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Chemical Analysis of Processed Human Hair Extensions for Use in Forensic Casework

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Chemical Analysis of Processed Human Hair Extensions for Use in Forensic Casework

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Abstract

In forensic laboratories, hair analysis is performed in the trace evidence unit and the DNA unit. The completed analysis of hair can include a variety of tests, but generally, microscopic analysis is performed and is followed by mitochondrial DNA analysis. With the increasing use of hair extensions, it is possible that a hair sample discovered at a crime scene may be a processed human hair extension and has no physical or genetic connection to the individual wearing the hair extensions. This can be misleading in an investigation and result in a misuse of time and resources. Being able to identify a hair as a processed human hair extension is valuable in determining its entire evidentiary value and result in the forensic laboratory adjusting its approach to analysis.

Current literature is limited pertaining to hair extensions as forensic evidence. Only one study has focused extensively on processed human hair extensions for their probative value in forensic case work. This study involved the microscopic examination and genetic analysis of processed human hair extensions but found no features that differentiated natural human head hairs from human hair extensions. There are multiple articles that research chemical analysis of hair, but few are looking into applications into the forensic laboratory. The lack of literature proves the necessity for this research.

This research determined whether a chemical difference was present between natural human head hair and processed human hair extensions through ultraviolet-visible spectroscopy, gas chromatography/mass spectrometry, and nuclear magnetic resonance spectroscopy. Three different hair extension brands and five different natural human hair samples were used in this research. Multiple organic solid-liquid extractions were completed using hexanes, Colorist Secrets™ Hair Color Remover, methanol, 1-octanol, and chloroform-d. The extraction solvents

were then used for instrumental analysis by ultraviolet-visible spectroscopy, gas chromatography/mass spectrometry, and nuclear magnetic resonance spectroscopy. The resulting spectra were analyzed and compared to determine differences between processed human hair extensions and natural human head hairs.

This study proves that there is a chemical difference between processed human hair extensions and natural human head hair. This was indicated by the differences observed in spectra on the UV-Vis, GC/MS, and NMR between the natural human head hairs and processed human hair extensions. The processed human hair extensions have a higher absorbance, when analyzed with UV-Vis spectroscopy than the natural human head hair samples. In addition, there are specifics in the processed human hair extensions that are visualized by GC/MS and NMR analysis that are not present in the control natural human head hair samples. The higher absorbance and peaks show a chemical difference between the samples. This chemical difference can be used to differentiate samples in a forensic laboratory, which could change how hair is analyzed for casework.

This research is significant because it allows for the chemical identification of processed human hair extensions which may help in determining if the hair has probative value in a forensic case. The ability to chemically identify a hair extension collected from a crime scene would provide investigate leads and help to preserve time and resources. Also, chemical analysis might allow a processed human hair extension collected from a crime scene to be definitively associated with others from the same manufacturing package. This would be beneficial if the extensions were still attached to the suspect's head. This research has the potential to change the way that hair analysis is viewed and used throughout the forensic laboratory.

Further research needs to be completed: analysis of more hair extension brands, determine a concise method of analysis, and the best extraction solvent. The study is a precursor to all of this and proves that there is a necessity for further analysis and implementation to forensics. But before any of that can happen, a reliable and consistent method needs to be determined and validated. This study proves this need for further research in this area, and ultimately updating the process for hair analysis throughout forensic laboratories.

Table of Contents

List of Figures

Introduction

The use of processed human hair extensions has become common practice throughout the world. There are companies that profit immensely from selling natural human hair, with revenues ranging from $30 million to $100 million dollars each year per company (Biel, 2010; Hayt, 2006). In fact, hair extensions account for around sixty-five percent of all hair care purchases (Dawson, 2009). Although hair extensions have become increasingly popular, there has been little research about hair extensions as forensic evidence. Determining if there is a chemical difference between processed human hair extensions and natural human head hair will aid forensic investigators in determining whether the information gained from an unknown hair sample is valuable to the investigation. If the unknown hair collected from a crime scene can be identified as a natural human head hair and not a processed human hair extension, the hair sample can be used for comparison and potentially for mitochondrial DNA analysis. However, if the hair is a processed human hair extension, then the information will most likely be useless to the investigation. In addition to this, mitochondrial DNA analysis is expensive, time intensive to process, and susceptible to contamination. A chemical difference would allow for a processed human hair extension to be excluded as probative evidence.

To date, only one study has been completed on processed human hair extensions and their evidentiary value in a forensic case. This study established that there were no microscopic differences between natural human head hairs and processed human hair extensions. This study also determined that processed human hair extensions within a single package were from different donors with various regional affiliations (Porterfield, 2014). This means if a human hair extension was found at a crime scene and analysis was completed as if it were a natural human head hair, the results would be misleading to the investigation as well as a waste of time and

resources. To prevent this situation from occurring, it is necessary to consider another type of analysis for processed human hair extensions that might differentiate them from natural human head hair. As chemical additives may be used during manufacturing that are unique to the extensions, it is possible that there is a chemical difference between natural human head hair and processed human hair extensions. Additionally, it may be possible to link extensions back to a specific manufacturer based on chemical variations. Chemical analysis would be useful to forensic casework and investigations by eliminating evidence not pertinent to the case.

In this study, chemical analysis was completed on processed human hair extensions and natural human head hairs to determine if there is any chemical difference between them. Chemical variations between hair extensions produced by different manufacturers were also assessed. It is possible, that during the manufacturing process of human hair extensions, chemicals are added to the hair that are different than those that occur naturally or those that are found in products used on natural human head hair. The results of multiple chemical analysis methods were compared to determine if there is a viable, measurable difference between the chemical make-up of processed human hair extensions and natural human head hairs. The compounds present in the processed human hair extensions and the natural human head hairs were extracted using an organic solvent extraction method. Multiple solvents were used to account for differences in the molecular structure of chemicals present on the hairs. The solvents were then analyzed using three different chemical techniques: ultraviolet visible spectroscopy, gas chromatography/mass spectrometry (GC/MS) and nuclear magnetic resonance spectroscopy (NMR).

Hair Extensions

Individuals today are using processed human hair extensions for a variety of reasons including length, volume, and to mask hair loss (Hayt, 2006). The many uses of hair extensions and the frequency of use invoke the need to examine them further. As the use of processed human hair extensions increases, it is necessary to consider the possibility that they could be collected at a crime scene as a source of forensic evidence.

Human hair extensions are made of natural human head hair that is processed and treated with proprietary chemicals. Companies are able to buy natural, untreated hair in order to create human hair extensions (Biel, 2010). The human hair that is needed to make the extensions is collected for profit from many different areas of the world. One country that sells human hair for profit is India. When people attend temples to worship, often they will receive a haircut as an offering to the gods. The cut human hair is collected, sorted, and cleaned before being sold to human hair extension manufacturers.

