5-2011

Post-operative Extraction of Oxycodone from Human Hair

Sarah Clickner
University at Albany, State University of New York

Post-operative Extraction of Oxycodone from Human Hair

Sarah Clickner

Objective:

The purpose was to extract oxycodone in human hair using gas chromatography-mass spectrometry, and to determine what other compounds could be extracted and the complications they can cause with a routine drug screening.

Introduction:

Oxycodone is an opiate drug prescribed for pain relief (U.S. Department of Justice). As pain medications such as oxycodone increasingly appear in drug screening, a need exists to detect and quantitate them (Moore, Marinetti and Coulter). The analysis of human hair can provide insight about a person's drug use. Hair has the benefits that it is simple to collect, difficult to alter and serves as a longer detection window (Barroso, Dias and Vieira). Extraction of opiate pain medications with methanol provides for adequate extraction and without the need for a pH adjustment. The results of extraction with methanol can be improved by incubating the solution overnight (Barroso, Dias and Vieira). Hair was collected from a subject that had anterior cruciate ligament (ACL) reconstruction and prescribed oxycodone for pain relief after surgery. Hair was harvested, cut into four centimeter sections, and diced. The extraction procedure involved incubation of each section in a 25:25:50 mixture of methanol:acetonitrile:ammonium formate (2 mM). Oxycodone was not detected in the first 20 cm of hair. The extraction solvent proved useful for extracting several understandable compounds from the complex matrix of hair that were identified as fatty acids and components of cosmetics.

Background:

Oxycodone is an opiate drug prescribed for the treatment of moderate to severe pain. Pain relief is provided by acting on opioid receptors in the spinal cord, brain and possibly tissue. Oxycodone produces euphoric effects similar to heroin making it prone to abuse (U.S. Department of Justice). The abuse of oxycodone was first noted as early at the 1960's, when it was first placed on the Drug Enforcement Administration's Schedule II drug list. The reports of abuse increased when Oxycotin, the long acting formulation, was introduced at 1996. The larger dose of oxycodone present in Oxycotin increased the street value making it more profitable to sell (Kral). As the abuse levels increase, pain medications are increasingly found in drug

screenings. This created the need for methods to test for, and quantitate pain medications (Moore, Marinetti and Coulter). Human hair is one matrix that can be tested for pain medications.

The analysis of human hair can also provide evidence of chronic drug abuse. Compounds become trapped in hair during growth and remain in the mature hair strand. A strand of hair consists of the root, which lies below the skin and the keratinized shaft, which extends above the surface. Trace amounts of compounds found in blood circulation are transferred to the follicle. Any medication present in circulation will diffuse into the rapidly growing root and deposit in keratin structures in the cortex of hair surrounded by the cuticle (figure 1 in the appendix). The compound is trapped in the follicle and carried in the hair shaft as it grows (Wong and Tse). A compound, such as prescription medication, will remain in a band without diffusion through the shaft. This band makes it possible to estimate both the time of use and trends of use over periods of time (Tsanaclis and Wicks). The average rate of hair growth is approximately one centimeter per month with some individual variation. This estimate of growth can provide retrospective information about when use took place (Society of Hair Testing).

Hair is easy to collect, is difficult to alter, and has a long detection window when compared to urine and blood. Hair's structure makes it difficult to affect the distribution of compounds in the shaft (Barroso, Dias and Vieira), and length is the only limiting factor for the detection window (Moore, Marinetti and Coulter). However, individual variations and chemical treatments damage hair, and cause it to hold less compounds than it would otherwise. Damage to the shaft can cause a loss of up to one half the compound concentration it could originally hold (Tsanaclis and Wicks). Multiple samples can be taken after the initial sampling that have the potential to be identical with the exception of the difference in length caused by growth between sampling (Kintz, Bioanalytical procedures for detection of chemical agents in hair in the case of drug-facilitated crimes). Hair analysis is not able to detect recent use and takes typically five to six days for the compounds to appear above the scalp (Tsanaclis and Wicks).

Individual strands of hair are in different stages of growth in different places on the scalp. Dues to these differences, the sample is best taken from the back of the head, where the growth is most uniform (Kintz). The sample size taken must be adequate to allow for all necessary testing, enough to perform any screening and confirmatory testing needed (Moore, Marinetti and

Coulter). The sample should be the width of a pencil or several locks of hair in thickness. After collection, the samples should be stored to minimize decomposition and contamination (Society of Hair Testing). The preparation and extraction steps are the most important steps in analyte recovery (Eser, Potsch and Skopp).

A necessary precursor step in sample preparation is to wash the hair to prevent false positives from external contamination (Tsanaclis and Wicks). Passive contamination is caused by traces of the substance depositing on the surface of the shaft exterior, which can lead to a positive test when the actual result should be negative (Poletti, Stramesi and Vignali). The Society of Hair Testing recommends decontaminating samples with an organic solvent followed by aqueous washes (Society of Hair Testing). Skender et al. recommend performing two washes to ensure the third wash solution was always negative for drugs (Skender, Karacic and Brcic). Minimal analyte is lost from the interior of the sample due to such washing (Tsanaclis and Wicks).

The extraction technique plays a major role in the quantity of analyte recovered (Poletti, Stramesi and Vignali). Extraction is the transferring of an analyte from one phase to another. In solid-liquid exactions such as hair analysis, the solvent will selectively dissolve the analyte, and leaves behind components of the solid matrix.. An extraction method must be reproducible, and not decompose or react to the compounds of interest. The extraction solvent must have the ability to gain access to the interior of the hair shaft where the analyte is located in order for the extraction to be successful (Eser, Potsch and Skopp). The three main extraction methods for opiates are digestion in a basic solution, incubation in an acidic solution, and extraction in neutral solvent. Basic digestion will completely decompose acylated opiates, and allow for measurements of only equivalents of core structures, such as morphine and codeine. The acidic method does not completely destroy the hair sample but it can also decompose acylated opiates. Both the acid and basic methods have poor reproducibility (Balikova and Habrdova). Solvents such as methanol often has lower yields and can have large amount of background contamination from the hair matrix. Often times, a solvent mixture can be used to extract opiates from hair and give a cleaner spectrum. One of these solvent mixtures is a combination of methanol, acetonitrile and formate buffer (Nielsen, Johansen and Dalsgaard).

Once the drug of interest is extracted from the hair sample, they must be detected and identified. The detection of prescription drugs can be done using gas chromatograph/mass

spectrometry. Gas chromatography, GC, involves the separation and analysis of organic compounds that can be vaporized without decomposition. GC separates the components on the basis of their boiling points and/or volatility. The solvent used must not have a boiling point similar to the sample to avoid interference with the separation. The temperature for the gas chromatograph column should be approximately equal to the average boiling point of the sample for the results to have a reasonable elution time. When a sample has a broad boiling range, it is often desirable to employ temperature programming, which increases the temperature continuously or in steps as the separation proceeds (Crouch, Holler and Skoog). The column is packed with a stationary phase, which is usually a liquid, wax or low melting solid. The liquid used for the stationary phase is stable under the highest temperature required. In capillary columns, the stationary phase is a film of liquid a few tenths of a micrometer thick that uniformly coats the interior of the tubing (Crouch, Holler and Skoog). The sample is injected into the injection port of the chromatograph and immediately vaporized in a heated chamber. In split-sampling, a portion of the sample can run through the column as opposed to the entire sample, which enables the operator to control the amount of sample injected. The vapor is introduced into a moving stream of gas called the carrier gas. The carrier gas must be chemically inert, and must not react with either the sample or the stationary phase. Examples of carrier gases are nitrogen, helium and argon. The flow of the carrier gas can be adjusted to improve the separation of the components. The sample is swept into a column coated with a liquid adsorbent. As the sample passes through the column, it is subjected to numerous interactions between the liquid and the gas, and these interactions allow for separation of the different components. The length of time required for a sample to move through the column is a function of its volatility and how much time it spends interacting with the liquid phase. By selecting the correct temperature program and column, the components travel through the column at different rates and are separated. .

The detector that indicates when the different components leave the column is called a flame-ionization detector (FID). The FID ionizes the components as they leave the column. The resulting chromatogram is a recording of the electronic signals from the individually separated ionized components. A chromatogram plots the response as a peak, or peak area, on the vertical axis and time on the horizontal axis. The time required for each peak to emerge is the retention time. The retention time is characteristic of the compound under the specific conditions used for

separation (Forensic Chemistry Lab Manual I). One disadvantage of gas chromatography alone is that it does not provide information about the absolute identity of the components, although retention times of unknowns can be compared to reference standards as a good first indication. The chromatograph is often linked to a mass spectrometer for absolute identification.

