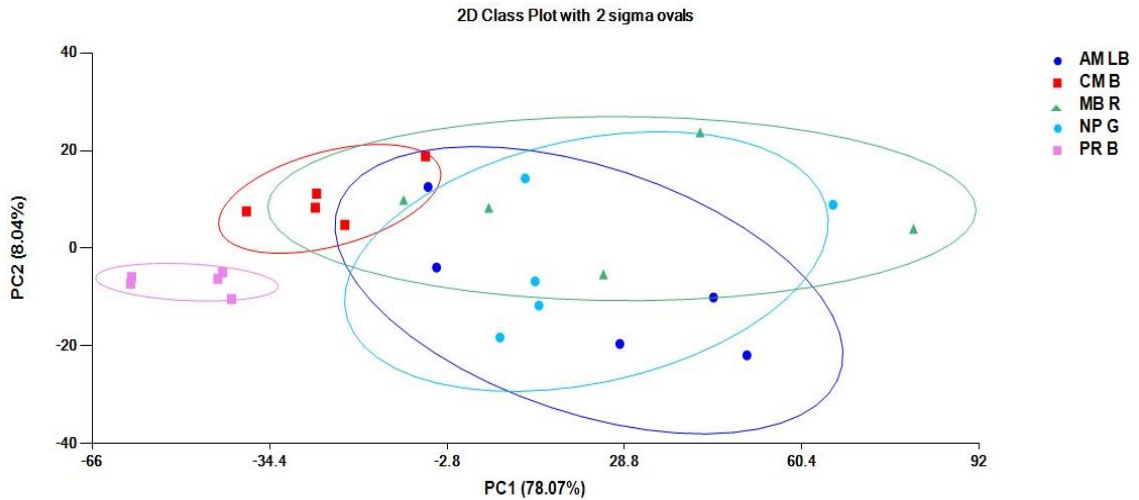


Figure 12. 2D Principal Component Analysis, Unsmoked Cigarette Paper, Side A. Generated with Mass Mountaineer™ Software version 7.01.5.0.

Similar to the data reflected in figure 11, certain brands covered a wide range. This was seen again in the American Spirit Light Blue, Marlboro Red and Newport Green ovals of figure 12. There was significant overlap among these three brands in particular. Camel Blue intersected with the Marlboro Red and American Spirit Light Blue ovals, but did not intersect as greatly with the Newport Green oval. It is possible that, if more replicates were added, a case of discrimination could be made to distinguish between Camel Blue and Newport Green cigarette paper. It is interesting to note that although Newport and Camel are both owned by the same company, there may be slight differences in the specific material chosen for the cigarette line.

Parliament Blue remained in its own unique oval, and was differentiated from the other brands when analyzing the cigarette paper. However, this can also be visually

determined, as side A of the Parliament Blue cigarettes is white, while side A of the other cigarettes observed in this research is brown.

To conclude the data collected with the unsmoked cigarettes, 3D and 2D PCA plots were generated from side B of the unsmoked cigarette papers, depicted in Figures 13 and 14, respectively.

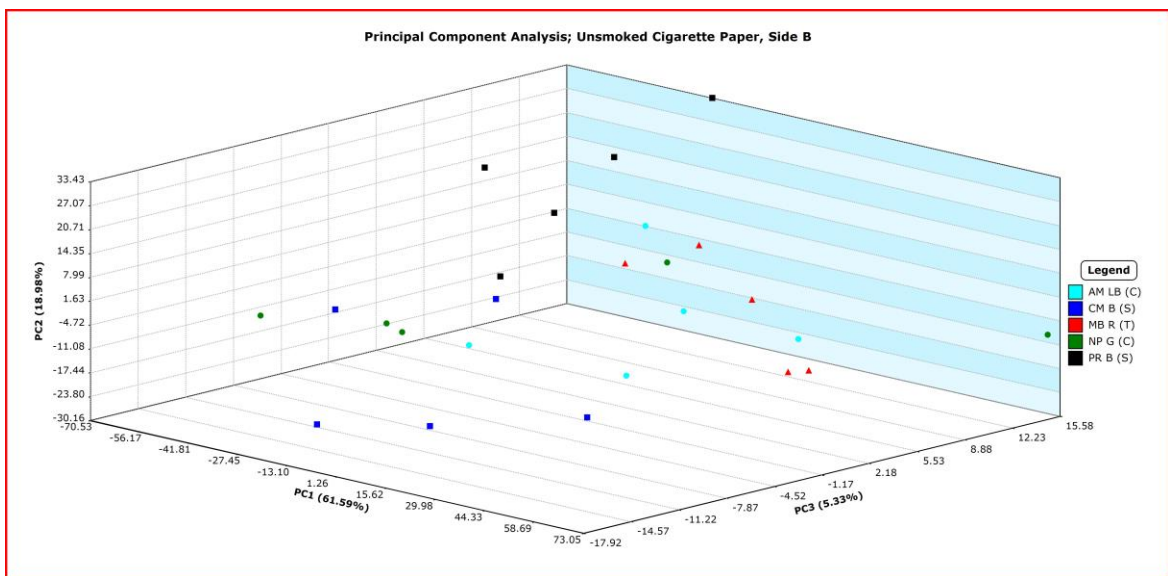


Figure 13. 3D Principal Component Analysis, Unsmoked Cigarette Paper, Side B. Generated with Mass Mountaineer Software™ version 7.01.5.0.

Compared to the 3D PCA plots generated from the other components of the unsmoked cigarettes as seen in figures 7 and 11, there was no distinctive grouping present among the individual data points of each brand in figure 13. Some data points among brands in figure 13 were visually located in closer proximity to each other than other brands, such as Parliament Blue and Camel Blue, which was consistently seen in the previous PCA plots in figures 7 and 11. However, other brands such as Marlboro Red and

American Spirit Light Blue tend to be closer in proximity in figure 13 than they were in figure 7.

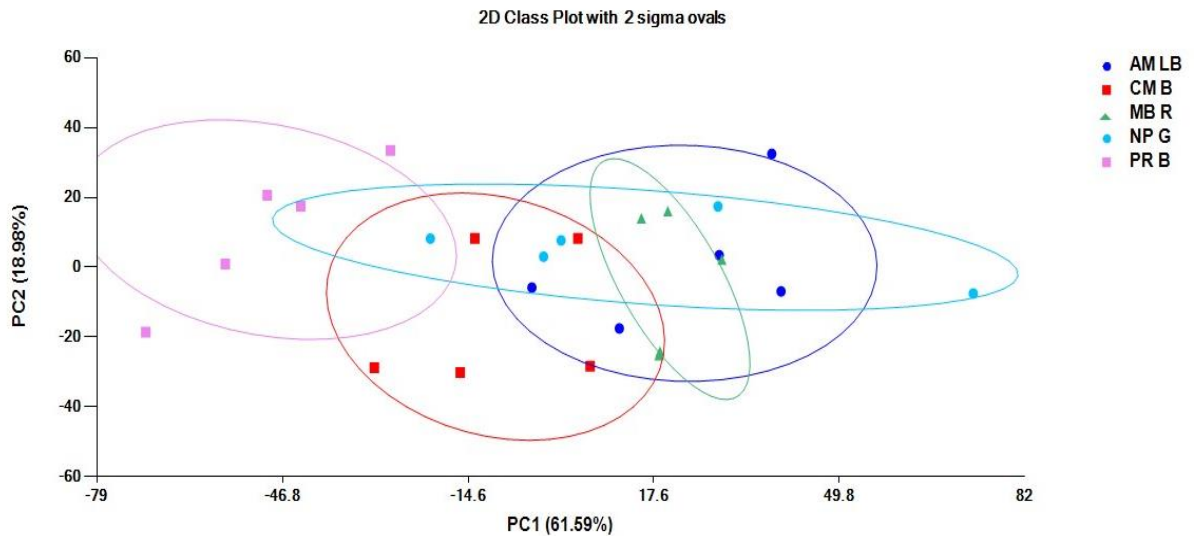


Figure 14. 2D Principal Component Analysis, Unsmoked Cigarette Paper, Side B. Generated with Mass Mountaineer™ Software version 7.01.5.0.