Hair extensions are used by a variety of individuals to include every ethnicity and gender, (Dawson, 2009). Whatever an individual's reason may be, cultural or for style, hair extensions have become a part of everyday life. With the abundance of use, forensic science must begin to consider ways to analyze processed human hair extensions and differentiate extensions from natural human head hair.

Hair Analysis

Hair analysis generally involves microscopic examination and DNA analysis. There is a lot of information that can be determined from the microscopic analysis of hair including: if the hair is human or non-human, what kind of damage has been done to the hair, and if the hair is affected by a disease. If the follicular tag of the hair is attached, nuclear DNA analysis can be

performed. If only the hair shaft is present, mitochondrial DNA can be extracted and sequenced (Houck & Siegel, 2011, p. 289-300).

For microscopic analysis, the hair is examined utilizing a microscope to visualize the three different layers of the hair shaft. These three layers are, from outermost to innermost, the cuticle, the cortex, and the medulla as shown in Figure 1 (Deedrick & Koch, 2004).

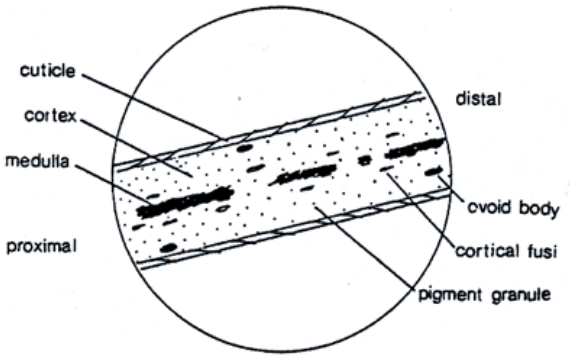

Figure 1: Components of a human hair shaft (Deedrick & Koch, 2004).

While microscopic hair examinations can be used for comparison purposes, they cannot be used to make identifications in case work. These analyses only lead to a conclusion of inclusion or exclusion. When observing hair for comparison analysis, multiple samples of known hair must be used to compare to an unknown sample, because every individual has the potential to have a variety of hair types. Using a comparison microscope, the entire length of each hair is compared to the unknown sample (Scientific Working Group on Materials Analysis, 2005). From the comparison, a conclusion is reached about whether the known hair could have come from the same individual as the unknown sample.

After microscopic examination and analysis, the hair sample can be processed for DNA, nuclear and mitochondrial. Since there are multiple mitochondria in each cell, there are multiple copies of mitochondrial DNA present in each cell, while there is only one copy of nuclear DNA.

If the follicular tag is still intact, then nuclear DNA analysis can be completed. However, if there is no follicular tag is present, mitochondrial DNA is extracted from the hair shaft for analysis. Mitochondrial DNA can be analyzed and used to determine the maternal lineage of an individual. Mitochondrial DNA is useful for forensic examinations, as it's present in high concentrations in the cell (Scientific Working Group on Materials Analysis, 2005). It is possible to extract mitochondrial DNA when nuclear DNA is degraded due to its high concentration within the cell. Nuclear DNA analysis allows for the identification of a specific individual and mtDNA analysis allows for exclusions and inclusions and connections to maternal lineages.

Hair analysis can be a useful tool in the forensic laboratory. There are many different analyses to help reach a conclusion about an unknown strand of hair collected from a crime scene. However, there are many improvements that need to be made with hair analysis to keep up with other analyses within the forensic laboratory. This does not mean that the information gained from hair analysis is not useful; nuclear DNA analysis can lead to identification. However, when microscopy and mitochondrial DNA analysis is used in tandem with other analyses, the combination of analyses can aid in the conclusion of a case.

Spectrophotometry

Spectrophotometry is the use of light radiation to measure chemical concentrations within a sample (Harris, 2013, p. 387-389). Spectrophotometry measures the absorbance of light through of a sample and is generally used for very small samples. Spectrophotometry does not destroy the sample since the sample itself is not being manipulated (Wade, 2010, p. 510-513). This type of analysis can be done with any wavelength of the electromagnetic spectrum, as shown in Figure 2. The reaction of the molecule depends on the wavelength of light that is being used (Harris, 2013, p. 387-389).

X-ray wavelengths in allow for information about how bonds in the molecule break as well as how they ionize. The ultraviolet and visible wavelengths give feedback about electronic excitation as well as information about concentration of the sample. Wavelengths within the infrared spectrum give insight into how a bond vibrates and stretches. The microwave wavelengths of the electromagnetic spectrum observe how rotation occurs around a bond. These are just a few types of spectral analyses and some of the information that can be obtained using light (Harris, 2013, p. 388).

A molecule's reaction from exposure to light wavelengths is measured using a spectrophotometer. If a sample can absorb light at a specific wavelength, then the radiant power of the light beam decreases after interaction with the sample. This change in radiant power is known as transmittance. This means that there is less light on the opposite side of the sample from where the light entered (Harris, 2013, p. 390). Transmittance, T, is detected and then is converted to absorbance, A, by:

$$A = -\log T$$

Absorbance relates to how much light a sample is able to absorb (Harris, 2013, p 391) However; absorbance differs for a sample depending on the light path length, b, or how far light is able to travel through the sample. Beer's law is used to correct for this situation, as well as give insight into the concentration, c. Beer's law states that:

$$A = \varepsilon bc$$

Molar absorptivity, ε, is the amount of light that is absorbed at a specific wavelength by a certain substance. Using Beer's law, absorbance is directly related to the concentration of the substance being measured (Harris, 2013, p. 390-393).

One type of spectroscopy that relies heavily on Beer's law is ultraviolet-visible (UV-Vis) spectroscopy. This is a specific type of spectrophotometry that is completed using only wavelengths within the ultraviolet and visible fractions of the electromagnetic spectrum. This is done by using a spectrophotometer to measure the change in the transmittance value. Once the change is measured, it is possible to compare multiple samples and determine the change in concentration.

Spectrophotometry is a useful chemical technique that has a wide array of uses. Due to the variety of wavelengths that can be used, there is quite a bit of information about a molecule that can be gained from this analysis. This can include any vibration, rotation, electronic excitation, or bond breakages. In this study, the ultraviolet and visible portions of the electromagnetic spectrum were used for chemical analysis to determine if there were any notable spectral differences between processed human hair extensions and natural human head hair.

