University of New Haven

University of New Haven
Digital Commons @ New Haven

Honors Theses | Student Works

12-18-2022

Analyzing Methods to Mock the Ingestion of Controlled Substances within a Hair Strand Utilizing LIBS Technology

Caitlin DeLuke

Follow this and additional works at: https://digitalcommons.newhaven.edu/honorstheses

UNIVERSITY OF NEW HAVEN
HONORS PROGRAM

2022-2023 Honors Thesis

Analyzing Methods to Mock the Ingestion of Controlled Substances within a Hair Strand Utilizing LIBS Technology

Caitlin DeLuke

A thesis presented in partial fulfillment of the requirements of the Undergraduate Honors Program at the University of New Haven.

Thesis Advisor:	_____	(Signature)
Department Chair:	Peter Valentin, Forensic Science Chair _____	(Signature)
Honors Program Director:	Matthew Wranovix _____	(Signature)

_____12-18-22_____

Date

University of New Haven

Honors Program

2021-2022 Honors Thesis

Project Title: Analyzing Methods to Mock the Ingestion of Controlled Substances within a Hair Strand Utilizing LIBS Technology

Student Researcher: Caitlin DeLuke

Faculty Advisor: Alyssa Marsico

Department: Forensic Science

Table of Contents

ACKNOWLEDGEMENTS

I have the utmost respect and gratitude toward Dr. Alyssa Marsico
for her consistent support and guidance throughout this
thesis research.
Special thanks to Dr. David San Pietro for allowing me to utilize
the UV cross-linker and to all of the people who fixed the LIBS every
time it showed an error.
This research would not have been as successful without those mentioned above
and without the support of my loved ones and friends.

LIST OF TABLES

LIST OF FIGURES

Abstract

The analysis of controlled substance metabolites within the hair strand have been relied on within the scientific community, particularly in regard to Forensic Science and Criminal Justice practices. A common conflict when analyzing a strand, however, involves manipulation of one's hair, be it through bleach and dyes or detox shampoos. Therefore, research must be conducted to determine how these manipulation efforts affect the concentrations detectable when it comes to lab analyses. This would allow for clarification within data analysis and potentially prove purposeful intent to cover up the ingestion of a controlled substance. In order to research these manipulation effects on concentrations recovered, a method to mock the ingestion of a controlled substance must first be established. This research will attempt to conquer both aspects, first, by determining if liquid or gaseous exposure to a controlled substance can mock its ingestion within a hair strand. Second, by determining how factors such as UV light, a high salt concentration, bleach, detox shampoos, and heat exposure affect the concentrations of Albuterol detectable through Laser induced breakdown spectroscopy (LIBS). The findings will aid in future lab research.

KEYWORDS: Forensic Science, hair, laser-induced breakdown spectroscopy (LIBS), bleach, UV, salt, heat, detox shampoo, drug exposure, Albuterol

Introduction

Hair strands are comprised of the hair shaft, on the outside of the head, and the follicle from which it grows, which is located inside of the skin. Hair grows in three phases, the first being a growth phase, or anagen phase. During this period of a hair strands life, the blood supply surrounding the follicle supplies nutrients and, by default, any other substances within the blood stream. This, alongside keratogenesis, which tightly binds the substances to the hair, is how drug metabolites get incorporated into a hair strand. The other two phases of hair growth, catagen and telogen, are the transitional and resting stages, respectively. Once the nutrients and outside substances are introduced to the strand, they stay in it for the duration of the hair strand's existence. [1]

The process of drug metabolites becoming part of the hair shaft is what makes it so relevant in the legal system and during criminal investigations. Metabolism occurs throughout the human body; it is the chemical conversion of an ingested substance into energy. This remains true when individuals ingest controlled substances. When a drug enters the body, it is broken down chemically into metabolites. [1] The hair shaft inadvertently serves as a timeline of all substances a person has ingested, be it cocaine, ibuprofen, or anything in between, as long as it can be detected in the hair. That being said, cosmetic changes are being made to individuals' hair more regularly than before. This poses disadvantages to previously reliable testing methods which are used to detect controlled substances in hair, as they alter the hair. The use of hair-altering products, specifically bleach, tend to decrease controlled substance marker concentrations, which can lead to false negatives during testing. Previous research shows that bleaching a hair strand significantly alters and degrades melanin, carnitines, amino acids and

their derivatives, purines, as well as nucleosides and their derivatives. Therefore, degrading evidence of drug metabolites within the strand. [2]

Within a standard Forensic Science laboratory, controlled substances are detected within hair samples in ways that are destructive to the hair strand. For example, hair samples suspected of containing controlled substance metabolites are often digested in harsh solutions, such as sodium hydroxide or hydrogen chloride. Ethanol and methanol are commonly used solvents to break the hair down further. These destructive digestion methods are generally followed by analyses using Immunoassays, or chromatographic methods such as Gas Chromatography Mass Spectrometry to determine what substances are present. Immunoassays are a screening test for the presence of drugs, but do not allow for the quantification of the possible drug. Prior to performing an immunoassay, the hair matrix has to be destroyed before being screened using radioimmunoassay, ELISA or fluorescence polarization immunoassays. Chromatographic methods, on the other hand, both confirm the presence of drugs and allow for their quantification. Components of a mixture are separated and detected, which allows for the drug and its metabolites to be quantified. This is commonly done utilizing Gas Chromatography Mass Spectrometry (GC-MS). GC-MS separates a mixture of compounds into individual components identified by a detector, the mass spectrometer. GC-MS analysis also requires extraction techniques, which destroy the hair sample. These processes, utilizing various laboratory instruments, are both time consuming and destructive towards the sample. [3]

In contrast to the commonly destructive and time-consuming methods, Laser Induced Breakdown Spectroscopy (LIBS) is a newer analytical method that has been investigated for use in the forensic science field. LIBS is a quick-working technology, which requires minimal sample preparation. It is also a fairly non-destructive technique, making it a better approach than

its more destructive counterparts. LIBS relies on a high-temperature plasma that is induced by a short laser pulse. Laser ablation at the surface of the sample ensues and the ablated material interacts with the laser pulse, resulting in an increase in energy, subsequently exciting atoms and producing ions from the ablated sample. This energetic plasma consists of free electrons, excited atoms, and ions that all arise from the sample analyzed. Once the plasma begins to cool, all excited atoms return to their ground state, which emit light with specific wavelengths that correspond to the identity of elements present. This is collected and a spectrograph reads the spectral analysis. Peaks produced in this analysis are unique to specific elements, which all together provide insight as to what may be present in the sample. [4]

The controlled substance that will be utilized throughout this study will be Albuterol, which has an elemental formula of $C_{13}H_{21}NO_3$. Albuterol belongs to the family of medicines referred to as adrenergic bronchodilators, commonly used to prevent and treat symptoms caused by asthma and chronic obstructive pulmonary disease (COPD). This type of medication works by relaxing and opening the air passages within the lungs. The inhalation solution comes packaged in its liquid form and is aerosolized by an inhaler. [5] The drug will be introduced to the hair strand through both gaseous and liquid exposure, in order to determine which best incorporates the drug into the hair strand.

While research and studies surround this topic, there are no set parameters on how to approach physically altered hair when trying to test for drug metabolites within a hair strand. The purpose of this research is to analyze the impact various hair alterations have on the detection of controlled substances and to approach what, if any, methods are more reliable in incorporating the metabolites. Conclusions from this research will promote clarification on how to first

approach an altered hair strand in the lab, therefore also aiding in forensic techniques and criminal investigations.

Lab analyses that allow for the detection of controlled substances within a hair strand can be vital within casework. Being able to mock the ingested concentrations of these substances found within an experimental hair strand is vital to perform further laboratory research. The purpose of this research is to determine if exposure to Albuterol, introduced in both liquid and gaseous forms, mocks its ingestion within a hair strand, and if alterations including heat, UV light, high salt concentrations, bleach, and detox shampoos both affect the resulting LIBS spectra when trying to identify if a drug is present in the hair and improve the incorporation of the drug into the hair.

Literature Review

Accomplished Methodology

Many techniques have provided successful results when investigating a hair strand for controlled substance metabolites. Common methodologies, completed by Boumba et al., included the use of immunoassays, capillary electrophoresis, and GC-MS. The study itself was to determine if hair could be validated as a marker for drug use, as well as understanding whether certain techniques had specific limitations. Their first step involved decontamination of the hair samples, which was completed in four different subgroups. One group was washed with methanol, ethanol, and acetone. The second group was washed with sodium dodecyl sulfate and other detergents. The third was washed with dichloromethane, and the final group was washed utilizing a combination of the other three groups relying on organic solvents and repetitive

washings with phosphate buffers. Any drugs left in the hair strand were then extracted, and to do so the strands underwent alkaline digestion, acidic extraction, or enzymatic digestion. The alkaline digestion had to incubate overnight, as did the acidic extraction. The results focused on which methodology worked the best at detecting the metabolites, as well as how things such as hair pigmentation affected the results. They concluded that the best methodology was variable based on which metabolite or controlled substance was being tested for, and also found that hair structure and pigmentation generally does not affect the concentration of bound metabolites, only cosmetic procedures would alter it. [1] These factors and observations are important to consider when performing individual research. The wash procedure they followed can be adapted to suit further research, and their results provide insight regarding the general idea that cosmetic procedures can in fact alter the hair strand.