Unlike the 2D PCA plot generated for side A, represented by figure 12, there was no data set that was contained within its own oval in figure 14. However, unlike the data generated for side A, the Marlboro Red data points were in a smaller, tighter oval, and the Parliament Blue data points were located within a larger oval that overlaps with two other brands; Newport Green and Camel Blue.

As seen in figure 13, when the datasets of Marlboro Red and American Spirit Light Blue were represented in a 2D space, it was clear that these two brands are statistically

similar. This trend is similar to the trend observed in figure 12 where Marlboro Red and American Spirit Light Blue datasets overlapped as well.

There were some brands in figure 14 that appeared to not overlap with each other, being Parliament Blue with American Spirit Light Blue, and Parliament Blue with Marlboro Red. A case can be made that the data could potentially be used to differentiate Parliament Blue from these other brands. No data set was definitively different enough in this case for brand identification. Camel Blue and Marlboro Red overlapped slightly, which may help differentiation efforts in the future, should more replicates be taken to completely isolate the two datasets.

3.2.2 Smoked Cigarettes

As with the unsmoked filters, infrared spectra for the smoked filters were visually analyzed for any immediate differences amongst the cigarette brands. Figure 15 depicts a representative spectrum from each of the cigarette brands.

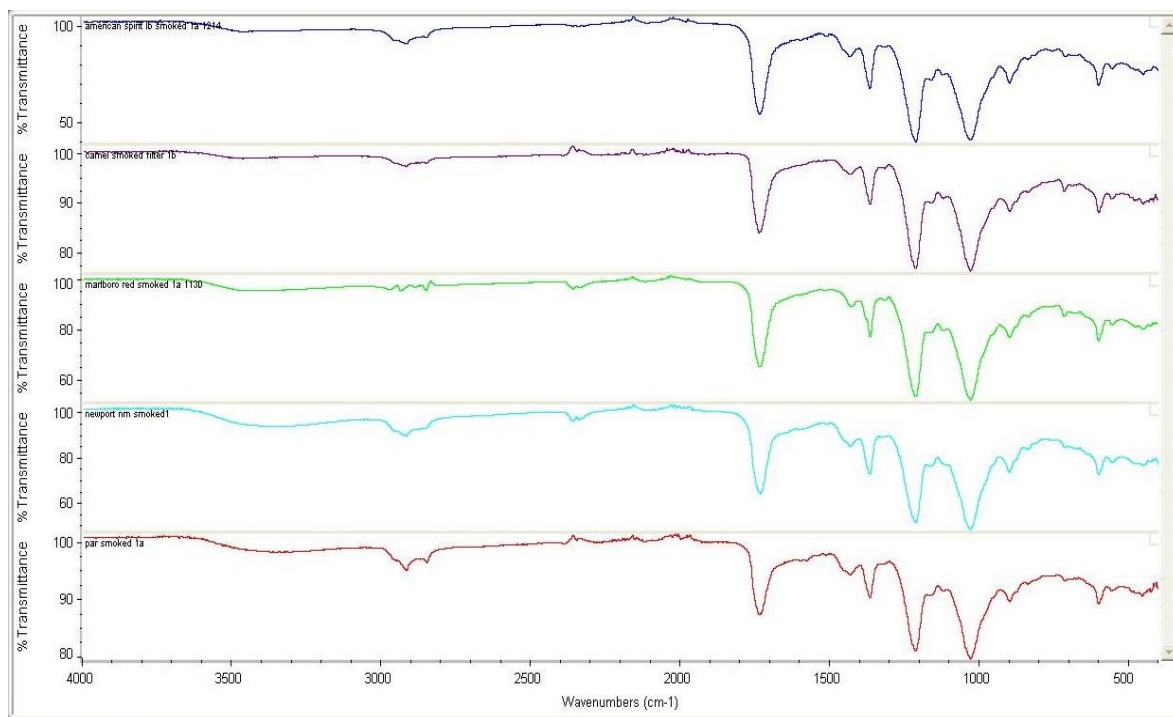


Figure 15. Infrared Spectra of the Smoked Cigarette Filters. Generated with OMNIC™ software. From top to bottom; American Spirit Light Blue, Camel Blue, Marlboro Red, Newport Green, and Parliament Blue. The Y axis of each spectra represents the % Transmittance, while the X axis depicts the wavenumbers in cm-1.

Similarly with the spectra of the unsmoked cigarette filters, represented in figure 6, no visual differences were noted between the spectra of the smoked cigarette filters. There seemed to be no visual difference between the spectra of the smoked cigarette filters and the unsmoked cigarette filters. Since each brand's spectrum looked similar, Mass Mountaineer™ software was employed to mathematically analyze the data.

The cigarette filters, as well as sides A and B of the smoked cigarettes were analyzed and 3D and 2D PCA plots were generated utilizing the Mass Mountaineer™ software. Figures 16 and 17 depict the 3D and 2D PCA plots generated for the smoked cigarette filters, respectively.

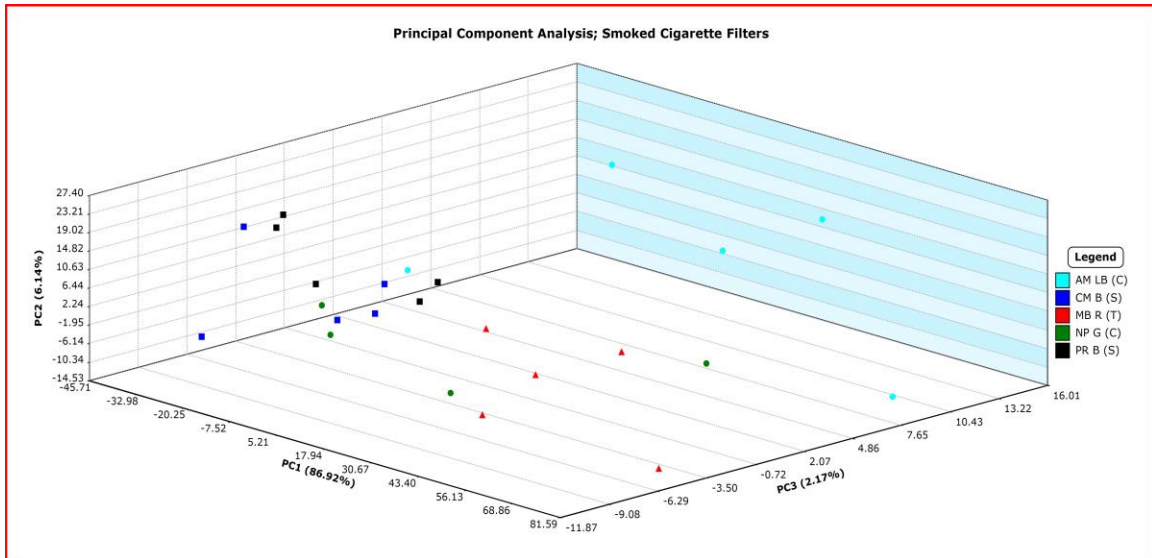


Figure 16. 3D Principal Component Analysis, Smoked Cigarette Filters. Generated with Mass Mountaineer™ Software version 7.01.5.0.