Chromatography

Chromatography is a method used in many chemical laboratories, as well as forensic laboratories. This technique separates chemical compounds into individual components by passing the sample through a column that separates compounds by a variety of methods. This includes separation by size, charge, or shape of a molecule and is accomplished using a chemical's natural propensity to move between mobile and stationary phases (Harris, 2013, p. 457). The stationary phase is what is contained within the column and the mobile phase is what is moving the mixture through the column (Harris, 2013, p. 456). There are two main types of chromatography used in laboratories for the separation of chemical components, gas and liquid chromatography. The name corresponds to the type of mobile phase being used, which correlates to whether the mixture is being pushed through a column by a gas or by a liquid. Determining

what method to use requires knowledge about the chemical and physical properties of the mixture itself and what would result in the best separation.

A chromatogram is created as the now separated sample elutes from the column. A detector sends a signal as a compound in the sample elutes from the column. The signal begins the moment a compound starts to elute to when only the mobile phase is eluting from the column. Another signal is sent from the detector when another compound begins to come out of the column, later than the compound before it. The moment a compound elutes from the column is referred to as the retention time and correlates to the amount of time the compound travelled through the chromatography column (Harris, 2013, p. 458). The chromatogram shows the amount of time that each compound present in the sample spent in the column, as well as how long the entirety of that compound took to elute.

From this point, it is possible to determine the components of the mixture, using the knowledge of the type of column and what is contained within the mixture. If there are no known elements of the mixture, then the detector needs to be able to determine the chemical makeup of each component of the mixture. While there are many types of detectors, one of the most common detectors is the mass spectrometer. When a chromatography column is coupled with a mass spectrometer, it is possible to determine structural information about the compounds of a mixture based on their retention time and the elution peaks on a chromatogram.

Mass Spectrometry

Following separation by chromatography, mass spectrometry can be used to determine the mass of each separate analyte in a mixture, which helps with identification of the mixture. A mass spectrometer can provide both qualitative and quantitative analyses. Qualitative analysis identifies what something is while quantitative analysis identifies how much of something is

present (Harris, 2013, p. 5). The ability for one instrument to possess qualitative and quantitative analyses options is useful because it eliminates the need for multiple analyses to be performed on a sample as it can be identified and quantified using the same instrumentation.

Mass spectrometry works by determining a compound's or fragment's mass-to-charge ratio. To identify this ratio, the compound is first ionized, a process that creates ions from the initial compound. Most commonly this is completed by electron or chemical ionization. With electron ionization, electrons encounter the molecules upon entering the mass spectrometer and break the molecules apart into smaller, charged fragments. With chemical ionization, a reagent gas is combined with electrons to create a variety of differently charged reagent gas products. These different charges allow for proton donation to occur to the molecules entering the ionization chamber. Ionization fragmentation occurs due to the structure of the molecule, which gives consistency over time. With both methods of ionization, all the fragments are measured by their mass-to-charge ratio and then plotted in a mass spectrum (Harris, 2013, p. 467-468).

More accurate and sensitive, methods of mass spectrometry that can identify even more subtle differences between molecules One example of this is the time-of-flight mass spectrometer. These methods are helpful when there is little difference between the mass-to-ion ratios of the molecules being identified (Harris, 2013, p. 473). With mass spectrometry, each individual molecule fragments in a unique way. This fragmentation is consistent and allows for identification of the molecule using pattern recognition and a mass spectral library. The mass spectrum for a compound remains the same regardless of the variations in the sample the compound is contained in, the solvent used for extraction, or the composition of the mobile and stationary phases of chromatography. Identification of molecules, is possible because of the consistency that mass spectrometry provides.

Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a technique that can be used to determine the structure of an organic molecule. It can be used to observe a variety of nuclei but the most useful to organic chemists are carbon-13 and proton nuclei. This is due to the fact that most organic molecules are made up of carbon and hydrogen (Wade, 2010, p. 561).

NMR works by the analysis of odd numbered nuclei and monitoring how the nuclear spin of the molecule reacts when subjected to a magnetic field. The NMR spectrometer creates a large magnetic field around the sample. The odd numbered nuclei react in two different ways when the magnetic field is applied. They will either align with the field or against the field, both which create a change in energy for the molecule (Wade, 2010, p. 561-563).

The number of electrons surrounding the nuclei affects how much reaction each nuclei has to the magnet. The more electrons surrounding the nuclei, the stronger the magnetic force required to have an effect on the nuclei. Since the electrons are charged they are also affected by the magnetic field being applied to the molecule. Therefore, the external magnetic field must be stronger than the needed magnetic field to reach the resonant frequency for the nuclei (Wade, 2010, p. 561-565).

Proton NMR corresponds to the reaction of the proton once the magnetic field is applied. Due to the different amounts of shielding on each proton, there is a different energy change at each proton. This makes it is possible to determine the number of different types of protons by observing the differing chemical shifts, or the difference in resonance frequency of the sample protons and tetramethylsilane (TMS). TMS is a reference compound that contains silicon, rather than carbon, which is less electronegative. This allows the protons in TMS to be heavily shielded and therefore difficult to reach with the magnetic field used for proton NMR. This allows for the

chemical shift of all other protons that are affected by the magnetic field to be determined from a starting point of zero with TMS (Wade, 2010, p. 561-567).

NMR spectroscopy is a method that allows for observations of minor differences within molecules. The way that nuclei react to a magnetic field allows for distinct differentiation between the different protons within a compound. This allows for the structure of compound to be determined. The main issue with NMR is that it is difficult to analyze mixtures. While it is possible that multiple proton types have similar chemical shifts in a single compound, it is much more possible that this case occurs within mixtures. It is also difficult to determine which proton corresponds to the multiple compounds present in a mixture. However, NMR is able to aid in determining the complexity of mixed samples. The presence of fewer chemical shifts indicates fewer proton groups, and generally a less complex mixture.

Literature Review

There is no microscopic difference between a naturally shed human head hair and a processed human hair extension (Porterfield, 2014). A method to identify differences between the processed human hair extensions and natural human head hair must be determined to aid in criminal investigations and to save forensic laboratories time and resources on analysis. The literature review will address the current state of research for hair extensions and focus on the chemical analysis of natural human head hair. Much of the literature is not directly focused on the analysis of processed human hair extensions, but will give insight to the analysis that could be useful for differentiating them from natural human head hair. From this literature review, the necessity for a method of chemical analysis of processed human hair extensions is evident.

Hair Extension Analysis

Only a single study has focused on the forensic evidentiary value of processed human hair extensions specifically and it was determined that there is no microscopic or genetic difference. Porterfield (2014) evaluated three brands of processed human hair extensions microscopically and genetically for their probative value in forensic casework. Microscopic analysis of hair morphology determined that the internal and surface characteristics of the human hair extensions were consistent with human head hair and failed to have any distinguishing features (pitting, striations, indentations, internal variations) that differentiated the extensions from natural human hair. Mitochondrial DNA extracted from the hair extensions was sequenced and compared to the revised Cambridge sequence (rCRS) to determine single nucleotide polymorphisms (SNPs). SNPs were used to assign haplotypes and distinguish regional affiliations associated with the extensions to establish the ethnicity of the hair donor's maternal lineage (Porterfield, 2014).