The mass spectrometer (MS) provides structural information of the substances separated by the GC. Each mass spectrum is very specific to each component and allows for identification. As the components leave the GC, they are split: half of the sample goes to the GC detector and the other goes to the MS. When the compound enters the MS, it is sent under a vacuum. The sample is channeled into a beam of electrons that ionizes the sample (Forensic Chemistry Lab Manual II). Part of the MS is a mass selector, in this case, a quadrupole consisting of four parallel cylindrical rods that serve as electrodes. When sample ions enter the quadrupole, they are accelerated into the space between the rods. At any given time, all ions except those with a certain mass to charge ratio (*m/z*) strike the rods and reach the detector. The entire range of masses can be scanned to identify each component and their decomposition products (fragments). The detector converts the beam of ions into an electrical signal that results in a mass spectrum (Crouch, Holler and Skoog). The mass spectrum is a plot of the *m/z* on the x axis and abundance on the y axis. The spectrometer can either scan for all *m/z* or it can be set to scan a specific mass range. A scan for a specific mass range that is usually one or two mass units is called selective ion monitoring (SIM). SIM will detect and plot only the specified ions resulting in a more specific plot (Forensic Chemistry Lab Manual II).

The two major categories of ion sources are hard ionization and soft ionization sources. A hard source transmits more energy on the sample than a soft source and creates more sample fragments (daughter ions). Two common ionization methods are electron impact ionization and chemical ionization. In electron impact, electrons are emitted from a heated tungsten or rhenium filament. The electron beam and sample beam cross paths at right angles producing radical cations when the electrons come close enough to eject an electron from the sample. This radical cation represents the molecular ion and will lose energy by fragmenting into lower-mass daughter ions. All ions and charged daughter fragments are accelerated into the quadrupole and ultimately the detector. In chemical ionization, a reagent gas is co-introduced with a sample into the beam of ionizing electrons. The high energy electrons ionize the reagent gas as done in electron impact since it is found in greater abundance than the sample. The reagent ions interact

with the sample by the transfer of a proton or hydride. Electron impact was used for the data in this thesis.

The intensity of a given peak in the mass spectrum reflects the number of fragments having that specific *m/z*. The peak in the spectrum with the largest relative abundance is called the base peak. The intensity of the base peak is set at 100 and the intensity of every other peak is a percentage of the base peak. The base peak represents the most stable fragment for that compound, which is why it is found in the largest abundance. The largest significant mass that is found in the spectrum is the molecular ion, which is most often times the mass of the radical cation formed from the initial ionization of the molecule. The mass of a fragment can be subtracted from the mass of the molecular ion to get the *m/z* of a significant peak found in the spectrum (Forensic Chemistry Lab Manual II).

The chemical components extracted from hair were tested in this fashion. Hair is a complex and difficult matrix The trace components of opiates must be in high enough levels that they can be extracted and detected by these methods. Both the extraction and detection method play a critical role in the detection of medications in hair.

Case Study:

The hair was taken from twenty year old female who had ACL reconstruction, and prescribed oxycodone to aid in pain relieve after surgery. The surgery took place on July 30, 2009, and 7.5 mg oxycodone pills were taken for approximately a week for a total of 45 pills. A second surgery was required approximately four months later. After the second surgery, hydrocodone was taken for pain relief. The total dose of hydrocodone ingested was approximately 37.5 mg.

Two hair samples were collected approximately eight months after surgery. Each sample was the width of a pencil, and located on the back of the head.. The hair was cut as close to the scalp as possible with scissors, approximately 30 cm in length. The hair was stored at room temperature. Pictures of the hair can be found in the appendix (figures 2-5). Since oxycodone was the only controlled substance expected to be found in the hair, extraction procedures involving both plain methanol, as well as methanol, acetonitrile and formate buffer mixtures were performed.

Experimental:

A standard of oxycodone (Cerillant lot FE062707-01) was obtained. The standard had 1.0 mg of oxycodone present in 1 mL of methanol. Approximately 2 µL of the standard were manually injected into the Hewlett Packard 6890 series chromatogram and HP 5972A selective mass detector using the parameters found in table 1 of the appendix.

The hair was measured and banded in four centimeter increments as seen in the appendix. The sections were separated using a razor blade. Analysis began with the hair sections closest to the root. The hair was washed with 2 mL of each of dichloromethane, deionized water, and methanol for two minutes. The sections were diced, and placed in round bottom flasks with enough extraction solution added to coat the hair.

The extractions on the right side of hair were performed with methanol. The methanol extractions were injected directly into the Hewlett Packard 6890 series chromatogram and HP 5972A selective mass detector. The extraction of the left side of hair had an extraction solution of a 25:25:50 mixture of methanol:acetonitrile:2 mM ammonium formate with 8 % acetonitrile. The round bottom flask containing the extraction solution and hair was set up in a reflux apparatus, the solution was brought to a gentle boil and left overnight. The liquid was decanted from the hair and divided into four portions. Three of the portions were saved for later analysis. The one quarter was evaporated to complete dryness using the gentle stream of nitrogen. This was then reconstituted using approximately 0.5 mL of methanol. The methanol was injected into the GC/MS.

Results:

The chromatogram and mass spectrum of the standard are figures 11 and 12 in the appendix. The peak at 13.26 min was identified as oxycodone. The peak was matched to oxycodone with a quality of 99% using a NIST02 library search.

The chromatograms for all other extractions can be found in the appendix. The chromatograms for the methanol extractions had few peaks. None of the medications taken after surgery were detected after the methanol extractions. It was hypothesized the medications were left behind in the hair's interior or in low enough levels that they were not possible to detect. The extraction mixture of methanol, acetonitrile and formate buffer found many more peaks. The appendix has a comparison of the chromatograms from both extraction methods.

Several compounds were identified in the chromatogram of the extraction with the solvent mixture. Tetradecanoic acid, hexadecanoic acid, 1-hexadecanol and hexadecanamine were detected in each section of hair with relatively the same retention time. Tetradecanoic acid and hexadecanoic acid are both fatty acids that differ only in the length of their carbon chain. Tetradecanoic acid is shorter then hexadecanoic acid by two carbons (methylene groups). Tetradecanoic acid is commonly found in cosmetics and topical medications. Hexadecanoic acid is the most common fatty acid in plants and animals. 1-hexadecanol and dexadecamide are similar to hexadecanoic acid but with different terminal functional groups. 1-hexadecanol is an alcohol that has the same length as hexadecanoic acid. 1-hexacanol is used as a surfactant in shampoos or as an emollient, emulsifier, or thickening agent in creams and lotions. Hexadecamide has an amide group as opposed to the carboxylic acid group on hexadecanoic acid. Hexadecanamide is commonly used as an ingredient in low irritation shampoo and liquid detergents.

Discussion/Conclusions:

Several published papers had derivatized the extract from hair before analysis with GC/MS. A compound must be volatile enough to enter the gas phase in order for it to be detected using GC/MS. Derivitizations are used to make the compound of interest more volatile and detectable using GC/MS. The oxycodone standard had one large peak at approximately 13.26 min (see figure 11 in the appendix). The mass spectrum for this peak (see figure 12 in the appendix) was matched to oxycodone using a library search. The mass spectrum had a molecular ion peak of 315 *m/z*, which is the molecular weight of oxycodone. The fact that the oxycodone standard was detected by the GC/MS meant there was no need to derivatize the extract. If the oxycodone standard had not been detected, this would indicate the oxycodone structure was not volatile enough and all future samples would need to be derivatized. .

There are several methods cited in the literature in use for the extraction of opiate drugs from hair. Different methods were experimented with to determine the best extraction procedure, and the solvent mixture mentioned above gave the best results.

The right section of hair was involved in method development with methanol. Figures 13 and 14 in the appendix show the extractions for sections 1 and 2. Sections 1 and 2 did not show many peaks in the chromatogram. The lack of peaks in both chromatograms indicated the

solvent may not have had access to the interior of the hair shaft. A sonication step was added to the extraction method for the third section to try to disrupt the hair structure, giving the solvent access to the interior of the shaft. Sonication allowed several additional peaks in section 3 (figure 15 in the appendix). The appearance of more peaks proved that sonication was a useful step in extracting more peaks from the hair sample.