Similarly to the 3D PCA plot of the unsmoked cigarette filters in figure 7, there seemed to be no clear grouping among the data sets in figure 16. No one brand tended to cluster in a relatively close manner. The American Spirit Light Blue data sets seemed to be the most widespread amongst the 3D PCA plot. Other brands such as Camel Blue and Parliament Blue tended to cluster closer together, but not as close as in figure 7. Comparing the 3D PCA plot of the unsmoked cigarette filter data of figure 7 to the smoked cigarette filter data of figure 16, it appeared that the act of smoking differentiates the cigarette filter slightly, as the data points became more spread out.

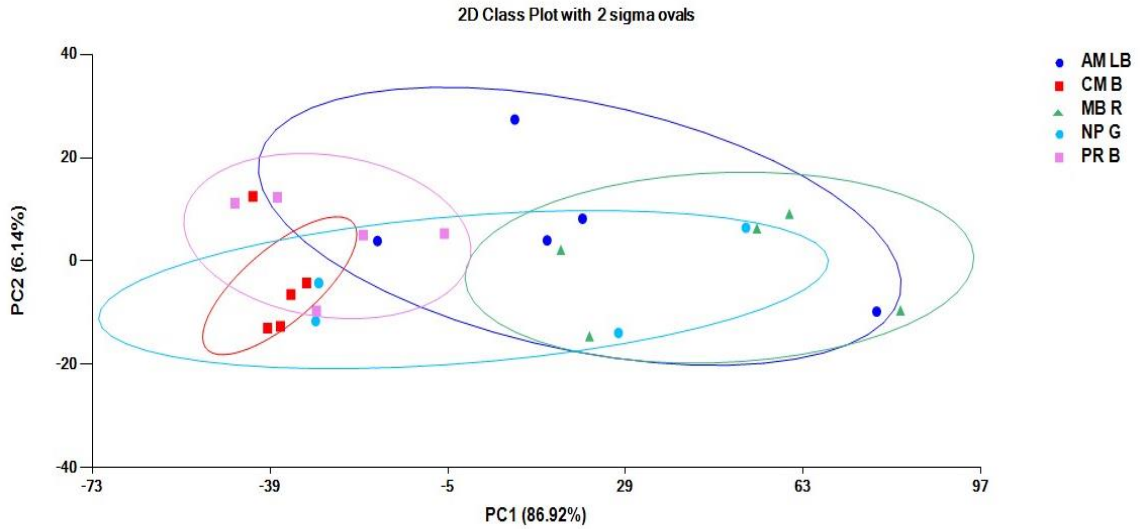


Figure 17. 2D Principal Component Analysis, Smoked Cigarette Filters. Generated with Mass Mountaineer Software™ version 7.01.5.0.

The findings above were seen in the 2D PCA plot generated, as the ovals in figure 17 did not completely stack on one another as they were in figure 8, but were more spread out. Again, American Spirit Light Blue seemed to be one of the widest spread data sets, as its oval encompassed every other cigarette brand. Newport Green also had a large oval, and encompassed all the other brands as well. There was not one brand that has its own distinctive oval, but there were some brands that did not intersect, being Camel Blue and Marlboro Red, and Parliament Blue and Marlboro Red.

Although Phillip Morris International owns both the Marlboro and Parliament cigarette line, it seemed that there were some differences among the cigarette filter when smoked that can make them distinguishable from the other. Camel and Marlboro cigarettes are manufactured by different brands, and the data set shows a clear distinction between

the Camel Blue and Marlboro cigarettes. This may make brand identification between these two cigarette filters possible when smoked.

Similar plots to figures 9 and 10 were generated with the Mass Mountaineer™ software, where the software was instructed to only include datapoints between 600 and 1400 cm⁻¹ in the calculations and generation of the PCA plots for the smoked cigarettes. The 3D and 2D PCA plots are depicted as figures 18 and 19, respectively.

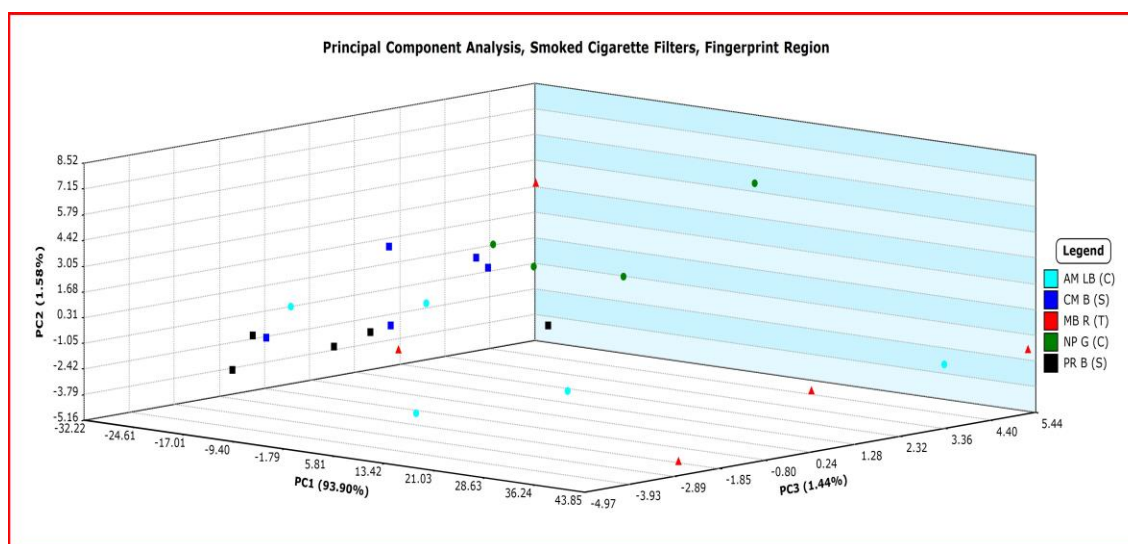


Figure 18. 3D Principal Component Analysis, Smoked Cigarette Filters, Fingerprint Region. Generated with Mass Mountaineer™ Software version 7.01.5.0.

Although the 3D PCA plot changed visually, compared to figure 16 where the entire dataset was included, the relationships between the data sets did not. Neither brand had data that was visually isolated from any other data. Instead, there appeared to be more outliers present when restricting the data between a certain range. Marlboro Red data points, in figure 18 became much more spread out, compared to figure 16.