Based on the above research that provided insight on cosmetic procedures altering drug concentrations, further experiments have been completed to determine which cosmetic alterations affect it, and to what degree. Research published by Eisenbeiss et al. within *The Analyst Journal* studied techniques that allow scientists to analyze if hair has been cosmetically altered to determine if an individual was attempting to cover up drug or alcohol usage. A control group consisted of untreated hair samples, while the experimental group's hair samples had been chemically treated in some fashion. All hair samples were subjected to various cosmetic treatments, allowed to dry, and were then tested using various techniques. The researchers bleached the experimental group according to the manufacturer's instructions and then rinsed the strands under running water and a 500 µL shampoo solution (1 mL shampoo, 9 mL H_2O). All of the strands were then washed with DCM, acetone, water, acetone again, and then left to dry overnight. Then, 31.5 mg of hair were weighed into a 2mL tube and pulverized for 10 minutes at

30 Hz using a bench-top mill. The samples were then extracted with 1 mL ACN/H_2O and 20 µL internal standard solution. They were then centrifuged for five minutes at 9000 rpm, and the supernatant was allowed to evaporate to dryness under nitrogen at 35 °C. The dried extract was reconstituted with 250 µL of ACN/H_2O before being centrifuged once more. Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS) was the primary technique utilized in this experiment. The results demonstrated that, in general, degradation of melanin and the alteration of carnitines within the hair strand were the most obvious signs of chemical treatment usage. [2] The results from this study are important to refer to when analyzing chemically altered hair strands within a laboratory research setting. During laboratory testing, watching for degraded melanin or altered carnitines can validate that the chemical treatment was successful in altering the strand itself.

Analyzing hair samples with Laser-Induced Breakdown Spectroscopy (LIBS) is a way to establish what elements are present within a hair strand. A study published in *Applied Optics* completed by Corsi et al. utilized LIBS technology to analyze hair tissue minerals. Analyzing the minerals present in hair is used as a screening test for metal poisoning and general health conditions. Their particular experiment tested the feasibility of using a calibration-free LIBS technique, since biological samples are highly variable and may not have reliable reference standards. In a normal LIBS experiment, spectra are taken in a singular laser shot at various time intervals. In their experiment, Corsi et al. took 20 LIBS spectra and then the average was taken. The values of elemental concentrations present were compared to the concentrations present in the sample-provider's healthy relatives. The ratios present in the LIBS results provided information as to which individuals had been exposed, either in longer duration or higher concentration, to harsh metals. The research also notes that darker hairs absorb laser radiation

more efficiently than lighter hairs, which is important to note when working with LIBS technology. [6]

In another research study published in the *Journal of Forensic Sciences*, Zhou et al. attempted to quantify and detect multiple substances, including amphetamines, opiates, ketamine, and cocaine, within a human hair sample. They attempted this using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and the samples were extracted with methanol via cryogenic grinding. This process takes the extracted sample, cools it, and then reduces it into small particles. When the reduced sample was analyzed using LC-MS/MS, calibration curves were produced that provided positive results for their methodology in terms of detecting multiple samples at once. The only drawback was the detection of amphetamines at the limit of quantification. [7] This study did not provide the most reliable results and thus suggests methodology that is not ideal when performing additional research, however, it remains valuable to refer to if additional attempts wish to be made in regard to analyzing multiple substances at one time.

External Contamination

An important factor in analyzing a hair strand for any controlled substance is knowing which techniques provide the best results. In a study completed by Sergi et al., fourteen major drugs were identified from hair strands utilizing liquid chromatography-mass spectrometry (LC-MS). In an attempt to aid their research, they also incorporated a decontamination step paired with a pressured liquid extraction, which showed good results in terms of avoiding false positive results where a drug was detected but was not actually present in the hair. [8] These results signify

the importance of ensuring not only proper laboratory techniques during analysis, but also the importance of decontaminating samples.

A separate study published by Tsanaclis et al. in the *Forensic Science International Journal* analyzed the importance of eliminating as much environmental contamination as possible prior to analysis of the hair strand. In an attempt to validate this idea researchers stressed the importance of decontaminating and washing a hair strand prior to analyzing it for controlled substances, while also suggesting a new methodology. This newer method would include analyzing wash residue and comparing it to detected concentrations within the hair strand. The methodology would factor in drug residue left behind after washing took place, as well as how much drug residue was originally present from environmental conditions. The amount collected could be analyzed and factored in to determine the quantity of drug metabolites in the hair strand that were derived from outside circumstances as opposed to actually ingested and metabolized internally. The proposal included using methanol as the solvent to wash out soluble compounds on the exterior of the hair strand, but only for a short soak time of 1 minute to prevent extracting drugs from the hair cortex. [9] This proposed technique was then experimentally tested.

In support of the new methodology proposed by Tsanaclis & Wicks, the same researchers ran a laboratory experiment to provide numerical datapoints. The experiment included analyzing hair samples collected from crack cocaine users and they compared concentrations detected within the strand to concentrations found from the wash residue. This, as they anticipated, provided insight as to whether the main concentration they were detecting was due to ingestion or external contamination. The results demonstrated that, in some cases, external contaminants could be excluded due to the wash residue, however, it did not work within every scenario. For example, if there was generally a lower concentration of cocaine present within the hair strand, it

was harder to detect which method the concentration was a result of. [10] This could be helpful in determining whether a concentration of an experimental sample was primarily due to external contaminants or internal ingestion and is important to keep in mind during any hair strand analysis.

Supplemental Resources

A key component in analyzing drug metabolites is having reference standards to compare results to. In a 2003 study, Welch and Tai generated two new standard references for determining potential drugs within a hair strand. Hair strands were soaked in a solution of water-dimethylsulfoxide containing target analytes and then GC-MS and LC-MS were utilized to determine the concentrations of analytes present. While utilizing GC-MS and LC-MS 0.1M HCl was used for all analytes aside from THC which utilized 1M NaOH. The two standards, SRM 2379 and SRM 2380 were both identified and verified. SRM 2379 is a standard reference comprised of cocaine, benzoylecgonine, cocaethylene, phencyclidine, amphetamine, and methamphetamine. SRM 2380 is a standard reference comprised of codeine, morphine, monoacetylmorphine, and tetrayhydrocannabinol. [11] These become standard references through the National Institute of Standards & Technology (NIST) which can be purchased and utilized in laboratory settings for research purposes. NIST then provides analyte information and preparation methods when working with a specific standard, including mass fraction values for the analytes within the reference. [12] Standards for reference, alongside control samples, are imperative when conducting laboratory research.

When working with experimental or positive control samples, having a list of known ingested drugs can be useful. That being said, individuals may leave out certain controlled substances be it unintentional or purposeful. In a study published by Palamar et al. within *The American Journal of Drug and Alcohol Abuse*, the identification of fentanyl within hair strands was reviewed. 40 individuals who had previously used heroin supplied the samples. UHPLC-MS/MS was the technique used to test for fentanyl and its metabolites. The results showed that despite only 27.5% of individuals reporting known fentanyl exposure and 67.5% suspecting exposure, 97.5% tested positive for fentanyl, and more tested positive for its respective metabolites. [13] This shows that even samples that come with a list of known ingested drugs may contain other drugs and their metabolites due to mixtures of street drugs or an individual leaving out their full ingested-drug history. This will have to be considered when collecting hair samples and reviewing the question about known drug use by the participants and stresses the importance of running a sample of their hair with the methodology proposed here prior to using each hair sample for treatments. This would verify that no drugs were present in the hair upon receival.

Aside from metabolites forming upon ingestion, chronic exposure to drugs environmentally can also lead to the incorporation of drugs within a hair strand. An experimental study run by Franz & Mußhoff investigated how, and to what extent, drugs become incorporated into children's hair strands if and when they are exposed to drug usage by other individuals. Their samples were from children up to the age of sixteen years old. The children were all living in homes of self-reported drug users for the research, therefore, most of the children had chronic exposure to gaseous controlled substances or were introduced to the drugs themselves through improper handling from the user, or accidental ingestion. They performed analyses such as liquid chromatography-mass spectrometry on the sample hair strands and compared the results to the

adult's hair sample concentrations. They also cross-compared with database drug concentrations. The results concluded that drug metabolites were highest in babies who may have been exposed in utero or during breast feeding. Generally, though, adult concentrations were higher than those found within children. The most vulnerable age groups were children less than a year old and between the ages fourteen to sixteen. [14] This overall observation is valuable in terms of knowing where external contaminants may come into play when it comes to children or young adults.

Another study published by Papaseit et al. within the *Journal of Medical Case Reports* focused on a single family in correlation to chronic crack exposure. Scientists focused on benzoylecgonine, a metabolite of cocaine, found within both a two-year old child's hair as well as her parent's hair. A segment of hair close to the scalp alongside a segment further down the strand was analyzed from both the parent's hair and the child's hair relying on forensic standards using an immunoassay. The child's segment from further down the strand showed a similar concentration to both of the mother's strand segments. [15] This showed that chronic gaseous exposure to drugs can mock ingestion in terms of concentration amounts and can remain on or in the hair for long periods of time.

Overall, the journals and articles reviewed supply vital information regarding techniques, setbacks, and things to consider when pursuing this line of research. Contamination must be avoided, and methods can be approached to decrease the risk of contamination even further. There are multiple approaches that can be taken whilst analyzing a hair strand, and some have better outcomes than others. There are also other factors to consider, such as if chronic exposure to other drugs has occurred, or if unknown ingestion of drugs, such as fentanyl, has transpired. All of this data and their respective conclusions will be considered during this research project.