The evaluated hair extensions were associated with multiple geographic regions. Sequencing also identified hairs from multiple donors in each package. This means that presently there is no way to properly conclude that a processed human hair extension as an extension and no forensic value in determining regional affiliations (Porterfield, 2014). From this research, it is evident that there is a need to identify a hair as an extension to aid in forensic investigations. Chemical analysis of the processed human hair extensions may be able to differentiate extensions from natural human head hair. It is possible that during manufacturing, unique chemicals are added to the hair extensions and may distinguish them from natural human head hair.

Ultraviolet-Visible Spectroscopy

UV-Vis spectroscopy is a technique that has been used for a wide variety of reasons. It is able to discern differences in concentration easily and quickly without disturbing samples. However, the use of this technique for hair analysis is scarcely mentioned in the literature. The few articles that discuss the application of this technique to hair are important to this study because they outline the types of issues that would be observed with UV-Vis analysis of hair (Barrett et al, 2010; Pires-Oliveira & Joekes, 2014). One of the articles focuses on determining hair damage while the other observed how hair dyes affect determining the natural color of the hair.

Washing hair and the use of hair care products affects how hair appears naturally, but also creates variations at the chemical level. Pires-Oliveira and Joekes (2014) placed natural human head hair into surfactants found within shampoos. The hairs were allowed to sit in these compounds for a variety of times and then were analyzed using UV-Vis spectroscopy. UV-Vis spectroscopy indicated that different compounds found in the shampoos removed different

substances from the hair as well as that some of the compounds caused damage to the hair shafts. They were also able to determine that after 72 hours within the surfactants, the hair samples created experimental errors. The study determined that the amount of color that the compounds removed from the hair sample was due to melanin and correlated to the amount of damage that the chemical did to the hair (Pires-Oliveira & Joekes, 2014). This gives insight into the best way to extract compounds from hair, as well as confirms that UV-Vis spectroscopy can be used natural human head hair.

Barret et al. (2010) in their study showed that hair presents distinct colors when dyed and that the amount of time a hair has been dyed can be determined by chemical analysis using UV-Vis spectroscopy. Over time and after multiple washes, the UV-Vis spectra weakened in terms of absorbance. The resulting spectra correlated to how strong the color was within the hair samples. The lower the absorbance, the lower the dye amount present in the hair. The greater the absorbance, the greater amount of dye was present in the hair. Chemicals from the actual hair appeared around 300-400 nm and the hair dye was around 425-550 nm on the UV-Vis spectra. In addition, the resulting color was different depending on the natural color of the hair that was dyed. It was suggested that this method should be added into the normal forensic processing of hair and should be completed after microscopic examination and before DNA analysis (Barrett et al, 2010). This would add another layer of class characteristics that could aid in including or excluding a sample prior to DNA extraction.

UV-Vis spectroscopy is a non-invasive technique that can be used to determine a wide variety of information. Both of the articles indicated how hair treatment decisions like shampoo or dye color can create notable chemical variations in hair simples UV-Vis spectroscopy is a

simple analysis process that has the power to potentially improve the forensic analysis of hair samples.

Gas Chromatography/Mass Spectrometry

Gas chromatography/mass spectrometry (GC/MS) may be the best chemical analysis technique suited for hair analysis, due to the specificity that this method provides. Using tandem GC/MS increases the likelihood of identifying a molecule as well as reduces the chance of error. Gas chromatography separates the molecules and the mass spectrometer can differentiate between the separated compounds. It is also possible that two molecules may have similar patterns of ionization in a mass spectrometer. However, it is extremely unlikely, that the two molecules will react the same, have the same retention times, and produce the similar spectrums in both instruments. Using both instruments in tandem allows for an almost definitive identification.

GC/MS is used throughout forensic laboratories for analyses such as to identify explosive residue compounds, perform arson residue analysis, and drug identification (Sahil et al., 2011). Within forensic laboratories, when hair analysis is completed using GC/MS, it is typically for drug detection. This method is non-invasive and observes an individual's drug use over time, (Aleksa et al., 2012). To analyze hair using GC/MS, an extraction process must first occur.

Prior to extraction, the hairs must first be prepared by washing and then rinsing with dichloromethane. The samples are then dried and soaked in methanol for further extraction. (Aleksa et al., 2012; Cabarcos et al., 2009; Pego et al., 2017). There are many types of extraction methods that can be used and little variation in the results based on the chosen method (Sachs & Kintz, 1998).

Solid phase extraction (SPE) and headspace solid phase microextraction (HS-SPME) are similar extraction methods. SPE uses a cartridge to purify the sample (Aleksa et al., 2012) and with HS-SPE the sample is "adsorbed at a moderate temperature on a coated silica fiber, which is placed in the vapor phase above the sample" (Cabarcos et al., 2009). Aleksa et al. (2012) used SPE and HS-SPME in tandem to be able to extract both acidic and basic drugs from hair and remove background noise in the spectra. Using these two methods in tandem led to a more purified result with a wider variety of samples (Aleksa et al., 2012). Drugs and their metabolites are not the only chemical that can be extracted from hair using HS-SPME. It is also possible to analyze the fatty acid ethyl esters that are used as alcohol markers (Cabarcos et al., 2009). Since analyses can be performed on natural human head hair to determine drug and alcohol abuse, it is possible that it will also work on an analysis of processed human hair extensions to identify chemical additives.

Another kind of extraction that is typically used for hair analysis is liquid phase microextraction (LPME). This method uses a hollow fiber filled with an acceptor phase on the inside of the fiber and an organic phase on the outside. Separation is achieved through a process that is similar to that of chromatography. When the fiber is inserted into the sample solution, the molecules become ionized as they travel into the fiber. Once this occurs, the molecules cannot travel back out of the fiber due to the phase and charge differences. The molecule travels through the organic phase and then into the acceptor phase. The acidic acceptor phase then ionizes the compound due to the change in pH. This change in charge does not allow for the molecule to travel back across the fiber. After this extraction, the sample is pure enough to test using GC/MS without any further purification steps (Pego et al., 2017).

In order to establish a baseline for quantitative analysis, internal standards must be added into the sample mixture (Aleksa et al., 2012; Cabarcos et al., 2009; Pego et al., 2017). Since a known amount of internal standard is added, it is possible to compare the values of the unknown samples to the baseline and determine their concentration. This is done before GC/MS analysis and proves especially important for drug analysis as there are legal limits on the amount or concentration of drugs allowed to be used by an individual.