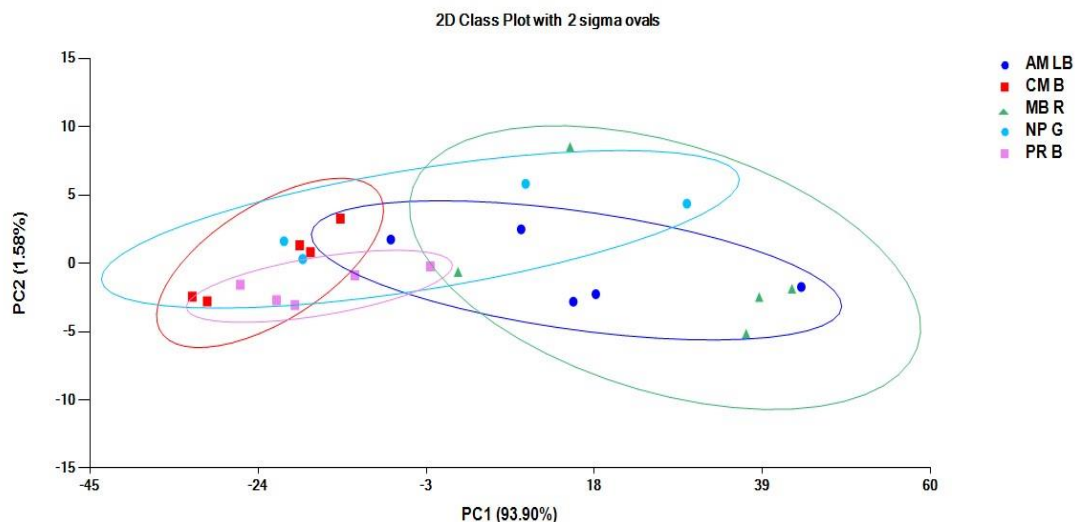


Figure 19. 2D Principal Component Analysis, Smoked Cigarette Filters, Fingerprint Region. Generated with Mass Mountaineer Software™ version 7.01.5.0.

Although the relationships between the datasets in figure 19 did not change, the sizes of the ovals generated for each brand did change, compared to figure 17. When the data was restricted to datapoints within 600 and 1400 cm^{-1} , the oval for American Spirit Light Blue shrunk, relative to all the others. The oval no longer overlapped with all of the other brands as it did in figure 17, but it did still intersect with the other brands. Camel Blue and Marlboro Red were still isolated and distinguishable from each other. However, when the data was isolated, rather than being distinguishable from each other, as seen in figure 17, there was a slight overlap between Parliament Blue and Marlboro Red, which may introduce some complications when trying to distinguish these two brands.

Ultimately, no definite change was seen among the datasets when the data was restricted to a certain region.

As with the unsmoked cigarettes, 3D and 2D PCA plots were generated for sides A and B of the smoked cigarette paper. Figures 20 and 21 below represent the 3D and 2D PCA plots of side A of the smoked cigarette paper, respectively.

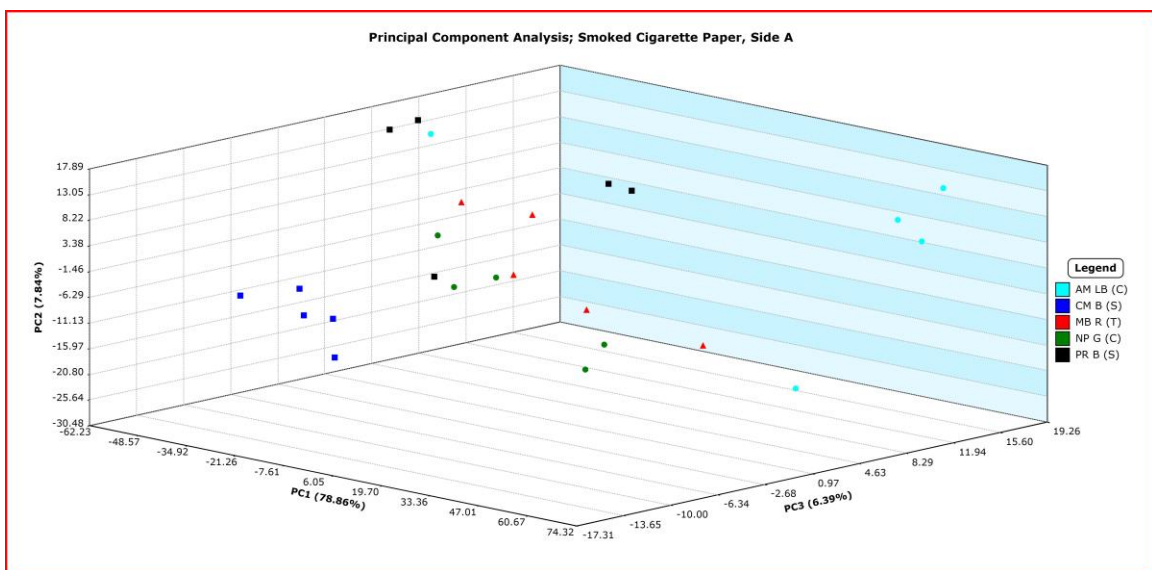


Figure 20. 3D Principal Component Analysis, Smoked Cigarette Paper, Side A. Generated with Mass Mountaineer™ Software version 7.01.5.0.

As with the data seen in figure 11, Camel Blue replicates tended to cluster closer together, where American Spirit Light Blue replicates were more spread out throughout the plot. Newport Green and Marlboro Red replicates clustered slightly closer together in figure 20, with some overlap between them. Parliament Blue datapoints almost remained

in their own group, but had an outlier that can be seen around Newport Green and Marlboro Red data points.

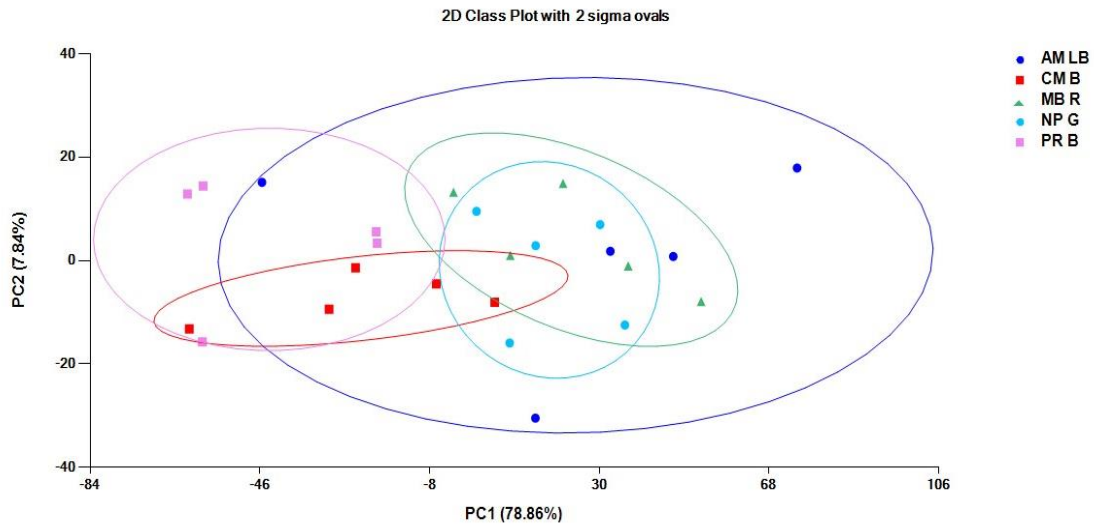


Figure 21. 2D Principal Component Analysis, Smoked Cigarette Paper, Side A. Generated with Mass Mountaineer™ Software version 7.01.5.0.