Description of Methodology

Materials

The ManicPanic At-Home Bleach Kit, the Zydot Ultra Clean Shampoo and the AmoVee Mini Flat Iron Cosmetic Hair Straightener were purchased from Amazon (Bellevue, WA). Certified ACS methanol was purchased from Fisher Scientific (Hampton, NH). NaCl was purchased from Sigma-Aldrich (St. Louis, MO). The Albuterol sulfate solution 0.083% was supplied by Dey Pharmaceuticals.

Hair Sample Preparation

Two hair samples were collected from consenting donors with regards to IRB Protocol #2022-021 via hair brushing or cutting. Hair sample 6 was medium brown hair, short in length, and thick in diameter. Hair sample 13 was medium brown hair, long in length, and thin in diameter. Neither hair sample was associated with controlled substance use, with the exception of birth control that was ruled out as affecting the results collected. The only cosmetic alteration was recurring straightening of hair sample 13. Each hair sample was separated into 20 mL disposable scintillation vials, with a total of 24 vials utilized. All strands underwent a decontamination step. The hairs in their scintillation vials were shaken and soaked in methanol for a duration of one minute prior to undergoing any damaging processes. After one minute they were rinsed with water and dried, and the hairs that were selected for treatment were treated accordingly.

To bleach the hair, one scintillation vial cap full of bleach powder and one scintillation vial cap full of developer was used. These were mixed together and massaged into the hair on a

plastic weigh boat. The hair strands were allowed to soak in the bleach for 20 minutes before being rinsed off with water.

To detox the hair using detox shampoo, one scintillation vial cap full of Zydot detox shampoo, and one scintillation vial cap full of Zydot purifier were used. These were poured directly into a vial containing the hairs and water was added to fill the vial. The vials were then shaken and were left to soak for 24 hours.

To expose the hair to high heat, the hair samples were exposed to heat directly with a AmoVee straightener for 1.5 minutes, after the straightener had warmed up for 5 minutes.

To expose the hair to UV light, the hair samples were placed in a UV cross-linker, SpectroLinker XL-1500, at the maximum wavelength of 254 nm for a total of 1200 seconds, or twenty minutes.

To expose the hair to salt, the hair samples were placed in a solution of 1.16 g of NaCl and 20 mL of water. These were shaken and allowed to soak for 24 hours.

Microscopic Hair Analysis

All hair strands were initially visualized under a Leica DM EP microscope prior to any cosmetic treatments. The two samples were observed at 100X and 200X magnifications to document color, general hair structure and thickness, medulla patterns, and pigment granule distribution.

After the hairs were treated, each sample was examined again under the Leica DM EP microscope to document any visible damage to the strand. They were visualized at 100X and 200X magnification to observe any differences in the characteristics listed above.

Controlled Substance Incorporation

Once the hairs were washed and treated, a controlled substance, Albuterol, was incorporated. All six of the subgroups, based on damage type, were split into two additional groups. One group was exposed to Albuterol in a gaseous state by using a nebulizer. The Albuterol was pumped directly into the hair's scintillation vial until it was full, and allowed to sit, sealed with parafilm, for twenty-four hours. The second group was exposed to Albuterol in a liquid state, by adding 3 mL of 0.083%, or 2.5 mg / 3 mL, liquid Albuterol solution into each of the hair's scintillation vial and allowing them to soak for twenty-four hours.

Instrumental Analysis

A ThermoScientific Nicolet iS10 ATR-FTIR spectrometer with the iTR Diamond attachment was first utilized to compare the samples. The parameters were 32 scans with a resolution of 4. It should be noted that in between FTIR runs, the liquid-exposed samples were washed with methanol and water to see if the signal changed based on surface contaminants being removed. This wash procedure may have altered subsequent results for the liquid samples.

Then, the hair samples, all 12 subgroups for each sample, underwent LIBS analysis to detect elemental changes. The Applied Spectra J200 LIBS and the Axiom software were

utilized to analyze the samples. The parameters for LIBS analysis mirrored research completed by Brady and can be seen in Table 1. [16]

Table 1: LIBS Parameters

Gate Delay	0.5 µs
Laser Energy	60%
Shots	15
Spot Size	40 µm
Rep Rate	10 Hz
Warmup Energy	100
Warmup Shots	50
Warmup Rep Rate	10 Hz

The intensities of each element detected in the samples were compared to determine which degradation and incorporation techniques best mocked the ingestion of the substance into the hair strand. The control groups for each degradation technique were also run through LIBS to analyze how the treatments affected the signals detected. Two runs were completed for each hair sample. A plain glass microscope slide and a microscope slide containing dried Albuterol were also run through LIBS to determine if any elemental intensities could be directly attributed to either of the two.

Data Analysis

The microscope images were compared to investigate any morphological changes that may have occurred to the hair strands during the cosmetic alterations. This included changes in color, thickness, medulla pattern, pigment granule distribution, and general appearance.

The FTIR-iTR results were compared for changes in peaks that were apparent in the spectra in addition to the intensities of these peaks.

The LIBS spectra were analyzed using the Clarity software, which searched the spectra against the NIST LIBS library database to identify elements present. The Clarity software also put out a table of the intensities of all identified elements, which was recorded. New data tables were then curated from the data obtained from Clarity to compare changes in intensities for each element per sample type. This was completed by taking the average of the two runs intensities and calculating their standard deviation. Bar graphs were then also curated to better visualize the comparison of elemental intensities between each sample, as well as between each deconstruction method and each drug incorporation method. The bar graphs were completed by plotting the summation of all like elements, with the addition of error bars formed by calculating error propagation. These were then compared by looking for overlaps of the plotted averages +/- the standard deviation between various samples. If the averages and standard deviation values overlapped, this was determined to not have changed. However, if there was no overlap, this was considered as a significant change in that element for the samples compared.

Results

Hair samples 6 and 13 were both visualized and photographed at 100X magnification (Figure 1) under a Leica DM EP microscope. Hair sample 6 was documented as medium brown in color, with a thicker diameter than sample 13. Sample 13 was longer in length than sample 6 and was similar to sample 6 in color. Sample 6 had a dark interrupted medulla pattern throughout some, but not all, of the strands. Sample 13 was consistently an absent medulla pattern. Sample 6 also had noticeably large pigment granules, while sample 13 was much smaller in pigment granule size.

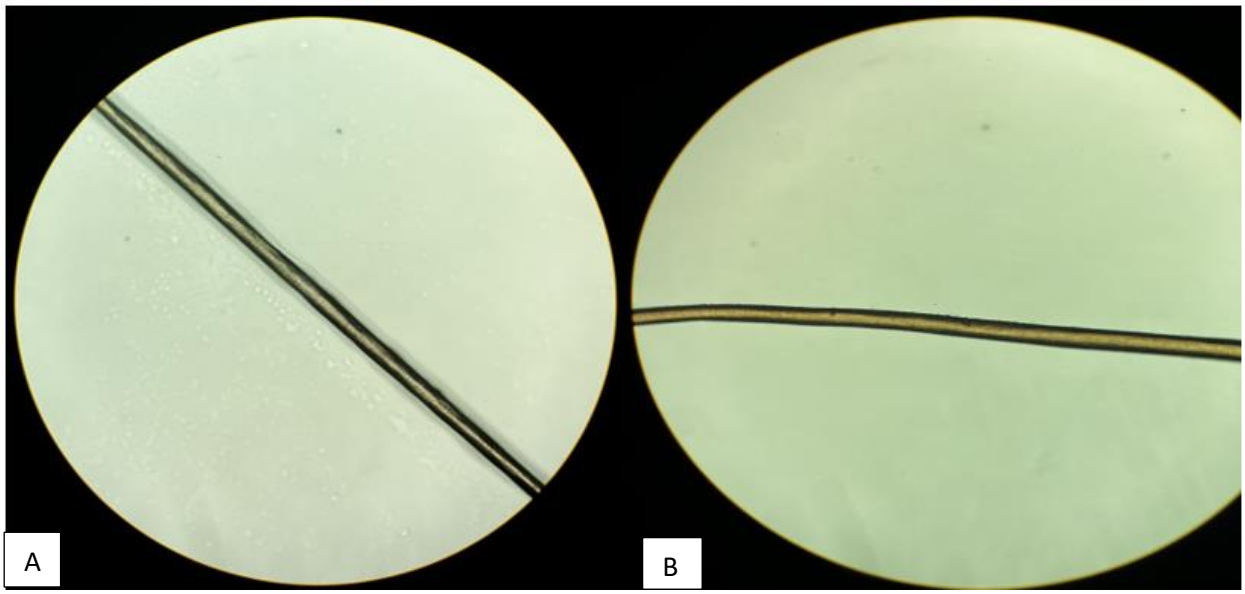

Figure 1: Sample 6 (A) Control Visualized at 100X Magnification; Sample 13 (B) Visualized at 100X Magnification

Both hair sample 6 and sample 13 were then observed again under the Leica DM EP microscope following their cosmetic treatments (bleach, UV, salt, detox shampoo, heat). The most noticeable difference in breakdown methods when compared to the control samples, was that the bleached samples appeared blonde in color, as opposed to their natural medium brown

hue. Images from each breakdown method were captured, but there were no other prominent changes, so only sample 6's images are provided (Figure 2).