After no oxycodone was detected, the left section was analyzed using the mixture of methanol, acetonitrile and formate buffer. The procedure incubated the solution overnight, decanting and evaporating the liquid, with reconstitution using methanol. The sonication step was left out to determine if the solvent alone could extract any peaks. Many more peaks were detected using this method, see figures 16-20 in the appendix. Although this method did not extract oxycodone, it did prove to be more efficient in extracting other compounds present in hair. One section of hair was sonicated with the solution after the initial extraction procedure was performed on it (figure 23). The sonication step did not extract any other compounds.

The chromatogram overlays (figures 21 and 22) demonstrate the great similarities in the peaks between the left side sections of hair. The chromatograms have peaks at similar locations that have similar mass spectra. The one difference between the peaks are their relative abundances. The differences in abundance could indicate changes in the person's habits over a period of time. More analysis on the actual identity of the peaks and their possible sources is needed to draw any conclusions about why there is a difference in abundance. The similarities in the retention times of all the peaks are an indication that there may be a number of compounds that are found throughout the entire length of hair.

The literature cites oxycodone to have a bioavailability of 60-80%. Bioavailability is the fraction of an administered dose of unchanged medication that reaches circulation. The total dose of oxycodone taken by the subject was 337.5 mg. The amount of unchanged oxycodone that reaches circulation due to the bioavailablity would be between 202.5 and 270 mg. Renal excretion of free oxycodone estimated to be up to 20%. There are several metabolic pathways that would further decrease the amount that would be found in blood circulation. The amount of unchanged oxycodone that would be available after metabolism and excretion would be between 50 – 125 mg (Kral). These numbers demonstrate that only a portion of the total dose will remain unchanged, and available to be incorporated into hair.

The literature states that only trace portions of medications are incorporated into hair, which further limited the amount that would diffuse into the shaft. A small portion of the original dose will remain unchanged after metabolism and excretion. Due to the mechanism that medication is incorporated into hair, only trace amounts of this already small amount will diffuse into the hair shaft. The fraction of hair analyzed was approximately one fiftieth the total amount of hair on a head. Trace amounts of the drugs would be incorporated into the hair and the fact that a small sample of hair was tested further diminishes the amount of oxycodone likely to be detected. Even if the entire amount of oxycodone was extracted from the hair sections, the limits of detection for the current instruments may not be low enough to detect that trace amount.

Detection of medications in hair can distinguish between one time use and habitual abuse. While a large dose of oxycodone was taken after surgery, it was not detected using the current method. Habitual abuse of oxycodone taken in even larger doses over an extended period of time would have a greater possibility of being detected.

Future Work:

Oxydocone was not extracted from any section of hair. Since oxycodone does exist in the interior of the hair, a method must be identified that can extract and detect the drug. The limits of detection should be improved to detect the smaller concentrations. The extraction efficiency must be tested to ensure that no oxycodone remained in the hair after the extraction was performed. This could be done by taking a section of hair known to not contain oxycodone, and spike it with a known concentration. The extraction procedure should be carried out, and the quantified. The concentration extracted should then be compared to the concentration that was originally used. This difference between these amounts would be revealing how efficient the extraction procedure was. In order to get a true measure of efficiency, it is preferable to use the hair from the same patient that the original sample was taken from, and using a section known to not contain pain medications.

The chromatogram had many peaks that still need to be identified. The peaks could be identified using a library search. A standard of the compound identified should be obtains and the mass spectra should be compared to confirm the identity. There were three wash steps used for each hair segment prior to dicing. The solvents decanted after each wash must be tested to

prove that there was no analyte loss due to the wash steps. The washes should also be compared to the chromatograms to determine if there were any similar compounds on the surface and on the interior.

The compounds that were currently detected in the extractions of the hair could also be compared to compounds found on the same patient's skin. If different compounds were detected on the skin, that would indicate that the compounds detected in hair were extracted from the interior of the shaft and not found in the surface. This comparison could be done by simply swabbing the patient's skin and extracting that swab. The extraction should be injected using the same GC/MS parameters as found in the appendix.

After the first surgery, 800 mg of Advil were taken several times a day for about two months after surgery. The chromatograms should be examined to determine whether or not ibuprofen, the active ingredient of Advil, is present in any of the hair sections. A muscle relaxer and a medication for nausea were also prescribed and taken in smaller doses. While none of these substances were yet identified, the improved extraction method had numerous peaks, some of which were identified and logical in the context of the biological matrix (hair). Controlled spiked serial dilutions would provide us our limit of detection and allow us to back calculate the probability that we could detect the medication based on the concentrations taken during recovery of surgery.

References

1. Balikova, M. A. and V. Habrdova. "Hair analysis for opiates: Evaluation of washing and incubation procedures." Journal of Chromatography B (2003): 93-100.
2. Barroso, M., et al. "Simultaneous quantitation of morphine, 6-acetylmorphine, codeine, 6-acetylcodeine and tramadol in hair using mixed-mode solid-phase extraction and gas chromatography-mass spcetrometry." Anal. Bioanal. Chemistry (2009).
3. Crouch, Stanley R., F. James Holler and Douglas D. Skoog. Principles of Instrumental Analysis. California: Thomson, 2007.
4. Eser, Hans P., et al. "Influence of sample preparation on analytical results: drug analysis (GC/MS) on hair snippents versus hair powder using various extraction methods." Forensic Science International (1997): 271-279.
5. Forensic Chemistry Lab Manual I. University at Albany, Department of Chemistry, Fall 2009.
6. Forensic Chemistry Lab Manual II. University at Albany, Department of Chemistry, Spring 2010.
7. Kintz, Pascal. "Bioanalytical procedures for detection of chemical agents in hair in the case of drug-facilitated crimes." Anal. Bioanal. Chem (2007).
8. Kintz, Pascal, et al. "Testing for GHB in hair by GC/MS/MS after single exposure. Application to document sexual Assult." Journal of Forensic Science (2003): 195-200.
9. Kral, Lee A. "Commonsense Oxycodone Prescribing and Safety." Pain Treatment Topics (2001).
10. Moore, C., et al. "Analysis of pain mangement drugs, specifically fentanyl, in hair: Application to forensic specimens." Forensic Science International (2008): 47-50.
11. Nakahara, Y., M. Shimamine and K. Takahashi. "Hair analysis for drugs of abuse. III. Movement and stability of methoxyphenamine (as a model compound of methamphetamine) along hair shaft with hair growth." Journal of Analytical Toxicology (1992): 253-257.
12. Nielsen, Marie Katrine Klose, et al. "Simultaneous screening and quantification of 52 common place pharmaceuticals and drugs of abuse in hair using UPLC-TOF-MA." Forensic Science International (2010): 85-92.
13. Poletti, Aldo, et al. "Determination of opiates in hair. Effects of extraction methods on recovery and on stabilitiy of analytes." Forensic Science International (1997): 259-269.
14. Skender, L., et al. "Quantitative determintation of amphetamines, cocaine, and opiates in human hair by gas chromatography/mass spectrometry." Forensic Science International 125.2-3 (2002): 120-126.
15. Society of Hair Testing. "Recommendations for hair testing in forensic science." Forensic Science International (2004): 83-84.
16. Tsanaclis, Lolita and John F.C Wicks. "Patterns in drug use in the United Kingdom as revealed through the analysis of hair in a large population sample." Forensic Science International (2007): 121-128.
17. U.S. Department of Justice. "Oxycotin Diversion, Availability, and Abuse." Intelligence Bulletin. 2004.
18. University at Albany, Department of Chemistry. Organic Chemistry Lab Manual. Cengage Learning, 2008-2009.