While drug analysis of hair samples is typical for forensic laboratories, other studies use the chemical analysis of hair for a variety of research interests. One group of researchers studied pesticide exposure in mothers using their hair. This analysis did not use any of the methods previously mentioned, but instead used a solid-liquid extraction and a liquid-liquid extraction of the pesticides. The solid-liquid extraction used hexanes and then separated the solution using centrifugation. Analysis was ultimately performed using GC/MS. The liquid-liquid extraction involved hydrochloric acid, methanol, and toluene. The toluene layer was separated out by centrifugation and then GC/MS analysis was performed. This method was able to identify pesticides and their metabolites (Posecion, 2006). While solid-liquid extraction and liquid-liquid extraction are older methods than SPE, HS-SPE, and LPME, they still can create results that are reliable.

The main issue with GC/MS is not GC/MS itself, but rather the extraction method used prior to GC/MS. The extraction method and choice of solvent is based on the chemical compounds expected to be identified. With processed human hair extensions, it is hard to know what chemicals are present since the chemical additives and manufacturing processes are proprietary. The simplest extraction method will be used first and adjustments in methodology will be made as needed.

Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance Spectroscopy is a powerful method for determining chemical structure. Polymers are large chemical compounds that are used in a wide variety of commercial products like plastics. Since they are so large, they have a complex proton NMR spectrum. This is shown in the work of Päch et al. They took monomers and then added a couple of compounds to the original monomer that contained TMS. After this addition, the monomer structure could be determined. Even though they were manipulating the polymer and could clearly identify the structure of the monomer, these created compounds create complex NMR spectra, which becomes obvious with the larger, more protonated monomer that was used (Päch et al., 2010). This study indicated the difficulty of NMR analysis of complex monomers. This suggest the analysis of polymers would be even more complex, since they are multiple monomers chained together.

Proton NMR is also used for molecular weight analysis of polymers. Izunobi and Higginbotham (2011) proved that by determining the number of peaks present in the molecule as well as having an idea of the end groups of the polymer, a mathematical system can be applied, and an estimate of the polymer's molecular weight can be determined. This approach also allows for the number of repeating units within the polymer chain to be determined. This research shows how complex a polymer proton NMR can be, as shown in figure 2 (Izunobi & Higginbotham, 2011).

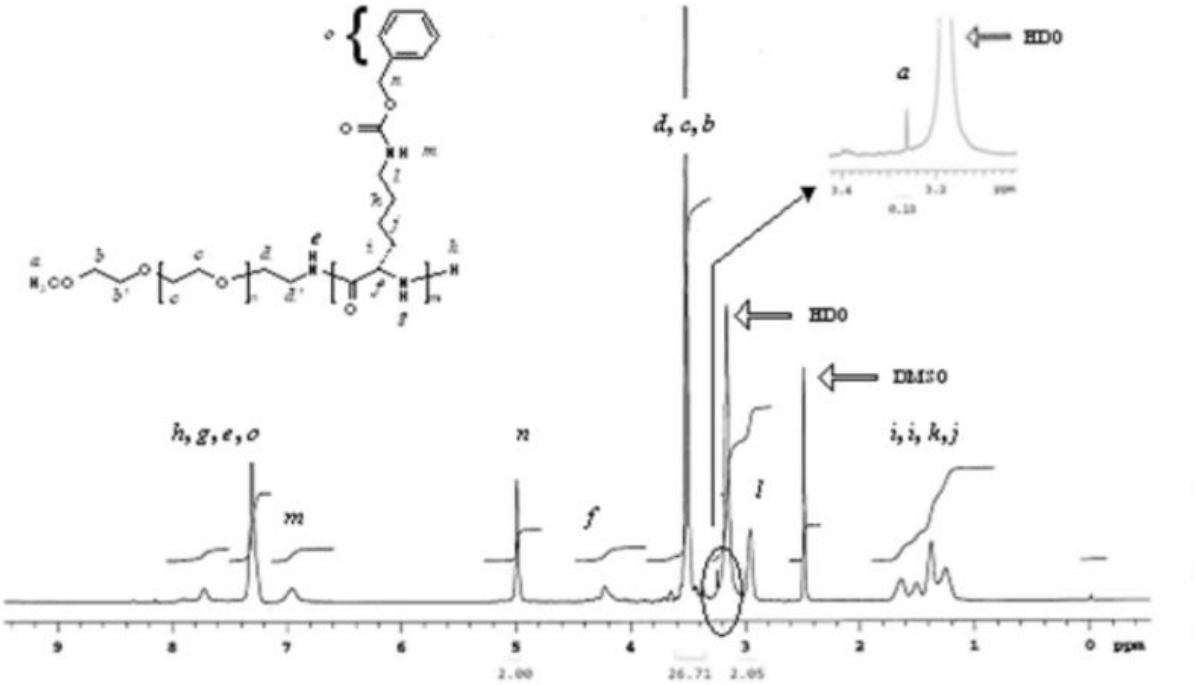

Figure 2: "NMR spectrum (DMSO-d6; 60° C) of the block copolymer, MPEG-b-PLL(Z)" that shows the complexity of a polymer analyzed by NMR (Izunobi & Higginbotham, 2011).

Figure 2 clearly shows the complexity of polymers and how evident it can be when one is present within a proton NMR. There are a large number of peaks present over a large area of the spectrum. Proton NMR is a powerful tool and is useful in determining if a polymer is present.

Proton NMR is an effective way to determine structure. With polymers, there are so many different protons that the resulting NMR spectra becomes difficult to analyze. In this research, it is particularly important that polymers be included or excluded as being added to the processed human hair extensions during manufacturing and NMR provides this level of discriminatory power. If the external hair shafts of processed human hair extensions are covered with a polymer, then there are different techniques and methods that will need to be used in future research. Being able to determine if polymers are present within processed human hair extensions can aid in determining the pathway to take with future research.

Methodology

Being able to identify a hair collected at a crime scene as a processed human hair extension is significant as it may exclude it as probative evidence in a forensic investigation. Previous research has shown that there is no microscopic difference between natural human head hair and processed human hair extensions. In addition, mitochondrial DNA (mtDNA) remains unaffected by the manufacturing of processed human hair extensions and provides no discriminating power in identifying a hair as an extension (Porterfield, 2014). In this research, multiple types of chemical analysis were performed to determine if there is any chemical variation between natural human head hair and processed human hair extensions. Comparative chemical analysis between the natural human head hairs and processed human hair extensions was completed using UV-Vis spectroscopy, GC/MS, and NMR spectroscopy. The natural human head hair samples were washed before use. An organic extraction was then completed in order to remove possible chemicals from both the natural human head hairs and processed human hair extensions. The extraction liquid was portioned and used for analysis with UV-Vis spectroscopy, GC/MS and NMR spectroscopy. The resulting spectra were then analyzed and compared to identify any chemical differences between all the samples. These three methods were able to visualize chemicals unique to the processed human hair extensions.