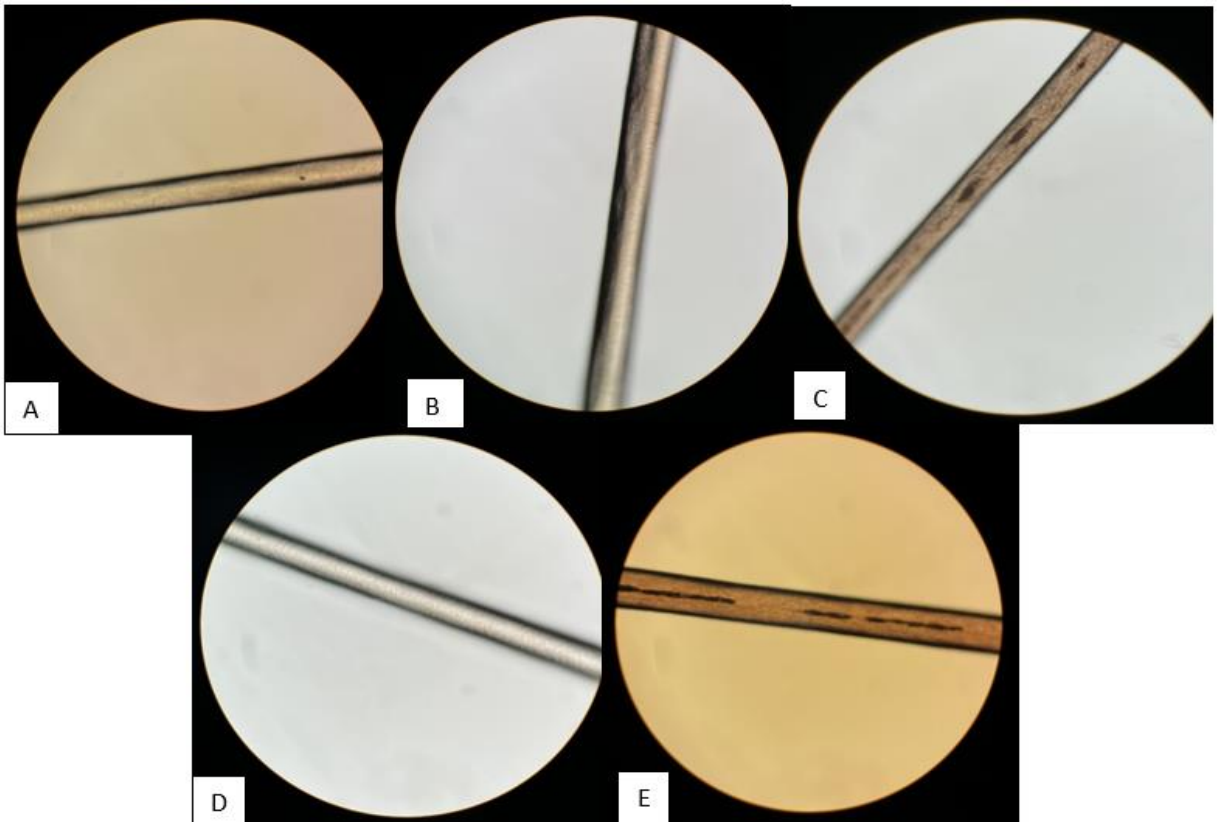

Figure 2: Hair Sample Images under Leica DM EP at 200X Magnification. (A) Sample 6 Bleach (B) Sample 6 UV (C) Sample 6 Heat (D) Sample 6 Salt (E) Sample 6 Detox Shampoo

The FTIR spectra did not show significant variation between present peaks and their respective intensities. Therefore, the FTIR spectra were not included in this report. Due to the washing step that took place in between FTIR analysis runs, the LIBS results for liquid exposed hair samples were also affected.

The spectra obtained from LIBS were saved to document which peaks and therefore elements to focus on (Figure 3). A manual noise of 2500-3500 was used to eliminate LIBS labeling insignificant peaks. The most common elements observed were magnesium, calcium,

sodium, oxygen, potassium, and hydrogen. For peaks with multiple elemental options, the element most commonly found within a hair strand was used. [17]

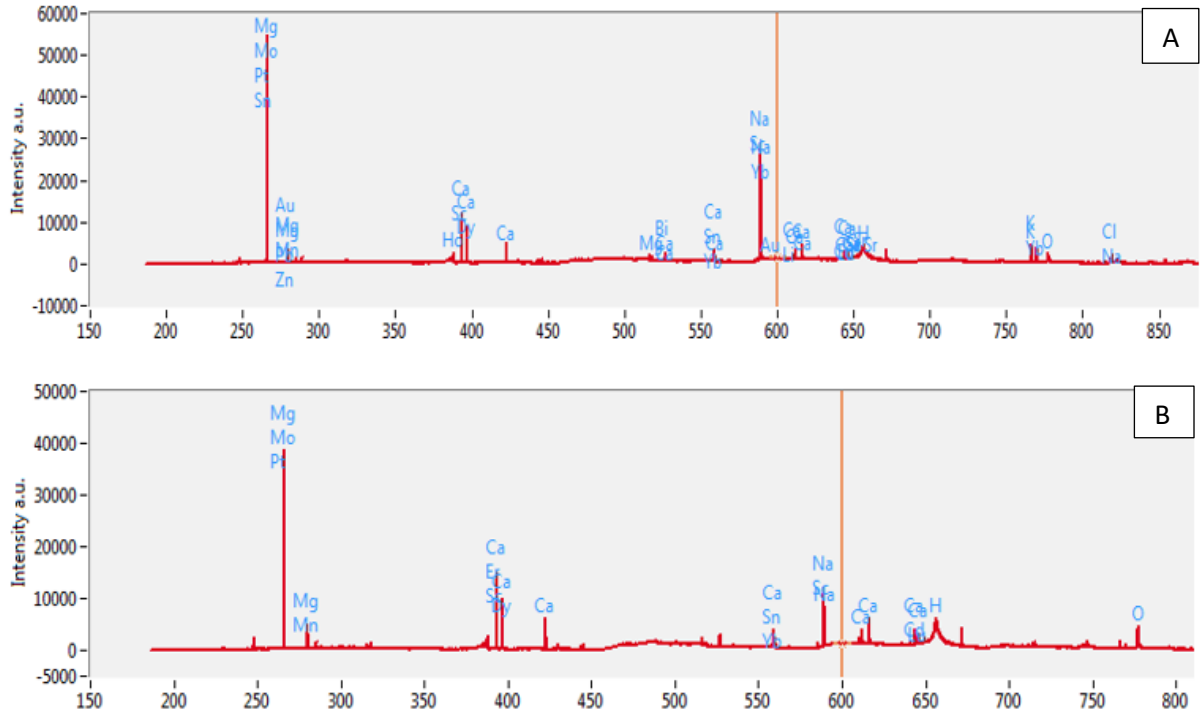

Figure 3: Sample LIBS Spectra (A) Sample 6 Control (B) Sample 13 Control

Spectra obtained for each damaging cosmetic alteration were compared to the control spectra. Changes in intensity, the disappearance of original peaks, or the appearance of new element peaks were recorded. When albuterol was introduced, the same comparisons were made to both the controls and the new controls for the damage techniques.

For each individual element, the control samples for sample 6 and sample 13 were compared regarding their initial measured intensities. From there, the change in intensities recorded for each damage method were compared visually. Finally, the change in intensities when the samples were exposed to albuterol were compared visually. Due to the initial FTIR

procedure and washing the liquid samples, liquid intensities for LIBS were unreliable and are not being utilized for definitive conclusions.

To first discuss elements commonly seen in these hair samples, calcium, and magnesium within hair in high intensities are generally an indication of hair treatments. The presence of magnesium can also be due to exposure environmentally or when ingested in higher levels than the body needs. Sodium levels within hair typically indicate repeated or recent shampoo usage. [18] Generally, though, hair is composed of carbon, oxygen, nitrogen, hydrogen, and sulfur. [17]

The damage methods used each affect the hair structure and composition differently. Salt not only dehydrates the hair, but it also exfoliates the strand. This not only damages the cuticle, but it also causes nutrients within the hair strand to be removed. UV light causes damage to the amino acids and proteins present within the hair strand, producing free radicals and weakening the hair strand overall. Bleach directly breaks bonds within the hair strand, including hydrogen and disulfide bonds, as well as salt bridges. High heat changes the protein structure within the hair, and similarly to bleach, breaks bonds present within the strand. Detox shampoos are used to eliminate toxins and build-up within the hair strand.