19. Wong, Raphael C. and Harley Y. Tse. Drugs of Abuse: Body Fluid Testing. Totowa, NJ: Humana Press, 2005.

Appendix Table of Contents

Appendix

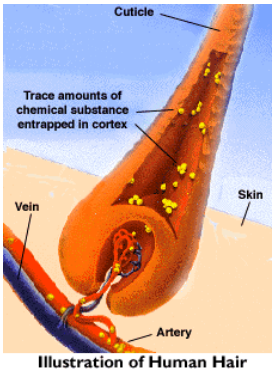

Figure 1: Illustration of Human Hair (http://www.drugtestwithhair.com/questions.php)

Figure 2: Right Side Sample

Figure 3: Left Side Sample

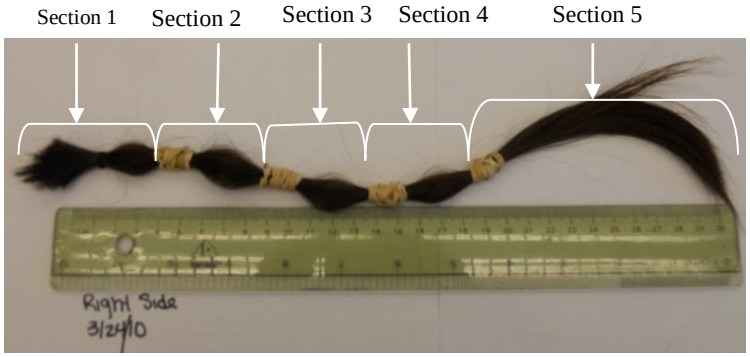

Figure 3: Right Side Sample Banded

Figure 5: Right Side Sample Cut

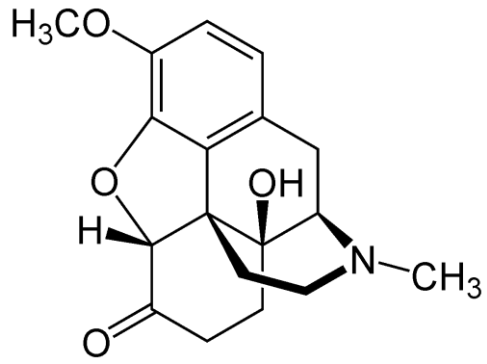

Figure 6: Structure of Oxycodone

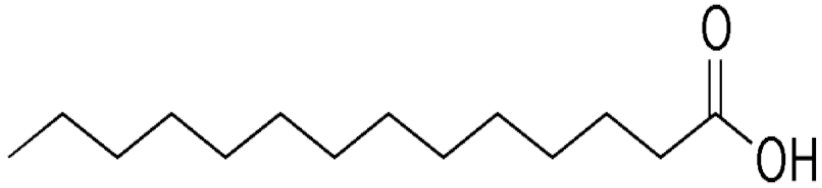

Figure 7: Structure of Tetradecanoic Acid

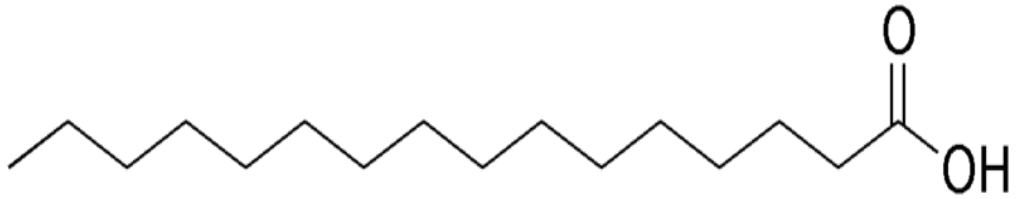

Figure 8: Structure of Hexadecanoic Acid

Figure 9: Structure of Hexadecanol

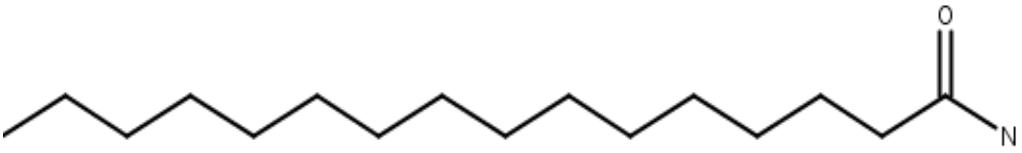

Figure 10: Structure of Hexadecamide

Table 1: GC/MS parameters

Inlet Temperature	280 °C
Injection Volume	1 µL
Gas Flow Rate	1 mL/min
Split Ratio	50:1 (25:1 for calibration curve)
Initial Oven Temperature	50 ˚C held for 1 min
Temperature Ramp Rate	20 °C/min
Final Oven Temperature	300 °/min held for 1 min
Transfer Line Temperature	280 ˚C
Solvent Delay	2 min