As with many other 2D PCA plots represented in this research, the American Spirit Light Blue oval encompassed all other brands tested. In figure 21, American Spirit Light Blue fully encompassed the Newport Green and Marlboro Red ovals. Thus, it would not be possible to differentiate between these 3, and brand identification is not possible.

Although hardly noticeable, there was a slight overlap between Parliament Blue and Newport Green. However, with more replicates, it may reveal that there is no overlap, which would make it possible to differentiate these two brands. The relationship between Parliament Blue and Newport Green continues to be apparent, as seen previously in figures

12, 14, 17 and 19. Parliament Blue data points overlapped greatly with other brands in figure 21 compared to figure 12, where they were contained in their own oval.

Three dimensional and two dimensional PCA plots were also generated for side B of the smoked cigarette papers. They are depicted in figures 22 and 23, respectively.

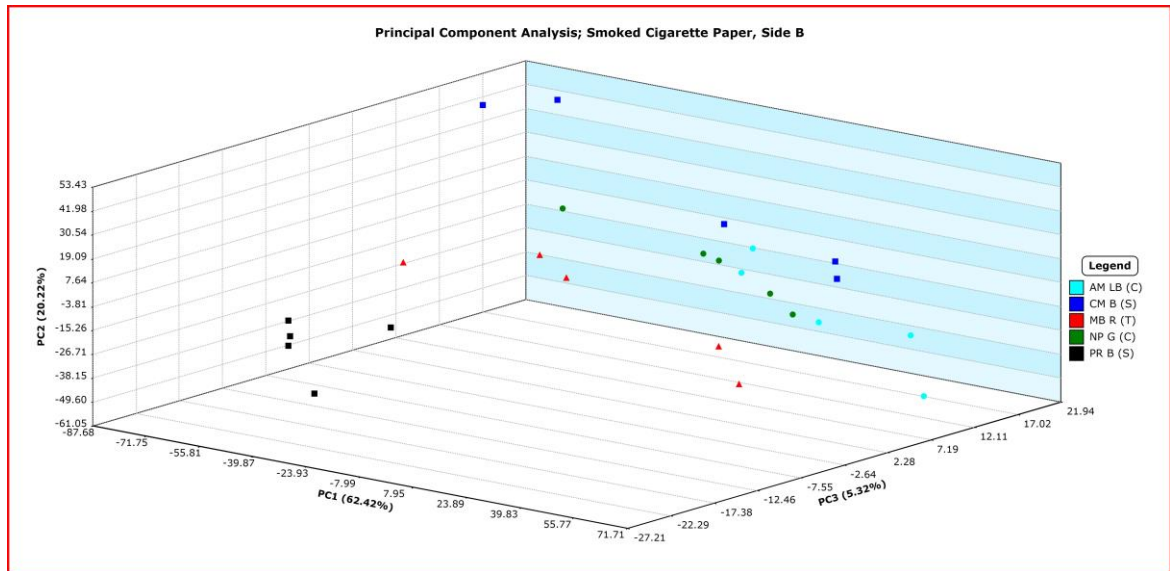


Figure 22. 3D Principal Component Analysis, Smoked Cigarette Paper, Side B. Generated with Mass Mountaineer Software™ version 7.01.5.0.

Parliament Blue replicates once again appeared to cluster together, which follows the trend of the previous 3D PCA plots seen in in this research. Compared to figure 13, which depicts side B of the unsmoked cigarettes, the brands depicted in figure 22 were dispersed over a smaller area, with more distinct groupings among the datasets represented in the 3D space. In particular, areas of overlap can be seen with the Newport Green, Camel Blue, American Spirit Light Blue data points.

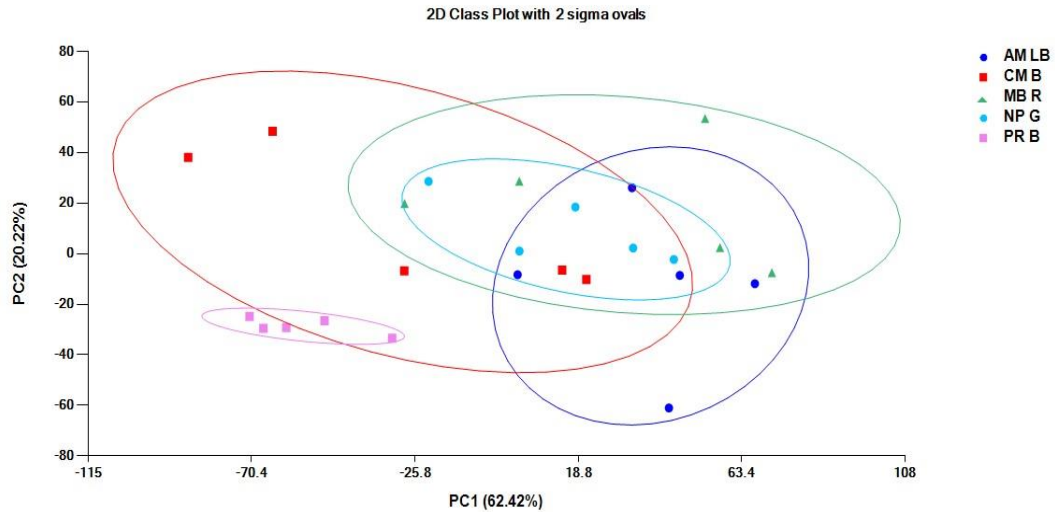


Figure 23. 2D Principal Component Analysis, Smoked Cigarette Paper, Side B. Generated with Mass Mountaineer™ Software version 7.01.5.0.

When looking at the 2D PCA plot generated from the data of side B of the smoked cigarettes it was evident that the ovals for the brands overlap with each other. Neither oval is fully encompassed within one larger oval, and instead, are partially encompassed by two or more brands.

This is the case with Newport Green, where it was encompassed within the Camel Blue, Marlboro Red and American Spirit Light Blue ovals. While Parliament Blue does not remain in its own isolated oval, it is only encompassed by one other brand, Camel Blue. This data can potentially be used to differentiate Parliament Blue from American Spirit Light Blue, Marlboro Red and Newport Green. Once again, although Parliament and Marlboro are manufactured by the same company, there may be a difference in materials used in the different cigarette lines.

3.3 Smoked Cigarettes Filter Extraction

Due to the variation among the cigarette lengths and widths as discussed in section 3.1, each cigarette filter was capable of absorbing a different volume of methanol. This resulted in a different level of liquid available to create the KBr powder. However, a consistent amount of sample extract, 150 μL , was used to create the powder for DRIFTS analysis of the smoked cigarette filter extraction. Although measures were taken to ensure the volume of sample extract was consistent for each sample, each sample was a different concentration.

Before mathematical analysis of the data was conducted, a visual analysis of the infrared spectra among the brands was performed. Figure 24 depicts representative spectra from the extract of each of the cigarette brand's filters.