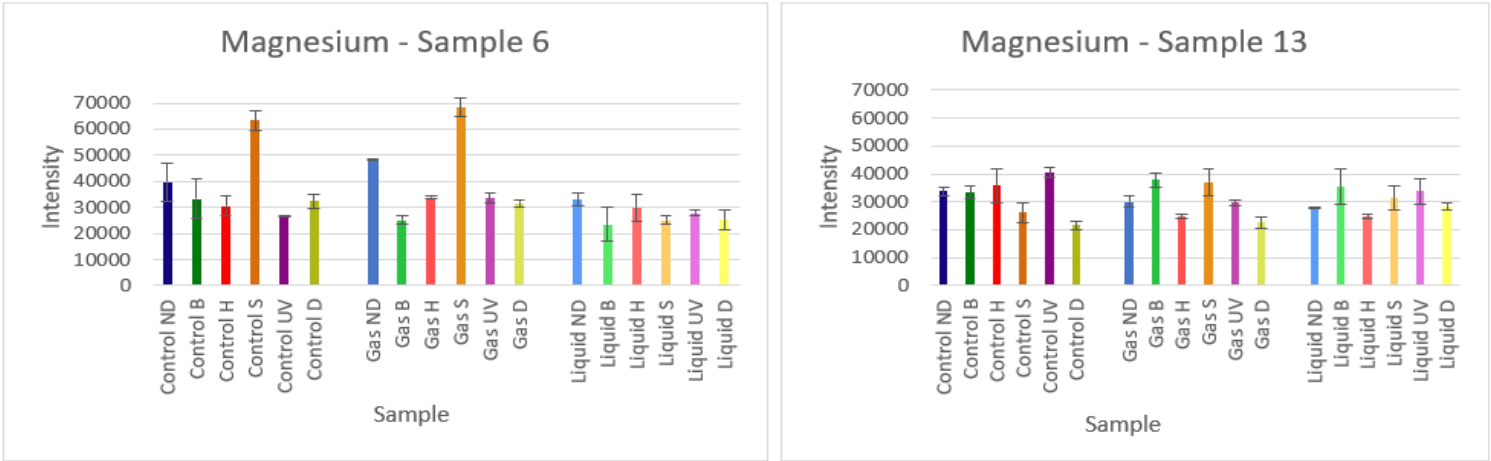

Figure 4: Magnesium Detected in Sample 6 (Left) and Sample 13 (Right)

The first element compared was magnesium (Figure 4). The levels of magnesium were close in range for both sample 6 and sample 13's control runs. Meaning, the amount of magnesium in each original hair strand was just about consistent. When the hair strand was soaked in a high salt concentration, the level of magnesium increased by a very high amount in sample 6 without, and with gaseous exposure to Albuterol. The salt could be provoking water present in the hair to leech out, increasing the levels of magnesium detectable by the laser resulting in a higher signal. Sample 6 also saw an increase in magnesium when the hair strand was exposed to high heat and UV, but not as drastically as the high salt solution. In sample 13, magnesium levels decreased for the control and albuterol gas exposure when the hairs underwent high heat exposure, UV exposure, and the detox shampoo soak. This decrease is likely due to the damage techniques drawing magnesium out of the hair. While magnesium serves important functions within the body, none of them take place in hair. Magnesium is generally only indicated in hair when ingested in high amounts and the body rids the excess through the hair, or when maldistribution takes place during events such as chronic stress, or toxic metal or chemical exposures. [19]

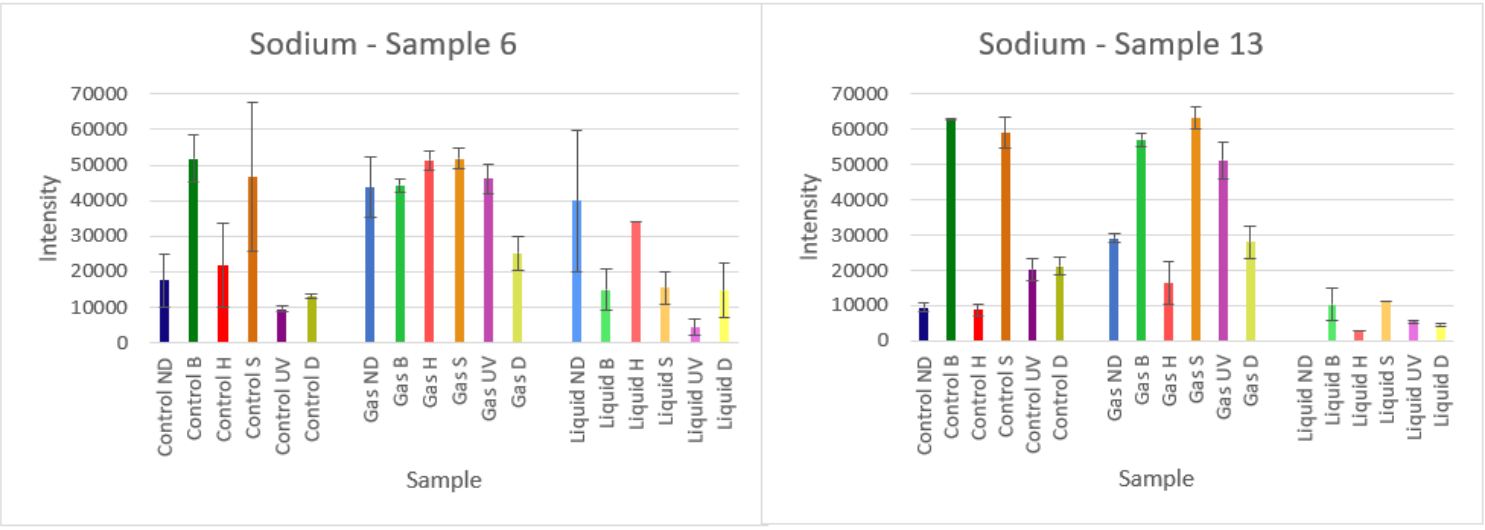

Figure 5: Sodium Detected in Sample 6 (Left) and Sample 13 (Right)

The second element was sodium (Figure 5). Sample 6 control had more sodium present initially. Without albuterol present the samples that were bleached and introduced to a high salt solution had a drastic increase in sodium for both sample 6 and sample 13. Gaseous exposure to albuterol increased the sodium intensities in both controls for sample 6 and 13. Gaseous exposure overall seemed to slightly increase all sodium intensities for both samples, which was particularly true for the samples that underwent UV exposure. The increase in sodium when exposed to albuterol can be explained either by the presence of sodium chloride within the albuterol solution and or the presence of sodium citrate in albuterol, both of which are commonly added to the solutions used in this research.

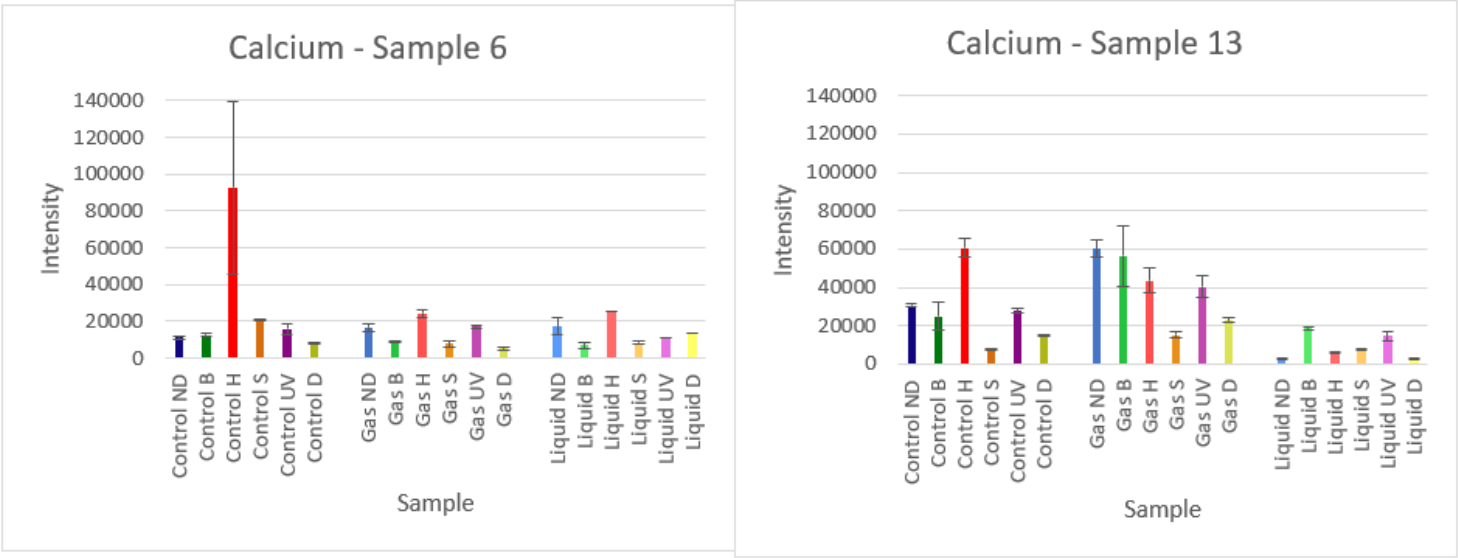

Figure 6: Calcium Detected in Sample 6 (Left) and Sample 13 (Right)

The third element was calcium (Figure 6). In terms of the control samples, sample 13 had a higher amount of calcium present initially. A study found that calcium concentration is related to age and sex, with females generally having a higher calcium concentration, as well as diet and general health of the individual, which may contribute to this difference. [20] Consistently for sample 6 and sample 13, high heat exposure led to a drastic increase in calcium

with no controlled substances present. During gaseous exposure, sample 13, with high heat, showed a decrease in calcium intensity. The increase could be explained by the ceramic coating present on the hair straightener. When in use, the straightener deposits calcium on the exterior of the strand, which is why an increased signal is detected. In sample 13, gas exposure paired with no damage, bleach, and UV exposures showed an increase in calcium. This could be due to the hair having been straightened in the past and could be attributed to supplements the individual may be consuming. This is the most likely scenario, as sample 6 does not show a drastic change in calcium concentrations with gaseous exposure, and sample 13 is known to consistently have been straightened. Sample 6 saw a slight increase in intensity with high salt and UV exposures when there was no albuterol present. The high salt for sample 6 saw a decrease in calcium when exposed to albuterol. Albuterol contains edetate disodium, which binds with calcium and other heavy metals within the body – commonly used to treat calcium overloads. This would explain why when albuterol is introduced, a decrease in calcium is documented, as it is bound to a component of the albuterol solution and may not be ionized as well during LIBS analysis. [21]