Figure 11: Chromatogram of Oxycodone Standard

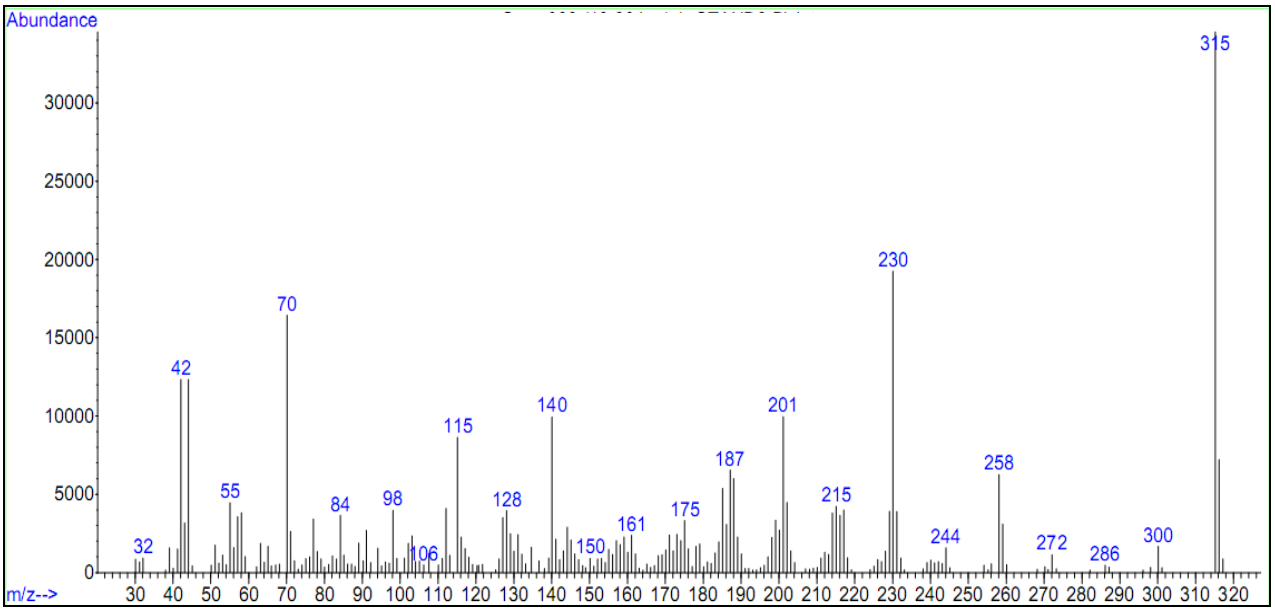

Figure 12: Mass Spectrum of Oxycodone Standard

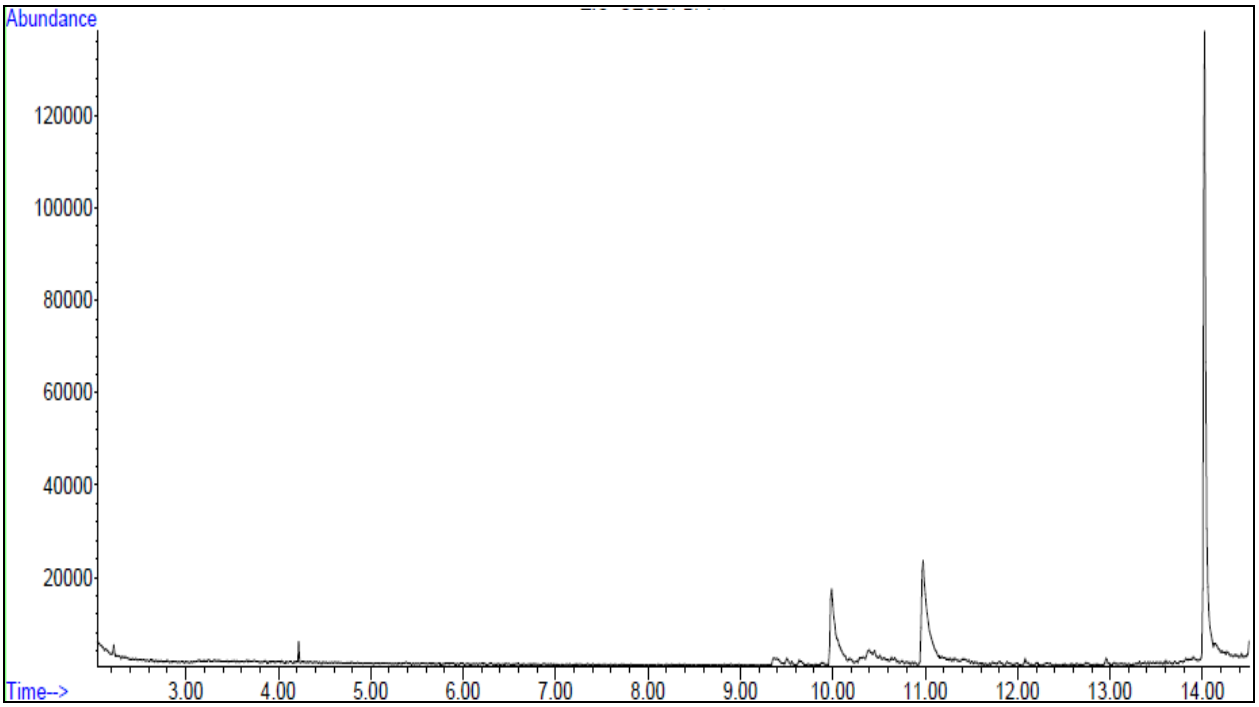

Figure 13: Chromatogram of Right Side Section 1

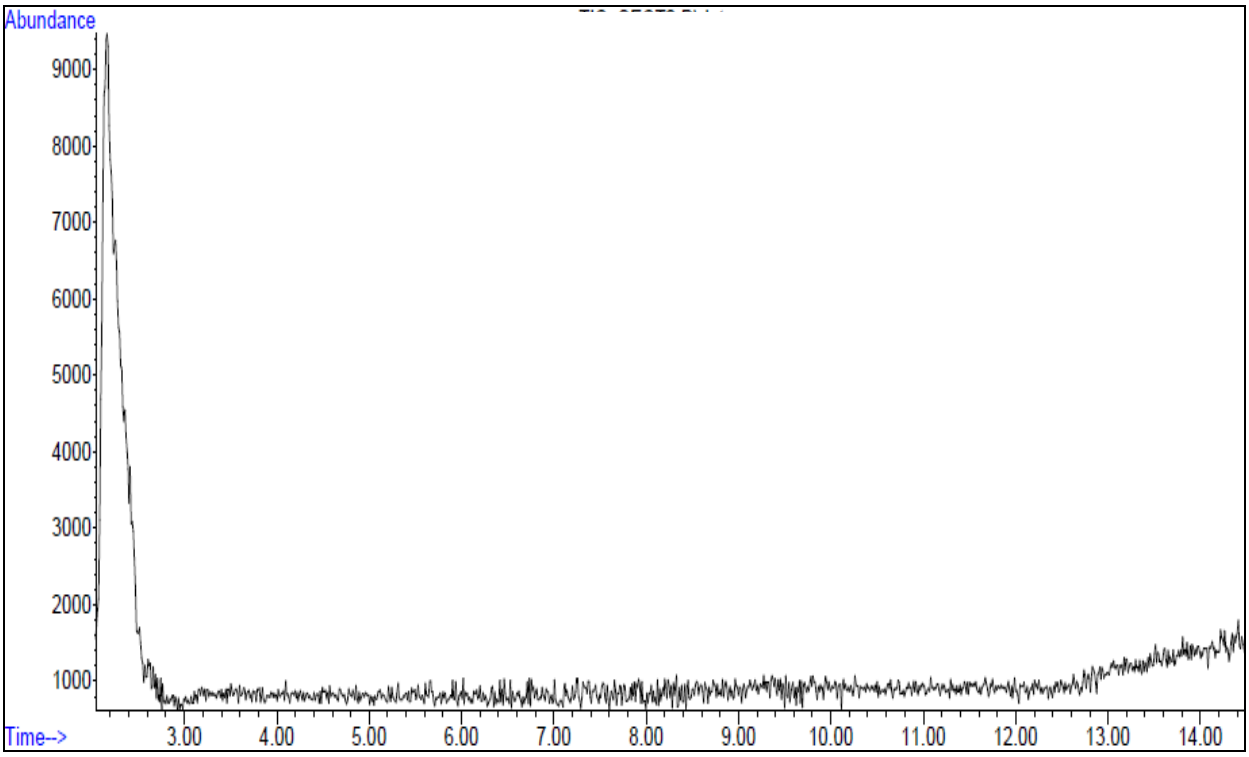