Processed Human Hair Extensions

Three brands of processed human hair extensions were used for chemical analysis. The three brands were: Cuticle Remy XQ - Fortified Cuticle High Quality - 2 Caucasian (100% Human Hair - High Quality), European Caucasian Weaving (ECW) - Black and Gold - 2 Caucasian (100% Human Hair - Medium Quality), and Que by Milky Way - 1 African American (100% Human Hair - Low Quality). These three processed human hair extension brands were

chosen for chemical analysis as a means of determining if the chemical composition varied based on quality and manufacturer.

Natural Human Head Hair Preparation

Natural human head hair samples were collected from willing and knowing volunteers based on hair type. This study was approved by the University of Central Oklahoma Institutional Review Board (IRB) on May 8, 2018, under study number 2018-034. The three different types of natural human head hair examined were: caucasoid, mongoloid, and negroid. In addition to the three kinds of hair analyzed, within in each type a dyed and undyed hair sample was obtained if possible. This led to five kinds of hair being analyzed; caucasoid undyed, caucasoid dyed, mongoloid undyed, mongoloid dyed, and negroid undyed. Negroid dyed hair was unable to be obtained. Prior to analysis, the natural human head hair was washed with warm water and TreSemme shampoo and air dried in order to ensure continuity of product on the hair shaft for each of the natural human head hair samples.

Organic Extraction

The chemicals used and the manufacturing method in the creation of human hair extensions are proprietary and thus may vary for each manufacturer. Compounds present within processed human hair extensions may include oils, alcohols, hydrocarbons, fatty acids, as well as other chemicals. Because of the variability in molecular structure and weight of the possible compounds, a variety of simple organic extractions were completed.

Each brand of processed human hair extensions and the natural human head hair controls, dyed and undyed, underwent a separate simple solid-liquid extraction using five solvents: hexanes reagent grade from Pharmco-Aaper, Colorist Secrets™ Hair Color Remover by L'Oréal, methanol from Fisher, 1-octanol from Acros, and chloroform-d from Acros. Hexanes, Colorist

Secrets™ Hair Color Remover by L'Oréal, methanol, and 1-octanol were used for UV-Vis spectroscopy and GC/MS, while chloroform-d was used solely for NMR spectroscopy. For non-polar compounds, hexanes was used as the extraction solvent. Colorist Secrets™ Hair Color Remover by L'Oréal was also used for extraction. This solvent is a reducing agent that uses ammonium chloride, persulfates, and peroxides to remove pigmentation from natural human head hairs. Colorist Secrets™ Hair Color Remover is a thick and viscous liquid, so it was dissolved in distilled water to create a solvent that was useable for chemical analysis. 59 mL of Colorist Secrets™ Hair Color Remover was created when mixed by the package instructions and it was diluted with 177 mL of distilled water. Methanol was used as an extraction solvent to remove polar compounds with low molecular weights. 1-Octanol was used to isolate polar compounds with high molecular weights. These four solvents accounted for any variability in the molecular structure of the unknown compounds present on the processed human hair extensions and natural human head hair controls during UV-VIS and GC-MS analysis. The final solvent used was chloroform-d, this solvent was used specifically for NMR spectroscopy. A deuterated solvent needs to be used for NMR analysis so that most of the hydrogen molecules being measured are from the questioned sample and not the solvent. There will be some hydrogens from the deuterated solvent, but they are known, can be easily identified, and will not overshadow the hydrogens in the sample. Approximately 0.1 gram of each hair sample was soaked in five milliliters of hexanes, and Colorist Secrets™ Hair Color Remover by L'Oréal, methanol, 1-octanol. Approximately 0.02 gram of each hair sample and one milliliter of solvent was used for the chloroform-d organic extraction. The sample weights can be found in Appendix A. After the solvent was added, the sample was vortexed for fifteen seconds and then allowed to

sit overnight. The next day, the hair was removed from the solvent and disposed. The extraction solvent was then portioned and used for chemical analysis.

Ultraviolet-Visible Spectroscopy

Each sample's extraction solvent was used for analysis and run in triplicate for confirmation of results. The sample was placed in a polystyrene cuvette and filled high enough to completely cover the testing window, which was approximately one milliliter of the extraction solvent. The blank was the original extraction solvent and was also measured in a polystyrene cuvette. The samples were then analyzed from 300 nm to 800 nm, and the resulting absorbance versus wavelength graphs were recorded and compared. The extraction solvents were analyzed using a Beckman Coulter DU720 General Purpose UV/Vis Spectrophotometer.

Gas Chromatography/Mass Spectrometry

All samples were analyzed using MSD ChemStation G1701EA E.02.00.493 and run in triplicate for confirmation of results. The extracted samples were placed in the autosampler from Agilent Technologies 7683B Series Injector. Methanol was used for both wash buffers of the injection needle. Splitless injection was used for the extractions containing methanol and Colorist Secrets™ Hair Color Remover by L'Oréal. However, split injection of 25:1 was used for the samples containing 1-octanol and hexanes. The sample was then volatilized in the GC system from Agilent Technologies 7890A and separated based on their volatilization temperature and their unique chemical and physical properties. A ramp temperature method was used with the starting temperature being 80°C and the final temperature being 250°C, a temperature increase of 10°C every minute. After column separation, the molecular components were individually analyzed by a mass spectrometer from Agilent Technologies 5975C inert XL MSD with Triple-Axis Detector. The resulting spectra were then analyzed using Agilent MSD

Productivity ChemStation for GC and GC/MS Systems Data Analysis Application. The analyzed spectra were then compared to reference spectra from the NIST reference library containing over 200,000 spectra.

Nuclear Magnetic Resonance Spectroscopy

The chloroform-d extraction was used for proton NMR analysis and only analyzed once. Approximately one milliliter of the extraction solvent was added to an NMR tube. The tube was then placed into the instrument. The samples were analyzed on an Eft-60 NMR Spectrometer from Anasazi Instruments. The frequency used was 60.010 megahertz and 32 scans were completed. The resulting spectra were analyzed using ACD/NMR Processor Academic Edition. The spectra was converted using Fourier transform and then a phase correction in order to properly visualize the resulting spectra. The chloroform-d has TMS added to the solution, so the lowest chemical shift peak was TMS and was therefore adjusted to 0. The rest of the peaks were labeled for chemical shift as well as integrated to see relationships between the peaks.

Results

Ultraviolet-Visible Spectroscopy

The individual spectra that were collected using UV-Vis spectroscopy are listed in Appendix B. Four different organic solvents were used for UV-Vis spectroscopy: hexanes, Colorist Secrets™ Hair Color Remover, methanol, and 1-octanol. For each extraction solvent solution, the blank used was the same as the original organic solvent.