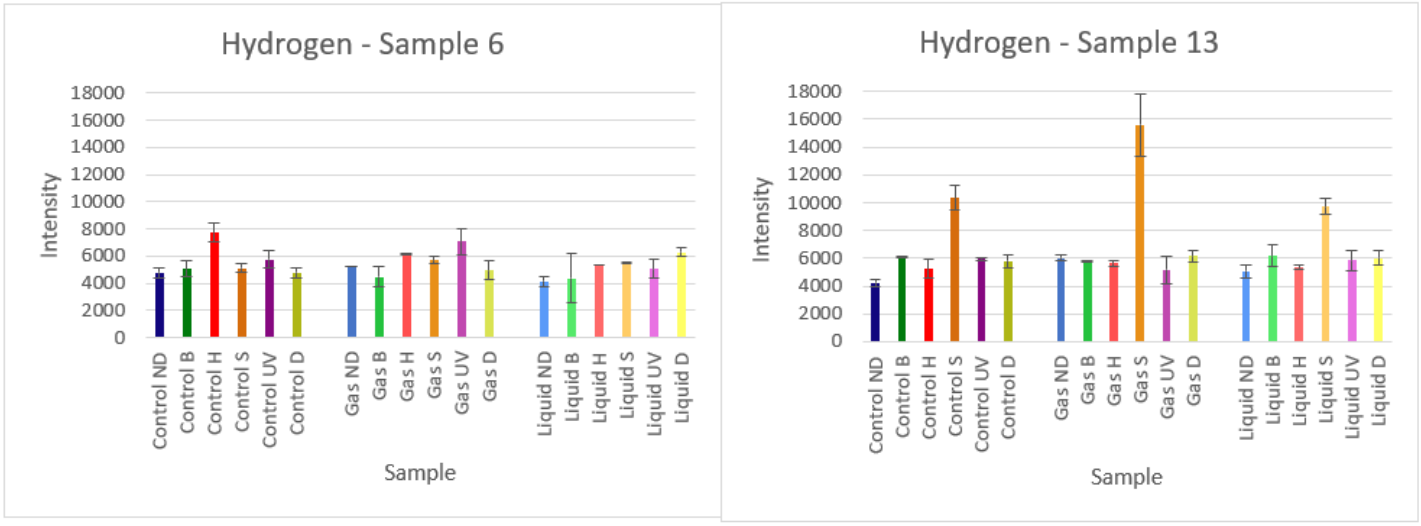

Figure 7: Hydrogen Detected in Sample 6 (Left) and Sample 13 (Right)

The fourth element analyzed was hydrogen (Figure 7). Sample 6 overall had a very low hydrogen presence, and sample 13 had much higher hydrogen intensities. For sample 13, all damage types slightly increased in hydrogen intensity, but the high salt solution increased it at a higher rate. Sample 13 did not see a drastic effect on intensity when exposed to albuterol in the gaseous state, with the exception of the high salt solution which skyrocketed hydrogen's intensity. This would suggest that the high salt solution allowed for albuterol to enter the hair strand, causing an increased signal in hydrogen, but it is not definitive, as hydrogen is far too common in both hair and other compounds that could be present on or in the hair sample, so it cannot be attributed solely to the Albuterol. Sample 6 saw an increase in hydrogen levels with the high heat and UV damages. With gaseous exposure to albuterol, sample 6 saw a decrease when heated, and an increase when exposed to UV and the detox shampoo. Although the liquid samples are unreliable, sample 13 with liquid exposure did retain its hydrogen intensity. This is likely due to the methanol used to wash the hair strands, and the hydrogen present in this, resulting in a higher intensity than the other elements investigated.

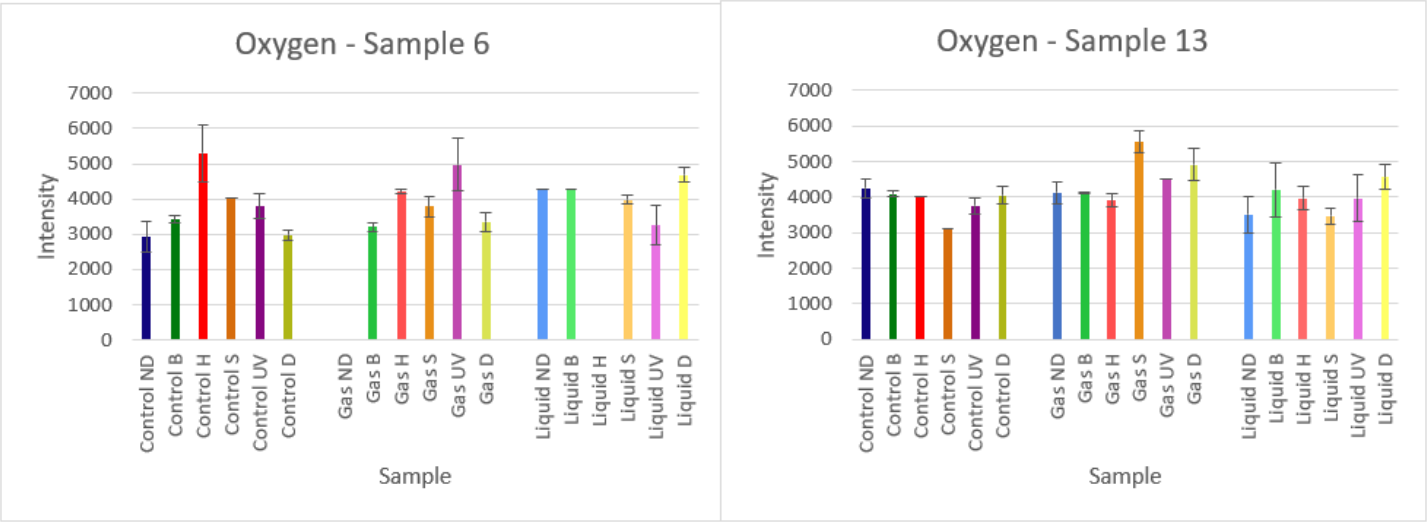

Figure 8: Oxygen Detected in Sample 6 (Left) and Sample 13 (Right)

The fifth element was oxygen (Figure 8). Since oxygen is present in the air, these values are not as significant, and overall are much lower in intensity than some of the other elements present. For sample 6, high heat, bleach, high salt, and UV all lead to an increase in oxygen. When paired with gaseous exposure to albuterol, UV damage increased the presence of oxygen in both sample 6 and sample 13. Sample 13 also saw an increase due to high salt and detox shampoo damages. Without any damage method, sample 6 had no oxygen present when exposed to albuterol via gaseous exposure. So, while UV and salt seem to increase oxygen's signal the most, oxygen is too common to attribute it solely to the albuterol.

Another element compared, but not plotted in a bar graph, was potassium. Potassium was present in sample 6 without damage and was increased by bleach and high heat exposure. When exposed to albuterol in its gaseous state, potassium increased in intensity for the no damage, bleach, and heat groups of sample 6 (Tables 2, 3, 4). Sample 13 only had potassium detectable when exposed to bleach, which increased slightly with gas exposure, and UV with no albuterol present (Tables 9 & 12). The Manic Panic bleach contained potassium persulfate, which explains the appearance and increase in potassium concentrations.

The final element that was not plotted in a bar graph was ytterbium. This element only showed up for sample 13's bleached samples, without albuterol and with albuterol gas exposure (Table 9). This is likely a result of the laser and can be ignored in terms of damage techniques and drug incorporation effects.

Thinner hair samples will be better penetrated by the laser and would also be more permeable to gaseous exposures. Any lowered intensity of elements commonly associated with control hair samples during the comparisons could be due to the laser focusing more on detecting the albuterol on the surface of the hair, rather than the elements present in the hair strand below

the surface contaminants. An aspect of this research that needs to be accounted for is the uncertainty of where the drug's elements are being picked up within the hair strand. The LIBS laser at the laser energy used was destructive to the hair strand, picking up signals from both the external surface of the hair, and the inside. This, alongside sampling placement can have a direct effect on what was picked up by LIBS.

Conclusion

Understanding methods that may work to mock the ingestion of controlled substances within a hair strand can be of importance within the forensic science field. This would allow laboratories and scientists to perform further research on drug metabolites within hair and how cosmetic alterations affect said metabolites, without necessarily needing hair samples from a controlled substance user. While there are techniques surrounding the collection of this information, many are destructive. Laser induced breakdown spectroscopy is a method to understand a change in elemental intensities present.

Within this study, two hair samples were compared in a controlled manner, as well as with various breakdown methods, bleach, UV, high heat, high salt solution, and detox shampoo, both visually and microscopically. The element signals and intensities for samples that were treated with these various breakdown methods were compared between each other, and then Albuterol was introduced both in its liquid and gaseous states. The difference in elemental intensities picked up between the two different types of drug introduction were also compared.