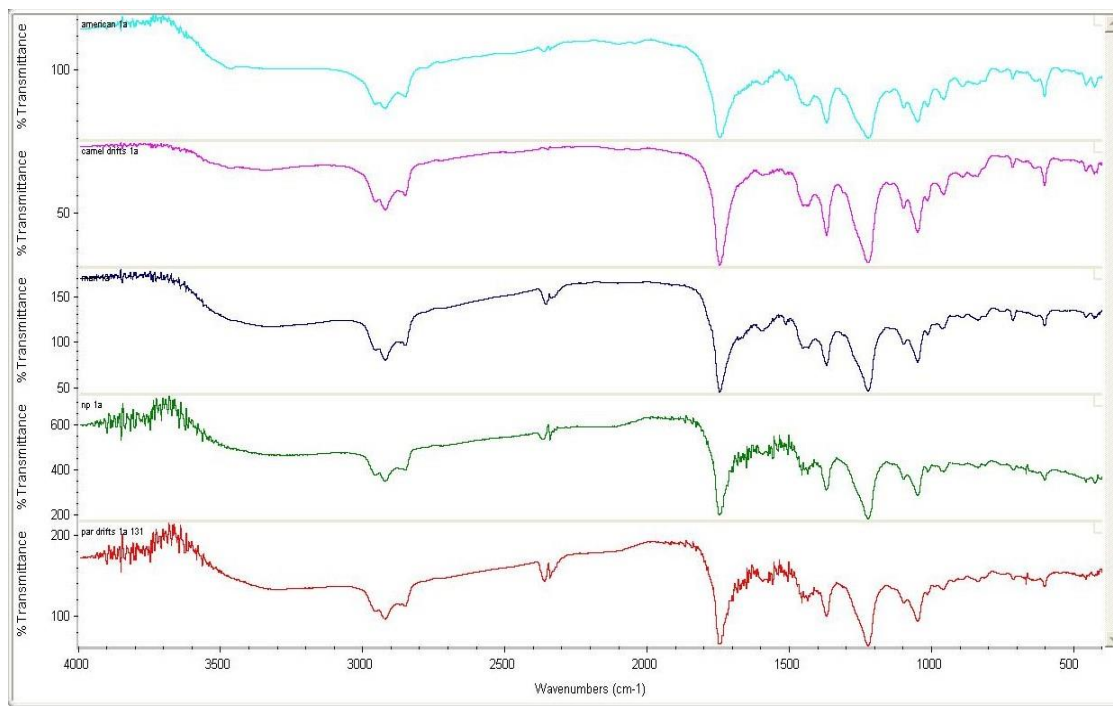


Figure 24. Infrared Spectra of the Smoked Cigarette Filter Extraction. Generated with OMNIC™ software. From top to bottom; American Spirit Light Blue, Camel Blue, Marlboro Red, Newport Green, and Parliament Blue. The Y axis of each spectra represents the % Transmittance, while the X axis depicts the wavenumbers in cm-1.

Ultimately, aside from differences seen in the % Transmittance, there were no visual differences noted between any of the extractions. Thus, Mass Mountaineer™ was employed to mathematically analyze the data.

Three dimensional and two dimensional PCA plots were generated for the data from the smoked filter extraction. They are represented in figures 25 and 26, respectively.

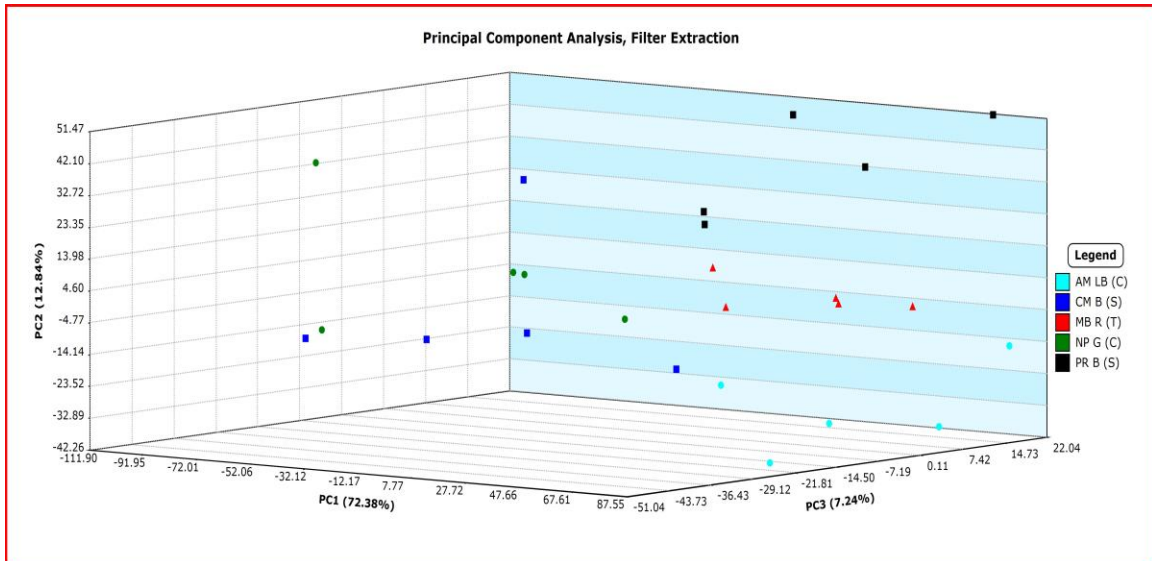


Figure 25. 3D Principal Component Analysis, Filter Extraction. Generated with Mass Mountaineer™ Software version 7.01.5.0.

Much like the other data sets represented by 3D PCA plots, the data points were highly spread out throughout the graph. However, there were areas in figure 25 where the individual brands tend to cluster, as seen with Newport Green and Parliament Blue. Camel Blue and American Spirit Light Blue showed some slight overlap of the data. Compared to datasets such as figures 16 and 18 which depict the data collected from direct analysis of the smoked cigarette filters, American Spirit Light Blue data points did not demonstrate a large spread of datapoints in the 3D space in figure 25.

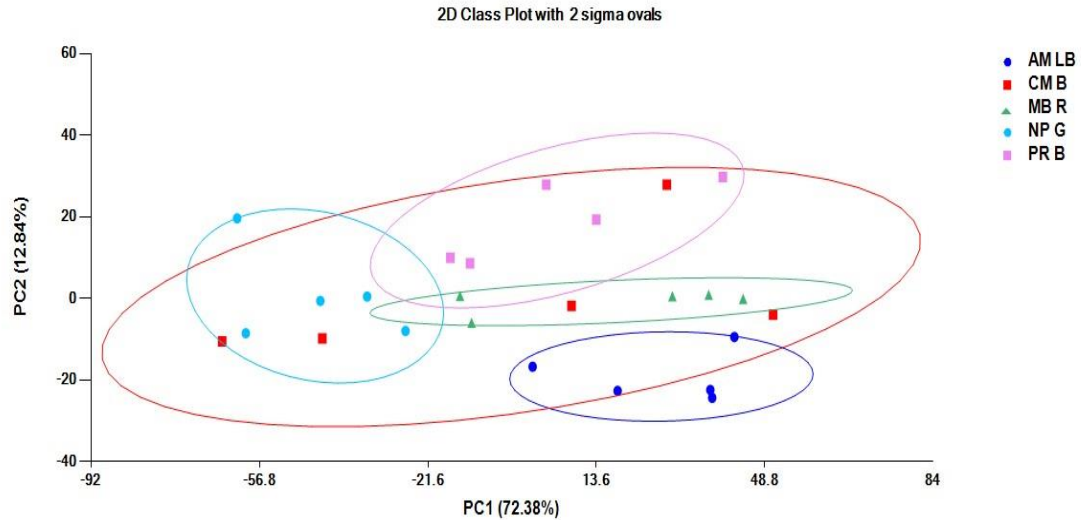


Figure 26. 2D Principal Component Analysis, Filter Extraction. Generated with Mass Mountaineer™ Software version 7.01.5.0.