Figure 14: Chromatogram of Right Side Section 2

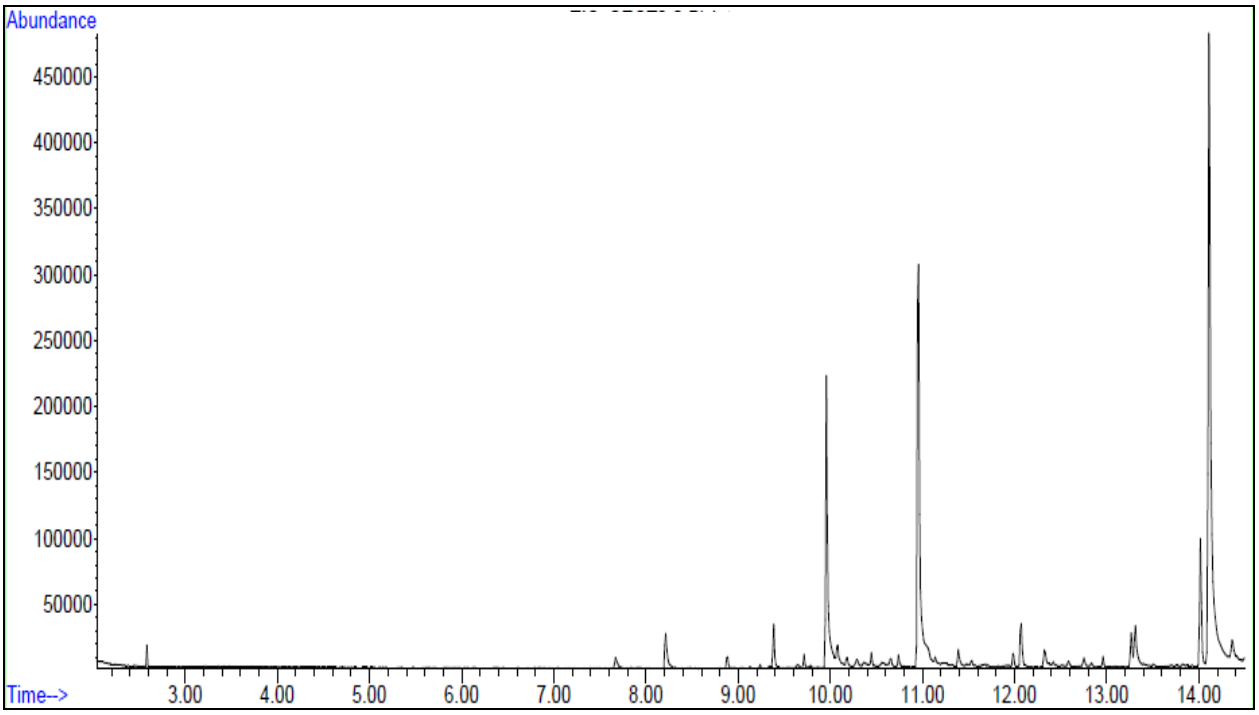

Figure 15: Chromatogram of Right Side Section 3

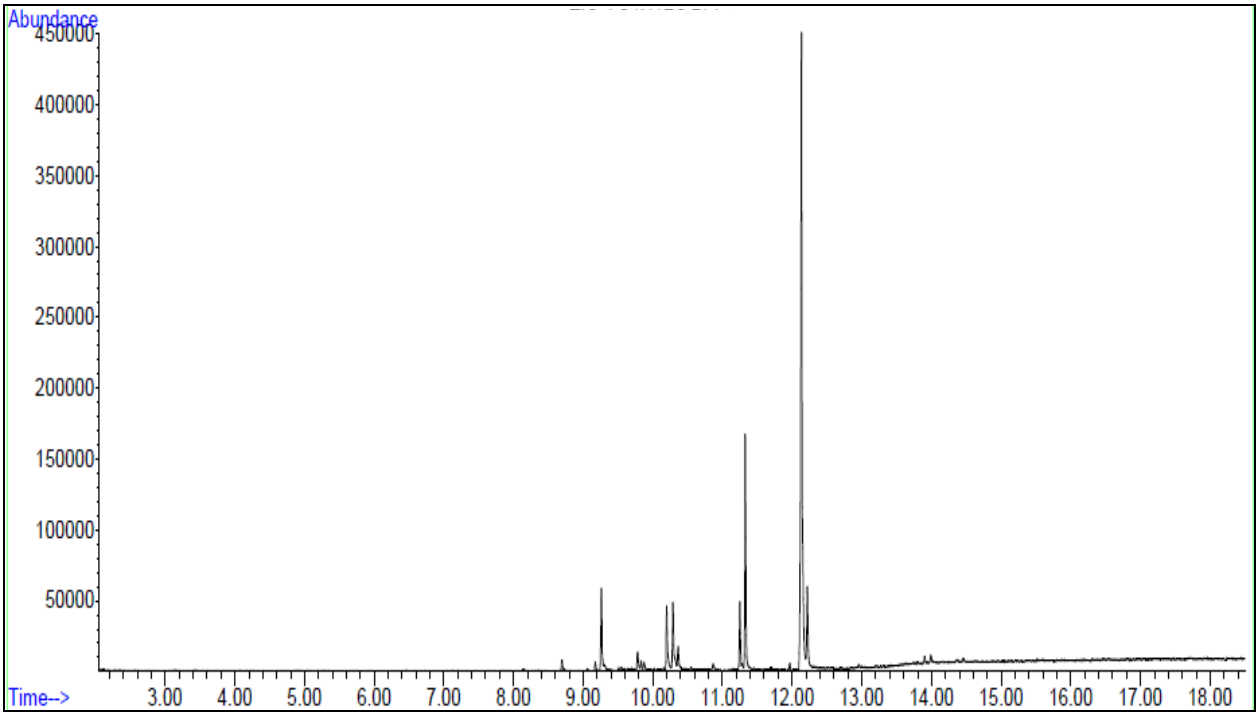

Figure 16: Chromatogram of Left Side Section 1

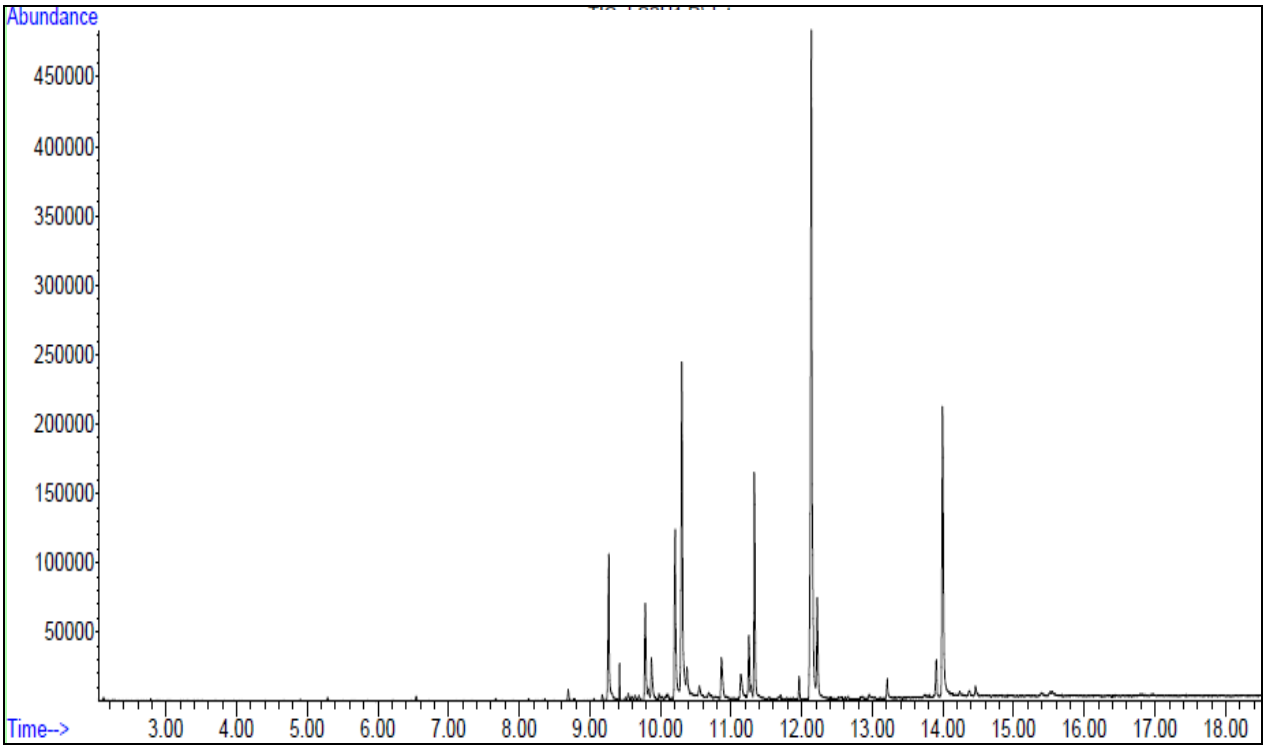

Figure 17: Chromatogram of Left Side Section 2