The first objective was to determine what type of external damage to the hair would best allow the incorporation of a controlled substance into the strand. UV exposure seemed to

have an effect on producing an increase in elemental intensities across the board for each element. Bleaching produced an increase in some elemental intensities, but generally due to ingredients present in the bleach as well as elements within the hair strand, which could pose issues regarding which intensities are related to the hair, controlled substance, or bleach in future research. The detox shampoo generally had little effect, or in some cases decreased the intensities detected. The high salt soak generally had a large effect on the elemental intensities, but similar to bleach, some of those, such as sodium, were directly due to the salt itself being detected, rather than exposing the hair elements. High heat had contrasting effects, increasing and decreasing various elements, or having little to no effect at all. Drug incorporation in this specific study should be primarily based upon elements such as sodium, hydrogen, and oxygen, since these are present in albuterol, the drug used in these experiments. The other elements, such as magnesium and calcium only provide information regarding the elements present in the hair strand, providing insight as to which damaging cosmetic treatments affected the hair strand more or less. Other changes in signals for magnesium and calcium and the elements commonly present in hair strands could be due to chemical interactions with the drug.

Overall, heat and UV are the most reliable damage techniques in terms of not introducing new elements from active ingredients present in other solutions (bleach, detox shampoo, salt). In addition, these did result in increases in elements that can be attributed to Albuterol, indicating that the damage caused by these treatments did allow the Albuterol to enter the hair. The high salt and bleach still had helpful results but may complicate the results based on increased signals in elements that they introduce to the sample. The detox shampoo was the least effective.

The second objective was to determine whether liquid or gaseous exposure to a substance best mocked the ingestion of it. It was definitively concluded that gaseous exposure was more effective. The liquid exposure remained at the surface level, so, when washed or rinsed, the substance was removed and mostly undetectable. Since a simple washing technique has such a drastic effect on the intensities measured for the liquid samples, it should be acknowledged that liquid controlled substance exposure is not as accurate in mocking the ingestion of said substance within a hair strand. The gaseous exposure allowed the albuterol to penetrate the hair strand, and changes in elemental intensities were better observed. The relationship between Albuterol and calcium indicates a way to detect albuterol incorporation into the hair, as the presence of albuterol would be documented as a decreased calcium signal.

Overall, it seems that breaking down the hair strand with high heat or UV and then introducing the controlled substance of choice in a gaseous manner is the best pairing to mock the ingestion of a controlled substance within a hair strand. These breakdown methods most reliably disrupt the structure of the hair strand, allowing the controlled substance and its corresponding elements to become incorporated into the hair strand. These new elements, or increased elemental intensities, can be detected by LIBS.

Future Work

The main objective of this research was to determine both a breakdown method, as well as a drug incorporation method to best mock the ingestion of a controlled substance within a hair strand. The results show that UV light paired with gaseous exposure to a substance best incorporated the substance within the hair samples. Further research could be done to test if this remains true with varying UV wavelengths, as well as various controlled substances to see if those results differ.

Elemental signals picked up with LIBS were dependent on sampling locations. To strengthen the results obtained, an optimal sampling location within a hair strand should be studied and determined. For this research, when an elemental signal was detected at multiple peaks, an average was taken, and error propagations were calculated. In future research, these differing peaks could be considered separately to determine if specific breakdown methods have a specific effect on whether an element is represented by its atomic or ionic form.

Since this was a preliminary study, there are many other aspects that can be reworked to derive more specific results. This includes testing the wash solution after washing the hair strands, to see if the elements were incorporated internally or just externally. Other hair textures and colors could also be tested, as the thickness of hair directly affects how easily penetrated the hair strand can be. In addition, various other drugs could be tested, as they are chemically different, and there may be better methods to incorporate other types of drugs into a hair strand based on their chemical properties.

References

1. Boumba, V. A., Ziavrou, K. S., & Vougiouklakis, T. 2006. “Hair as a Biological Indicator of Drug Use, Drug Abuse or Chronic Exposure to Environmental Toxicants”: International Journal of Toxicology, 25(3), 143–163.
2. Eisenbeiss, L., Binz, T. M., Baumgartner, M. R., Kraemer, T., & Steuer, A. E. 2020. “Cheating on Forensic Hair Testing? Detection of Potential Biomarkers for Cosmetically Altered Hair Samples Using Untargeted Hair Metabolomics”: The Analyst, 145(20), 6586–6599.
3. Usman, M., Naseer, A., Jamshaid, T., Shahwar, M., Khurshuid, S. 2019. “Forensic Toxicological Analysis of Hair: A Review”: Egyptian Journal of Forensic Sciences, 9(17).
4. Anabitarte, F., Cobo, A., Lopez-Higuera, J.M. 2012. “Laser-Induced Breakdown Spectroscopy: Fundamentals, Applications and Challenges”: ISRN Spectroscopy, 1-12.
5. American Society of Health-System Pharmacists, Inc. 2022. “Albuterol Oral Inhalation”: MedlinePlus.gov
6. Corsi, M., Cristoforetti, G., Hidalgo, M., Legnaioli, S., Palleschi, V., Salvetti, A., Tognoni, E., & Vallebona, C. 2003. “Application of Laser-Induced Breakdown Spectroscopy Technique to Hair Tissue Mineral Analysis”: Applied Optics, 42(30), 6133-6139.
7. Zhuo, Y., Wang, X., Wu, J., Zhang, S., Deng, H., Liu, W., Wu, H., Xiang, P., & Shen, M. 2019. “Simultaneous Quantitative Determination of Amphetamines, Opiates, Ketamine, Cocaine and Metabolites in Human Hair: Application to Forensic Cases of Drug Abuse”: Journal of Forensic Sciences. 65(2), 563–569.

8. Sergi, M., Napoletano, S., Montesano, C., Iofrida, R., Curini, R., & Compagnone, D. 2012. “Pressurized-liquid Extraction for Determination of Illicit Drugs in Hair by LC–MS–MS”: Analytical and Bioanalytical Chemistry. 405(2-3), 725–735.
9. Tsanaclis, L., & Wicks, J. F. C. 2008. “Differentiation Between Drug Use and Environmental Contamination when Testing for Drugs in Hair”: Forensic Science International. 176(1), 19–22.
10. Tsanaclis, L., Andraus, M., & Wicks, J. 2018. “Hair Analysis When External Contamination is in Question: A Review of Practical Approach for the Interpretation of Results”: Forensic Science International. 285, 105–110.
11. Welch, M. J., Sniegoski, L. T., & Tai, S. 2003. “Two New Standard Reference Materials for the Determination of Drugs of Abuse in Human Hair”: Analytical and Bioanalytical Chemistry. 376(8), 1205–1211.
12. Wise, S., & Watters, R. 2012. “Certificate of Analysis: Standard Reference Material 2379. Drugs of Abuse in Human Hair I”: National Institute of Standards & Technology. 1-3.
13. Palamar, J. J., Salomone, A., Bigiarini, R., Vincenti, M., Acosta, P., & Tofighi, B. 2019. “Testing Hair for Fentanyl Exposure: A Method to Inform Harm Reduction Behavior Among Individuals Who Use Heroin”: The American Journal of Drug and Alcohol Abuse, 45(1), 90–96.
14. Franz, T., Skopp, G., & Mußhoff, F. 2021. “Findings of Illicit Drugs in Hair of Children at Different Ages”: International Journal of Legal Medicine. 135(2), 465–471.
15. Papaseit, E., Joya, X., Velasco, M., Civit, E., Mota, P., Bertran, M., Vall, O., & Garcia-Algar, O. 2011. “Hair Analysis Following Chronic Smoked-Drugs-of-Abuse Exposure in Adults and their Toddler: A Case Report”: Journal of Medical Case Reports. 5(1).

16. Brady, T. 2020-2021. “Amino Acid Composition Analysis as a Means to Differentiate Hair Samples from Individuals of Similar Demographics and the Effect of Hair Treatments”: University of New Haven Honors Thesis.
17. Beauty Tomorrow. 2015. “The Chemical Properties of Hair”: https://beautytmr.medium.com/the-chemical-properties-of-hair-955985908d05#:~:text=The%20overall%20chemical%20composition%20of,cells%20from%20the%20germinal%20layer.
18. BioLab Medical Unit. 2012. “Hair Mineral Analysis”: www.biolab.co.uk/docs/Hair_Mineral_Analysis.pdf
19. Meridian Valley Lab. 1999-2006. “Hair Elements Report”: http://meridianvalleylab.com/wp-content/uploads/2012/06/Hair-Minerals.pdf
20. Ha, B., Lee, G., Cho, I., Park, S. 2019. “Age- and Sex-Dependance of Five Major Elements in the Development of Human Scalp Hair”: Biomaterials Research, 23(1).
21. Cleveland Clinic. n.d. “Edetate Disodium, Disodium EDTA injection”: https://my.clevelandclinic.org/health/drugs/20773-edetate-disodium-disodium-edta-injection

Supplemental Information

Table 2: Sample 6 Control / No Damage

Element	Control	Gas Exposure	Liquid Exposure
Mg (266nm)	37531.5 ± 7316.5	48613.5 ± 146.5	28706 ± 2354
Mg (280nm)	2123	Not Present	4236
Ca (393nm)	9335.5 ± 804.5	11196.5 ± 2080.5	10159.5 ± 4527.5
Na (589nm)	15531 ± 7584	38637 ± 8648.5	33303 ± 19920
Ca (616nm)	2050.5 ± 299.5	5672	7135
H (656nm)	4778.5 ± 361.5	5194	4127.5 ± 421.5
K (766nm)	3013.5 ± 1213.5	5381.5 ± 499.5	3833
O (777nm)	2923 ± 426	Not Present	4271
Na (819nm)	1983	5125	6717