Hexanes

The three different types of processed human hair extension extractions, Remy XQ, ECW, and Que all read around zero absorbance, which means that there was little to no difference between the organic extraction solvent and the blank. Some of the natural human head hair extractions reacted differently. The extraction solutions from undyed caucasoid, dyed caucasoid, and undyed mongoloid all begin with a large drop in absorbance between 300-400 nm and then after 400 nm had an absorbance of zero. This is shown clearly in figure 3.

The other two natural human hair extractions, dyed mongoloid and undyed negroid, had an absorbance around zero across the spectra. From Figure 3, it is evident that some of the hair samples reacted similarly to hexanes while some reacted differently. The dyed mongoloid and undyed negroid had similar results to the processed human hair extensions. The undyed caucasoid, dyed caucasoid, and undyed mongoloid all reacted similarly in the presence of hexanes.

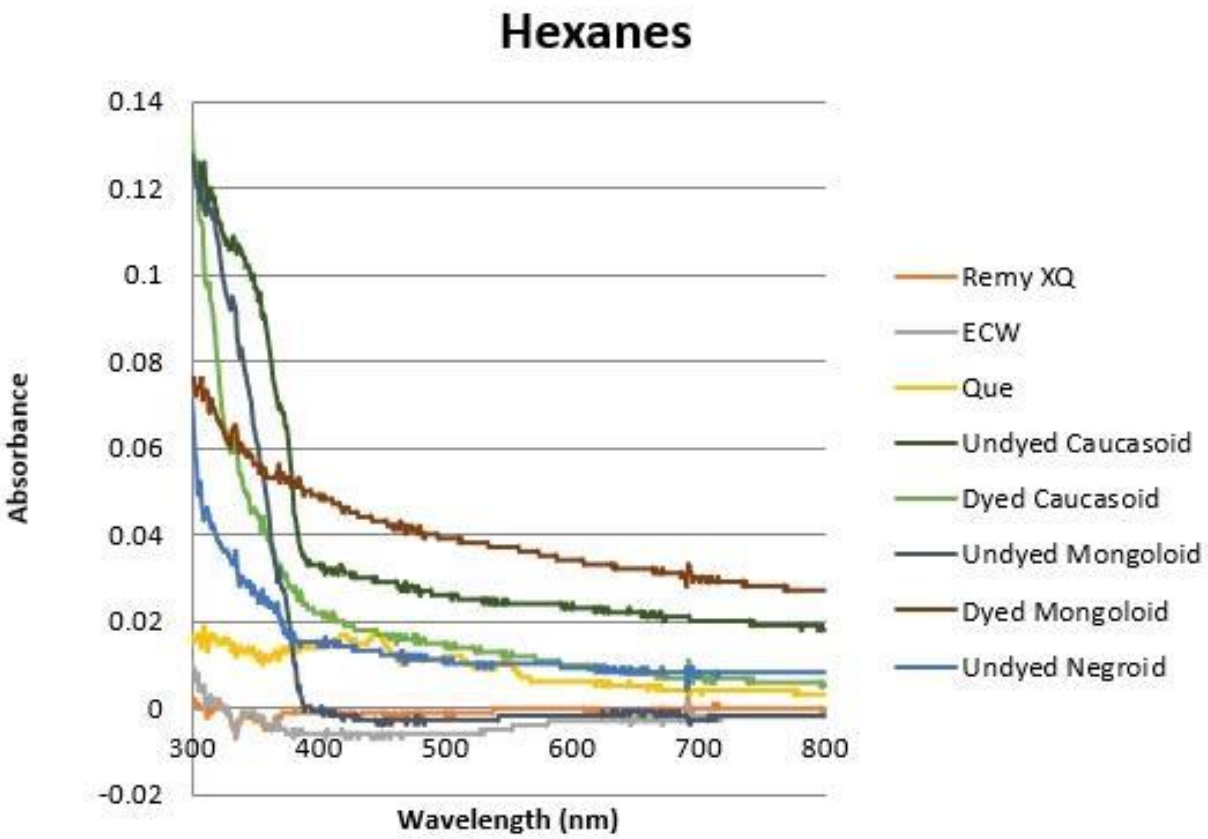

Figure 3: UV-Vis spectra from all hair samples when extracted with hexanes. This figure shows 0.1 gram of the eight different hair samples; three processed human hair extension samples and five natural human head hair samples, after being extracted, separately, overnight in 5 mL of hexanes. The extraction solvent was analyzed using UV-Vis spectroscopy from 300 to 800 nm.

Colorist Secrets™ Hair Color Remover

In Colorist Secrets™ Hair Color Remover, all the hair samples had a similar reaction. For all samples, the extraction solution started out as a cloudy blue color and did not change. They all had little to no detectable absorbance difference between the organic extraction and the blank. This is shown in Figure 4.