While American Spirit Light Blue tended to have the largest oval generated in previous 2D PCA plots, figure 26 shows a different trend. Instead, Camel Blue had the largest oval generated and encompassed all the other brands. This makes differentiation between Camel Blue and all other cigarette brands using this data impossible. Although American Spirit Light Blue was encompassed by the larger Camel Blue oval, American Spirit was isolated in its own unique oval from three other brands; Marlboro Red, Parliament Blue and Newport Green. Thus, brand differentiation may be possible between American Spirit Light Blue and Parliament Blue, Newport Green, and Marlboro Red.

Although Marlboro Red intersected with Newport Green and Parliament Blue, the area of intersection among these three ovals may be small enough to distinguish between these brands if more replicates were to be taken in future research.

3.4 Smoked Cigarette Tobacco Extraction

Each tobacco extraction utilized the same volume of liquid extract for DRIFTS analysis, which would ensure that the concentration remained the same for each sample. Due to the tendency of the tobacco to stick to the side of the vial, the use of a filter for the pipette was not necessary to ensure that large amounts of plant matter were not included in the sample.

The infrared spectra generated from the tobacco extraction were visually analyzed to note any immediately discoverable differences among the cigarette brands. Figure 27 depicts the infrared spectra from the tobacco extraction.

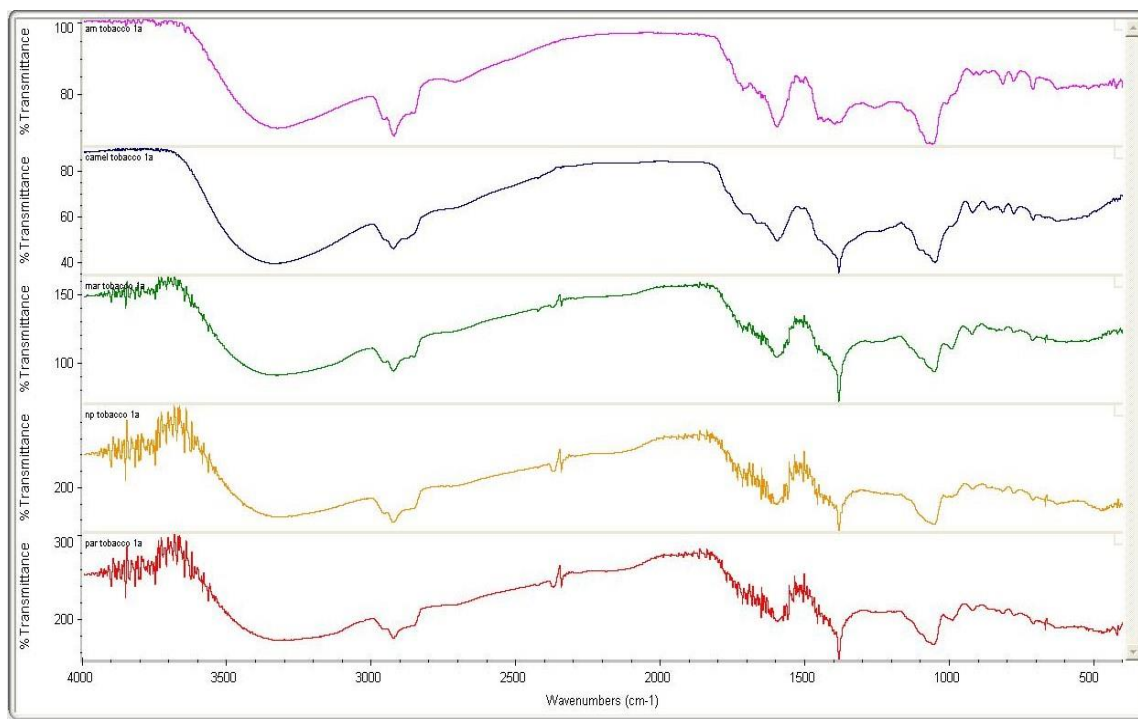


Figure 27. Infrared Spectra of the Smoked Cigarette Filter Extraction. Generated with OMNIC™ software. From top to bottom; American Spirit Light Blue, Camel Blue, Marlboro Red, Newport Green, and Parliament Blue. The Y axis of each spectra represents the % Transmittance, while the X axis depicts the wavenumbers in cm^{-1} .

Visually, all the spectra appeared to be the same, apart from American Spirit Light Blue, which lacked a sharp peak that is seen among the other brands at approximately 1400cm^{-1} . Additionally, between all the brands, the area between 1000 and 800cm^{-1} appeared to be different. Many of the prominent peaks remained similar between the brands, specifically at 3200 and 1600cm^{-1} . Compared to figure 24 which depicted the spectra of the smoked cigarette filter extraction, the tobacco extraction spectra featured a prominent, deep peak between 3500 and 3000cm^{-1} that was lacking in the filter extraction spectra.

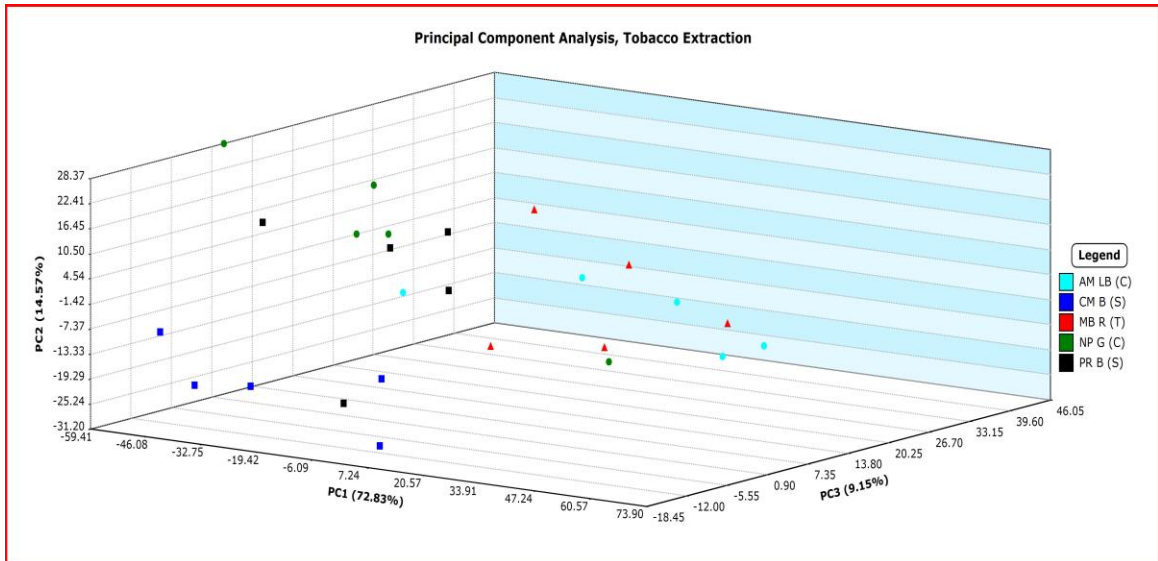


Figure 28. 3D Principal Component Analysis, Tobacco Extraction. Generated with Mass Mountaineer Software™ version 7.01.5.0.