Table 3: Sample 6 Bleach

Element	Control	Gas	Liquid
Mg (266nm)	33328 ± 7447	25177.5 ± 1778.5	23489 ± 6387
Ca (393nm)	7788.5 ± 379.5	6085± 122	3768.5 ± 1677.5
Ca (422nm)	4906 ± 905	3614	3350
Na (589nm)	46310 ± 6387	38836 ± 8051	14878 ± 5727.5
H (656nm)	5076.5 ± 595.5	4487 ± 709	4384.5 ± 1826.5
K (766nm)	19454 ± 4225	13925.5 ± 3721.5	7942
O (777nm)	3423.5 ± 112.5	3213 ± 118	4271
Na (819nm)	5568.5 ± 1458.5	5438 ± 1753	Not Present

Table 4: Sample 6 Heat

Element	Control	Gas	Liquid
Mg (266nm)	27288.5 ± 3716.5	33802 ± 492	30017.5 ± 5242.5
Mg (279nm)	3224 ± 1094	Not Present	Not Present
Ca (393nm)	70912.5 ± 46859.5	14766.5 ± 1753.5	11369
Ca (422nm)	8702.5 ± 4161.5	6345.5 ± 668.5	8346
Ca (527nm)	4452	Not Present	Not Present
Ca (558nm)	5973	3226	5566
Na (589nm)	21795.5 ± 11663.5	45792.5 ± 2782.5	34157
Ca (649nm)	2741.5 ± 487.5	Not Present	Not Present
H (656nm)	7737.5 ± 699.5	6106.5 ± 53.5	5306
K (766nm)	4806 ± 2574	3657 ± 626	5945
O (777nm)	5299.5 ± 798.5	4214.5 ± 46.5	Not Present
Na (819nm)	Not Present	5505 ± 10	Not Present

Table 5: Sample 6 Salt

Element	Control	Gas	Liquid
Mg (266nm)	63257 ± 3709	68325 ± 3493.5	25080 ± 1590
Ca (393nm)	13943 ± 263	5034.5 ± 1686.5	4770 ± 1068
Ca (422nm)	7155	3108	3726
Na (589nm)	38081 ± 20861	44991.5 ± 2753.5	15442.5 ± 4603.5
H (656nm)	5112 ± 291	5686 ± 251	5536 ± 32
O (777nm)	4046	3784 ± 290	3797 ± 131
Na (819nm)	8589	6804.5 ± 944.5	Not Present

Table 6: Sample 6 UV

Element	Control	Gas	Liquid
Mg (266nm)	26852 ± 79	33580 ± 1966.5	27744 ± 1074
Mg (280nm)	3261	Not Present	Not Present
Ca (393nm)	11461.5 ± 2907.5	11367 ± 734	8180
Ca (422nm)	4434.5 ± 384.5	5738 ± 562	3351
Na (589nm)	9580.5 ± 912.5	46109 ± 4302	4392.5 ± 2310.5
H (656nm)	5757.5 ± 649.5	7072 ± 935	5052 ± 708
O (777nm)	3786 ± 354	4979.5 ± 752.5	3250 ± 552

Table 7: Sample 6 Detox Shampoo

Element	Control	Gas	Liquid
Mg (266nm)	32289.5 ± 2839.5	31512 ± 1471	25268 ± 3888
Ca (393nm)	5616.5 ± 103.5	5445 ± 972	9210
Ca (422nm)	2941 ± 249	Not present	4658
Na (589nm)	13099.5 ± 516.5	25209 ± 4827	14711 ± 7593
H (656nm)	4807.5 ± 374.5	4962 ± 698	6308.5 ± 372.5
O (777nm)	2978.5 ± 151.5	3349 ± 265	4688.5 ± 201.5

Table 8: Sample 13 Control / No Damage

Element	Control	Gas	Liquid
Mg (266nm)	33675.5 ± 1426.5	30102 ± 2220	27835 ± 132
Ca (317nm)	Not Present	3619.5 ± 408.5	Not Present
Ca (393nm)	12477 ± 289	29531 ± 1097	2989 ± 264
Ca (422nm)	5578.5 ± 386.5	8186.5 ± 1358.5	Not Present
Ca (558nm)	3478.5 ± 121.5	5491.5 ± 1941.5	Not Present
Na (589nm)	9513 ± 1361	29139.5 ± 2096.5	Not Present
Ca (616nm)	5868.5 ± 226.5	8808.5 ± 3346.5	Not Present
Ca (646nm)	3305 ± 128	4632 ± 1200	Not Present
H (656nm)	6218.5 ± 9.5	5996.5 ± 216.5	5054 ± 469
O (777nm)	4245 ± 251	4132 ± 307	3507 ± 527

Table 9: Sample 13 Bleach

Element	Control	Gas	Liquid
Mg (266nm)	33227 ± 2321	37779.5 ± 2560.5	35427.5 ± 6167.5
Ca (393nm)	10888 ± 5412	27689 ± 12394	9000.5 ± 518.5
Ca (396nm)	7434.5 ± 3781.5	20520 ± 9326	6159.5 ± 296.5
Ca (422nm)	6585.5 ± 2888.5	8094.5 ± 1783.5	3435 ± 34
Na (589nm)	56279 ± 62	49474.5 ± 1014.5	10238.5 ± 4539.5
H (656nm)	6056 ± 36	5808 ± 33	6161.5 ± 780.5
K (766nm)	25749 ± 1005	28316 ± 5915	2790
Yb (769nm)	18880 ± 859	21724 ± 4580	Not Present
O (777nm)	4092.5 ± 92.5	4120.5 ± 22.5	4204.5 ± 768.5
Na (819nm)	6837 ± 68	7510.5 ± 1522.5	Not Present

Table 10: S13 Heat

Element	Control	Gas	Liquid
C (247nm)	Not Present	Not Present	2254 ± 52
Mg (266nm)	30647.5 ± 6049.5	20934.5 ± 277.5	24752.5 ± 659.5
Mg (280)	5170	3680.5 ± 713.5	Not Present
Ca (393nm)	17237 ± 3321	12516 ± 3329	3681 ± 313
Ca (396nm)	11847.5 ± 2074.5	8504.5 ± 2240.5	2418.5 ± 98.5
Ca (422nm)	7240.5 ± 1866.5	4920 ± 894	Not Present
Ca (527nm)	3868	2804	Not Present
Ca (558nm)	4378.5 ± 1210.5	3713	Not Present
Na (589nm)	8786 ± 1775	16427 ± 6187	2858
Ca (612nm)	5066 ± 1086	3447.5 ± 774.5	Not Present
Ca (616nm)	7099 ± 1405	4781.5 ± 1123.5	Not Present
Ca (646nm)	3742.5 ± 681.5	2920.5 ± 511.5	Not Present
H (656nm)	5253 ± 672	5627.5 ± 196.5	5350 ± 188
O (777nm)	4036	3912 ± 184	3972 ± 333

Table 11: Sample 13 Salt

Element	Control	Gas	Liquid
Mg (266nm)	26099.5 ± 3554.5	37069.5 ± 4881.5	31386.5 ± 4414.5
H (393nm)	5894 ± 876	7956.5 ± 2237.5	4514 ± 513
Ca (396nm)	3969 ± 426	5486 ± 1409	3042.5 ± 405.5
Ca (422nm)	3395.5 ± 264.5	5806.5 ± 413.5	2153
Na (589nm)	54072.5 ± 4351.5	56117.5 ± 2985.5	11219
Ca (616nm)	Not Present	3704.5 ± 496.5	2176
H (656nm)	4467 ± 205	7628.5 ± 252.5	5223 ± 280
O (777nm)	3119	5544.5 ± 301.5	3469 ± 233
Na (819nm)	4814.5 ± 672.5	7086 ± 811	Not Present

Table 12: Sample 13 UV

Element	Control	Gas	Liquid
Mg (266nm)	36385.5 ± 1663.5	26025 ± 826	33673.5 ± 4620.5
Mg (280nm)	4070.5 ± 41.5	3596 ± 500	Not Present
Ca (393nm)	11768 ± 1270	13858 ± 1279	5869.5 ±2057.5
Ca (396nm)	7951.5 ± 692.5	9310.5 ± 809.5	3984 ± 1317
Ca (422nm)	4561.5 ± 373.5	6091.5 ± 447.5	2807
Ca (558nm)	Not Present	3355	Not Present
Na (589nm)	20228 ± 3073	51262 ± 5290	5500 ± 370
Ca (616nm)	3544 ± 105	4518 ± 752	2015
Ca (646nm)	Not Present	3040	Not Present
H (656nm)	5943 ± 115	5172.5 ± 976.5	5851.5 ± 720.5
K (766nm)	6003	Not Present	Not Present
O (777nm)	3750.5 ± 223.5	4528	3969 ± 660

Table 13: Sample 13 Detox Shampoo

Element	Control	Gas	Liquid
C (247nm)	Not Present	Not Present	2286 ± 204
Mg (266nm)	21597 ± 1653	22615 ± 1691	28483.5 ± 1150.5
Ca (393nm)	7289.5 ± 63.5	9333 ± 1018	4578 ±422
Ca (396nm)	4896.5 ± 87.5	6164 ± 806	3131 ± 232
Ca (422nm)	3153	4109	Not Present
Na (589)	21238.5 ± 2338.5	27983 ± 4659	4533.5 ± 559.5
Ca (616nm)	Not Present	3330	Not Present
H (656nm)	5800.5 ± 444.5	6177.5 ± 411.5	6033 ± 564
O (777nm)	4057 ± 251	4908 ± 446	4563 ± 345