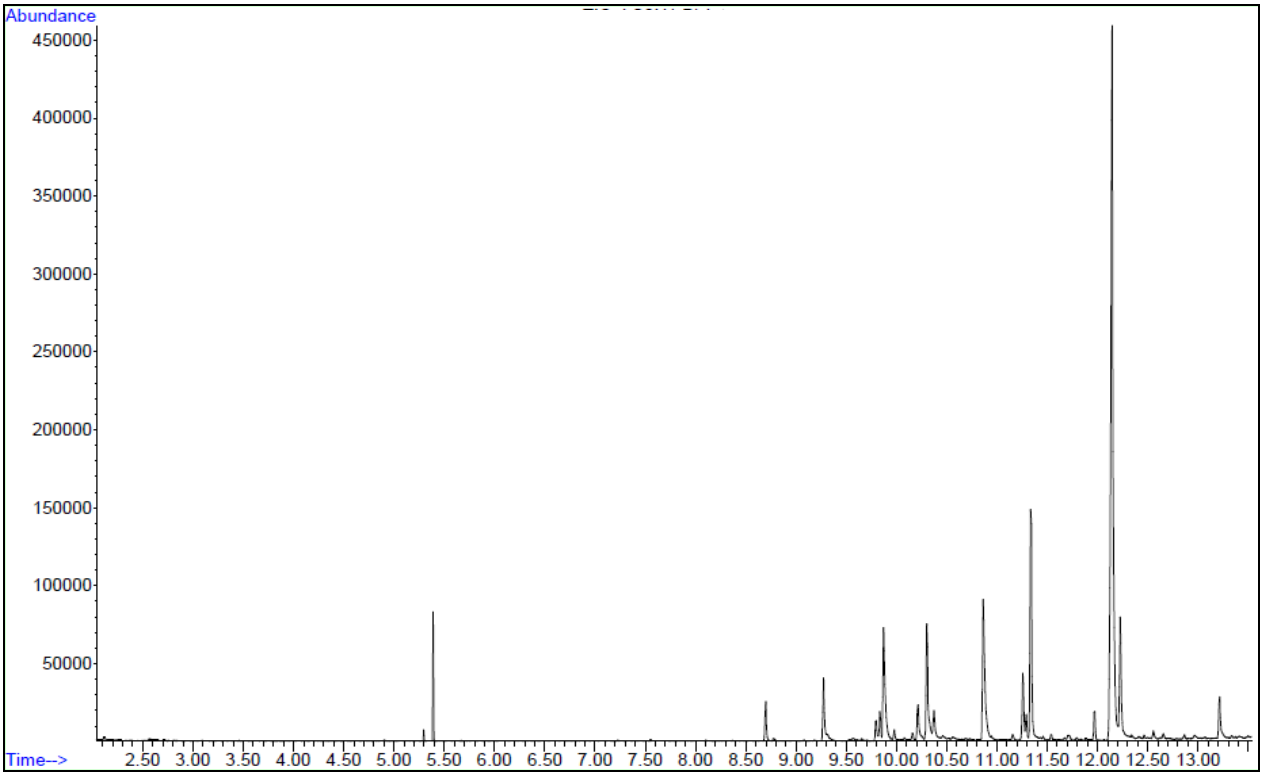

Figure 18: Chromatogram of Left Side Section 3

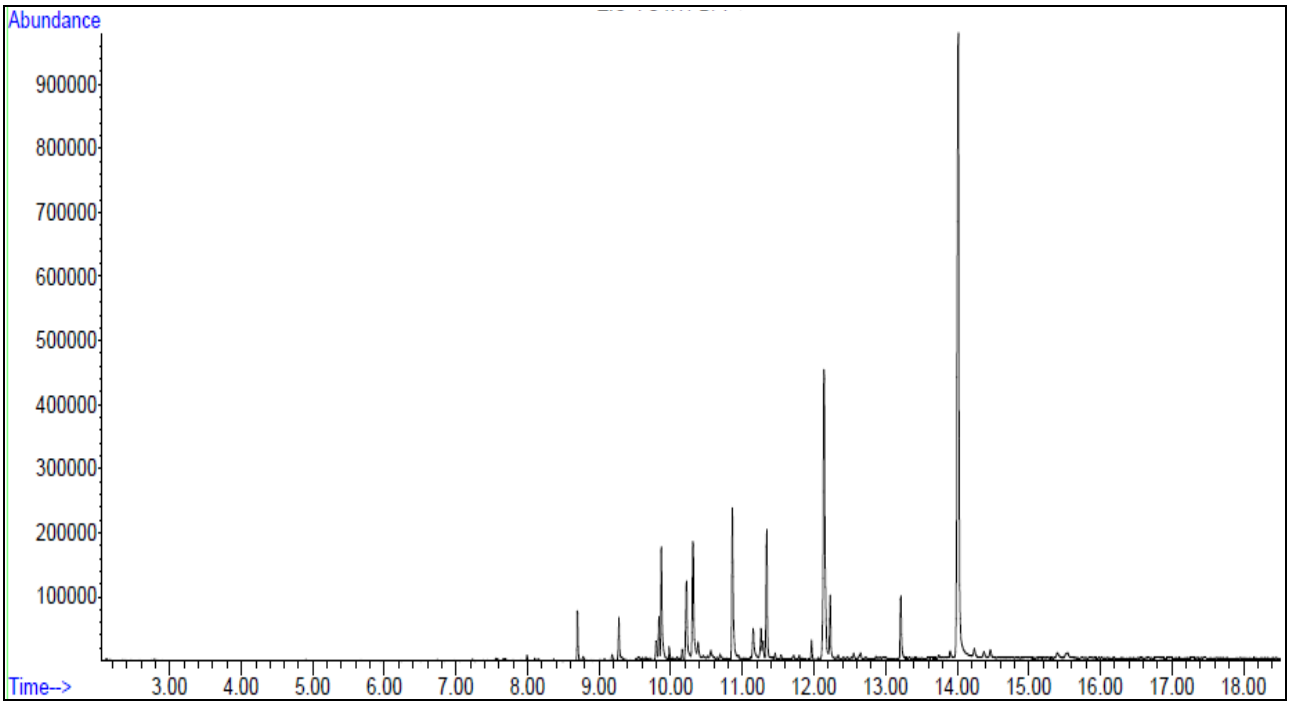

Figure 19: Chromatogram of Left Side Section 4

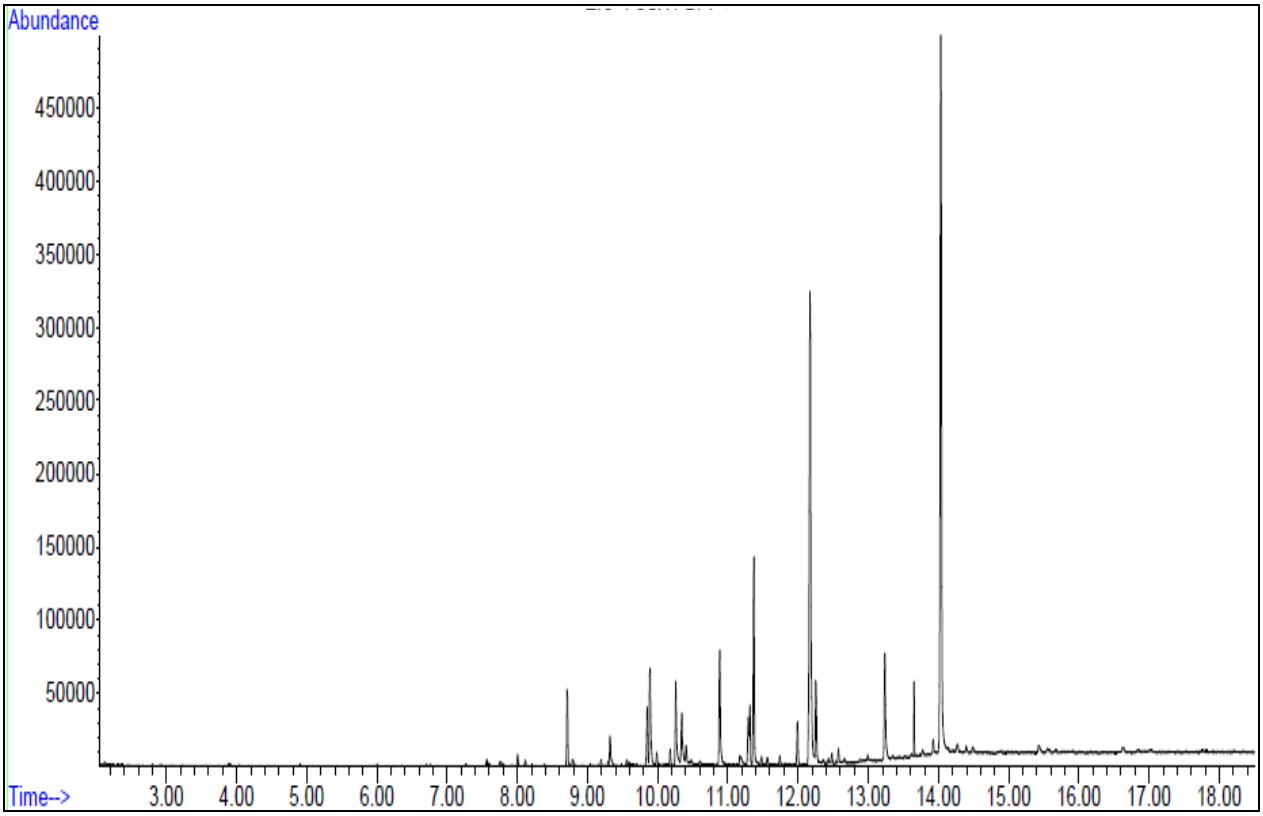

Figure 20: Chromatogram of Left Side Section 5