Compared to figures 25 and 26 from the filter extraction, the data points followed a similar cluster tendency. American Spirit Light Blue data points were again seen spread throughout the plot, with outliers near other brand data points. Compared to figure 25, Marlboro Red data points were still clustered together, but spread out further from each other in the 3D space. Newport Green and Parliament Blue data points were clustered closer together than other brands. The overall trend demonstrated in this plot indicates that many of the brand data points cluster near each other. However, there are fewer outliers compared to figure 25, as the spread of the data points is less extreme.

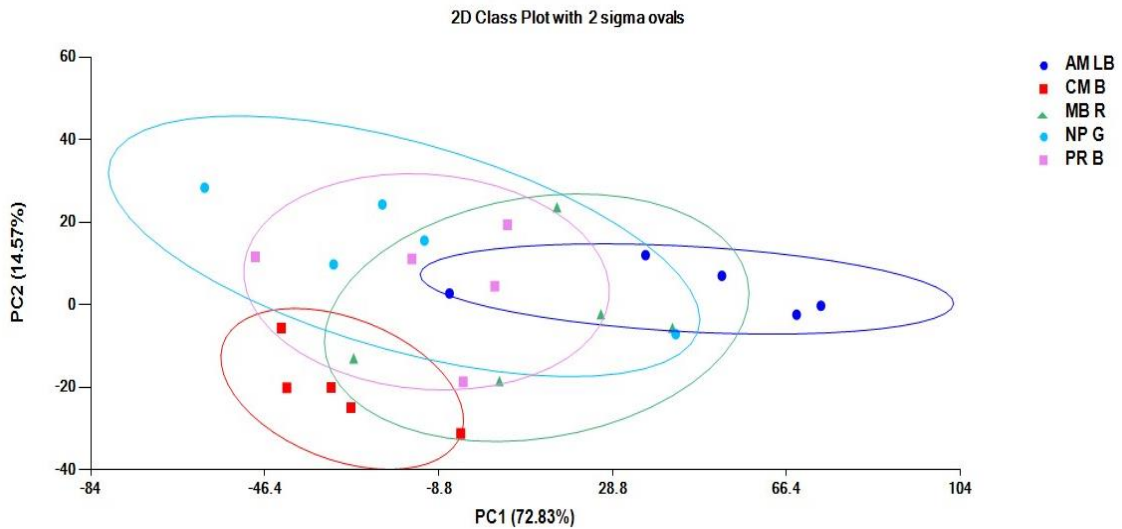


Figure 29. 2D Principal Component Analysis, Tobacco Extraction. Generated with Mass Mountaineer Software™ version 7.01.5.0.

Unlike the oval generated in the filter extraction data, depicted in figure 26, the oval generated for Camel Blue, as shown in figure 29, was smaller, and did not fully encompass any brands. Instead, Newport Green had the largest intersection with all other brands, as seen in figure 29. Although figure 28 would suggest that the brands overlap, there was some distinction between certain brands. American Spirit Light Blue and Camel Blue data points did not intersect at all, which may make brand identification for any tobacco extracted from a smoked cigarette possible. This was different from the tobacco extraction in figure 26, in which it was evident that differentiation between these two brands with the filter extract was not possible.

Camel Blue had a small intersection with Newport Green, as seen in figure 29, but it may be possible to differentiate between these two brands with more replicates. Due to the nature of the ovals generated in figure 29, it would not be possible to identify a brand among the Newport Green, Parliament Blue, and Marlboro Red tobacco extracts, as all the

ovals intersected with each other. Similarly, it would not be possible to distinguish American Spirit Light Blue from these brands, due to the fact that the oval from this plot intersects with all other brands, indicating a high degree of similarity among the data points.

4. CONCLUSION

When observing specific components of the cigarettes, such as the tobacco, the filter or the paper, whether it be smoked or unsmoked, certain brands were able to be discriminated. However, it was not possible to completely identify one brand from the remaining 4 brands tested. A different conclusion was derived from each component analyzed regarding brand identification. Certain brands were able to be distinguished from each other by using the information gathered from the extracted tobacco, but not from information gathered from the cigarette filters. Ultimately, it became clear that, with this scope of information gathered, complete identification of one brand from another was not possible, but differentiation between a select few brands may be possible.

One potential change to the research established in this paper would be to adjust the ratio of tobacco and methanol used in the filter and tobacco extracts. Certain filters were capable of absorbing more methanol than others, which may have caused some samples to be more dilute than others. Instead of using the whole filter in the extraction it may be more beneficial to use only the region where the filter was discolored after smoking, in an effort to obtain a more concentrated sample.

Due to the desire to keep everything consistent amongst the tobacco extractions, a similar amount of tobacco was used for all samples for the tobacco extraction. Each cigarette, when smoked to the designated area, resulted in a different amount of tobacco left over for testing. In the future, it may be possible to determine a specific ratio between amount of tobacco used and amount of methanol used in the extraction. An example of this may be a ratio of tobacco weight in mg to mL of methanol used in the extraction, that

would scale accordingly among cigarettes that had more, or less tobacco available for analysis, as presented in the materials and methods section. Since methanol is not the only solvent that nicotine is soluble in, it may be beneficial to try these methods with other solvents to observe any changes in the generated data (42).

Due to the nature of this research, there are many more avenues to explore when determining ways to differentiate among cigarette brands. Although this research focused on traditionally non-destructive methods of analysis, other methods of analysis could be employed, such as analysis by Gas Chromatography-Mass Spectrometry. By employing this method, it may be possible to identify certain components in cigarette brands that may not be present in other brands.

Since this is only the beginning regarding this research topic, more brands and flavors can be utilized in future research. Chemometric software is capable of learning from training datasets and predicting the identity of samples based on the training data it was provided. More replicates can be generated in order to build proper training datasets for chemometric software, such as Mass Mountaineer™. Should brand differentiation, or even identification be possible with a larger study, the next step may be statistical testing, in which a certain number of cigarettes are tested from a box, or several boxes of a specific brand, to observe if Mass Mountaineer™, or other chemometric software, is capable of correctly identifying the chosen sample.

Should enough information be gathered to identify certain brands, or differentiate certain brands from other, blind trials can be conducted, where various cigarette butts are

collected and tested with the training datasets that the chemometric software has been provided.

LIST OF JOURNAL ABBREVIATIONS

Am J Public Health	American Journal of Public Health
Anal Chem	Analytical Chemistry
Cancer Epidemiol Biomarkers Prev	Cancer Epidemiology, Biomarkers and Prevention: A Publication of the American Association for Cancer Research, cosponsored by the American Society of Preventative Oncology
Forensic Sci Int	Forensic Science International
Int J Legal Med	International Journal of Legal Medicine
Inhal Toxicol	Inhalation Toxicology
Microchemical Journal	Microchemical Journal: Devoted to the Application of Microtechniques in all Branches of Science.
Prev Chronic Dis	Preventing Chronic Disease

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