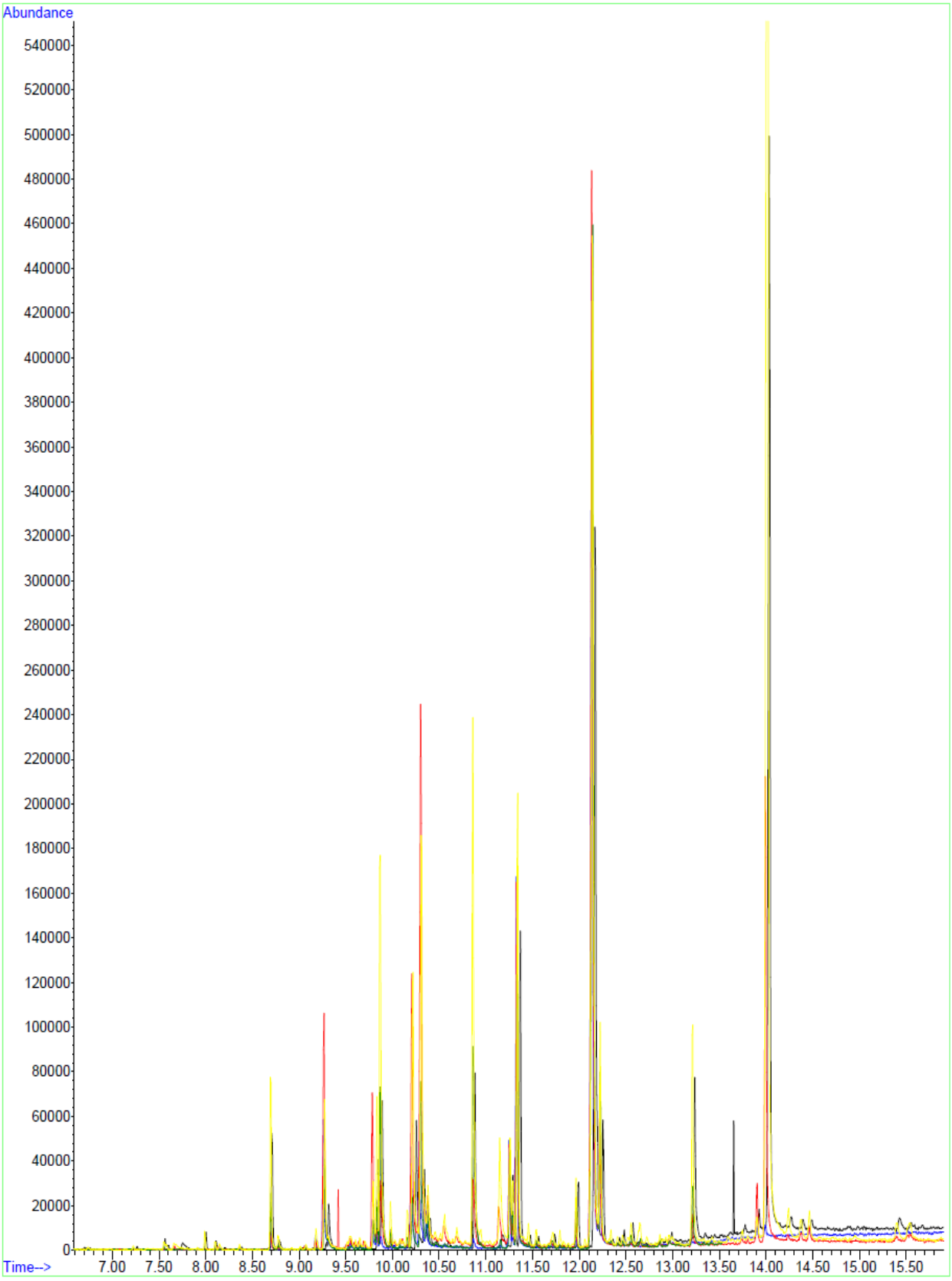

Figure 21: Left Side Extractions Chromatogram Overlay

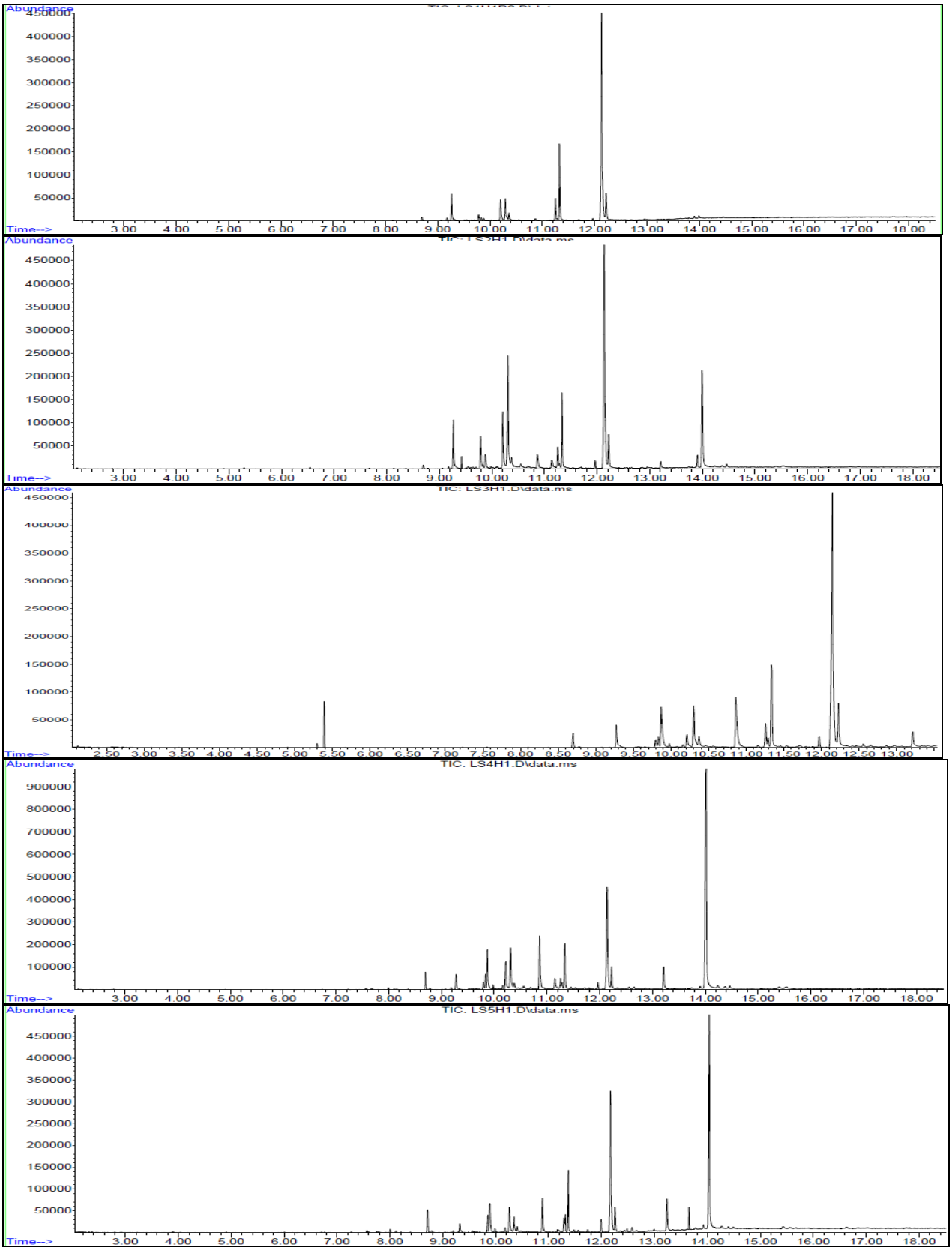

Figure 22: Left Side Extractions Expanded Chromatogram Overlay

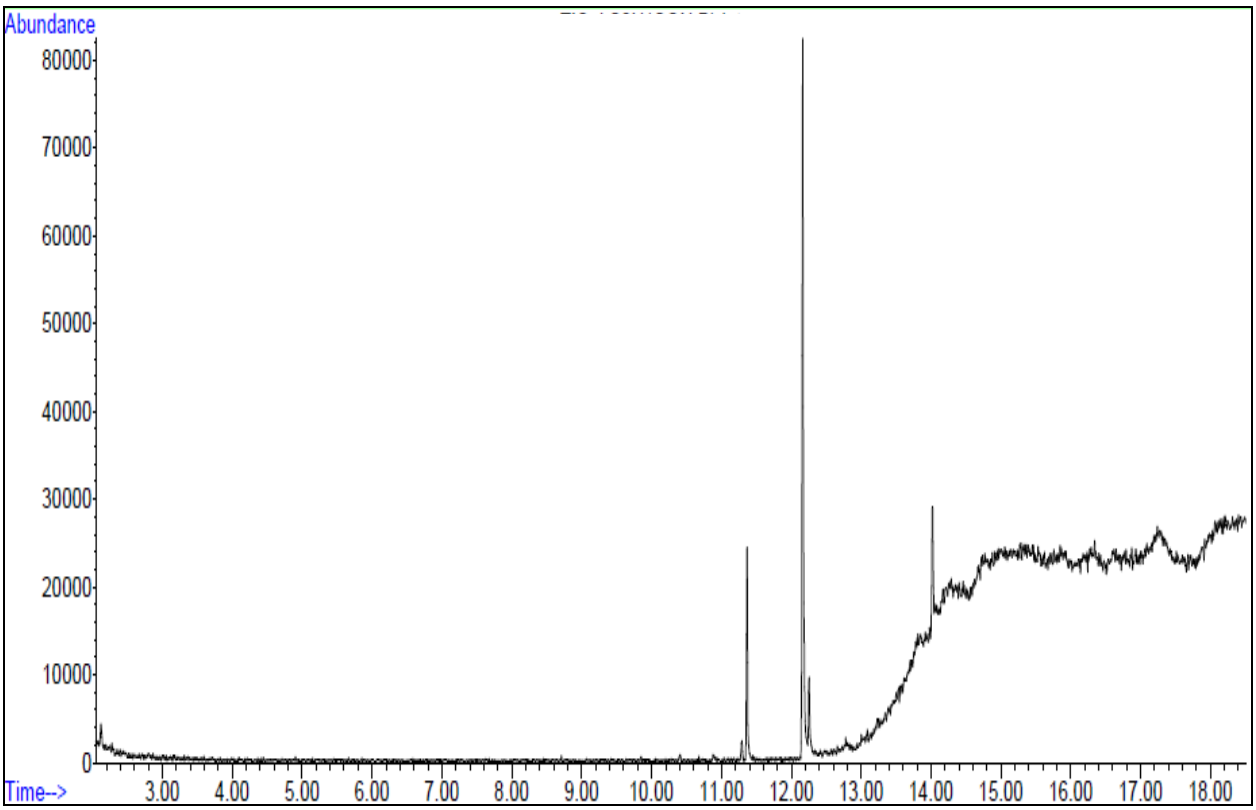

Figure 23: Chromatogram of Left Side Sonication