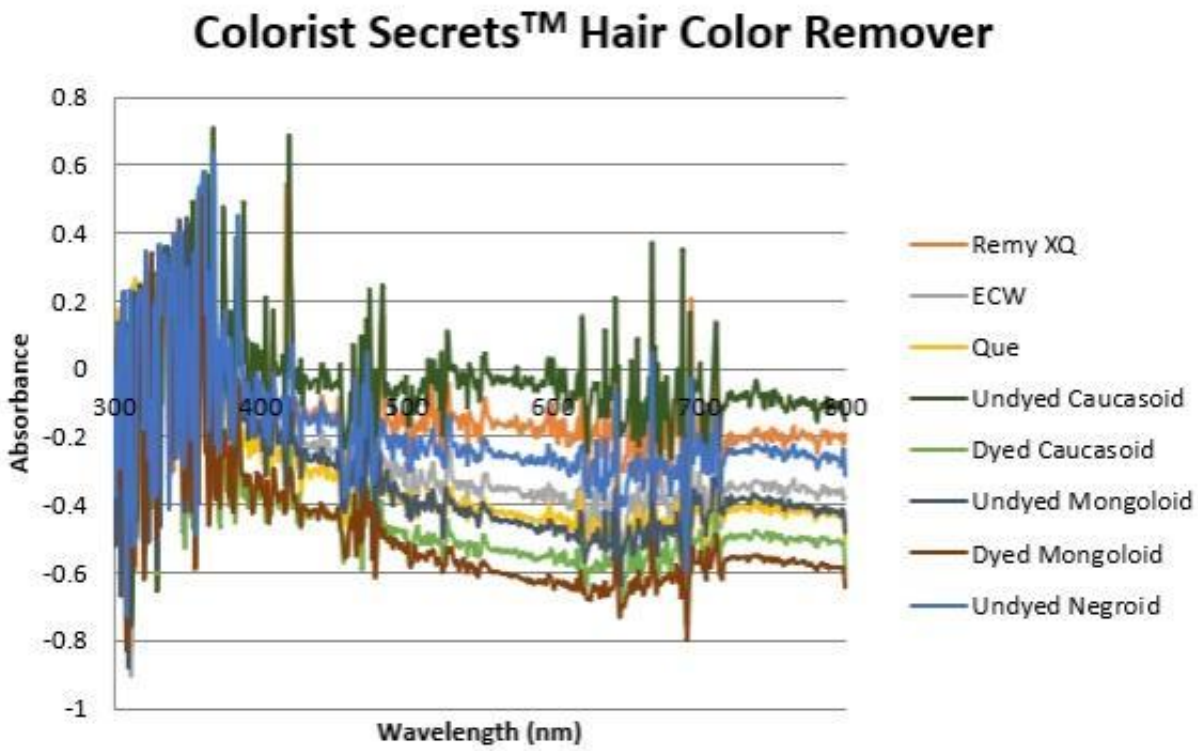

Figure 4: UV-Vis spectra from all hair samples when extracted with Colorist Secrets™ Hair Color Remover. This figure shows 0.1 gram of the eight different hair samples; three processed human hair extension samples and five natural human head hair samples, after being extracted, separately, overnight into 5 mL of Colorist Secrets™ Hair Color Remover. The extraction solvent was analyzed using UV-Vis spectroscopy from 300 to 800 nm.

Methanol

The hair samples that were extracted in methanol were the only samples that visually changed the color of the extraction solution. The color of the solution took on the color present within the hair samples. This made peaks in the visible wavelengths of the spectra much more apparent. The processed human hair extension samples reacted strongly in the visible wavelengths. The Remy XQ sample had a distinct peak around 575 nm, the ECW sample had two separate peaks around 375 and 575 nm, and the Que sample had multiple slight peaks at

approximately 425, 450, 525, 550, and 625 nm. Some of the natural human hair samples reacted similarly, but at a much weaker absorbance, while others had no difference in absorbance from the blank. The samples with undyed caucasoid hair, dyed caucasoid hair, and undyed mongoloid hair all had no change in absorbance while the samples containing dyed mongoloid hair had slight peaks around 400 and 600 nm, and undyed negroid hair had peaks at 400 and 600 nm. All of this is depicted in Figure 5.

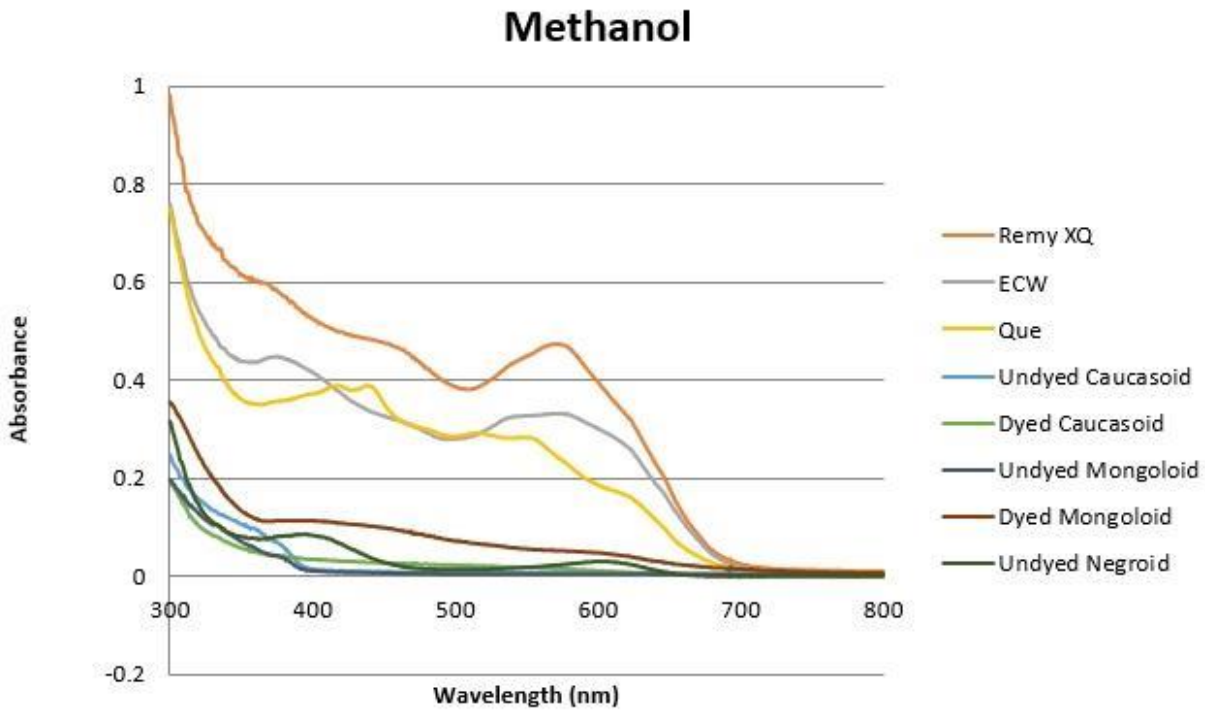

Figure 5: UV-Vis spectra from all hair samples when extracted with methanol. This figure shows 0.1 gram of the eight different hair samples; three processed human hair extension samples and five natural human head hair samples, after being extracted, separately, overnight into 5 mL of methanol. The extraction solvent was analyzed using UV-Vis spectroscopy from 300 to 800 nm.

Methanol was the only extraction solvent that lead to the solvent taking on the color that was visible in the hair. This was more evident with the processed human hair extension

extractions than the natural human head hair extractions. This is most likely because the processed human hair extensions had no wear or use prior to testing while the samples collected for natural human head hair would have exposure to elements that would wear down the color of the dyed hair. It is also possible that the dye that can be used on processed human hair extensions are harsher than the dyes that can be used on natural hair on an individual. The fact that the dyed mongoloid hair had a higher absorbance than the dyed caucasoid hair may be a result of the length of time since hair color application. These inconsistencies make it difficult to differentiate based on hair dye.

The processed human hair extensions have a significantly more complex spectrum than any of the natural human head hair spectra, and the peaks are located in different locations. When peaks were present in the natural human head hair, they were around 400 and 600 nm. The processed human hair extensions had much more variable peak presence. This is possibly because there is a wide variety of an initial hair color in each hair extension. While the dye applied by the company will visually cover all of the imperfections, the methanol could extract both the overlying and underlying hair dye, which would lead to a more complex spectrum. This UV-Vis spectroscopy using menthol as an extraction solvent appears to differentiate between processed human hair extensions and natural human head hair based on the complexity of the spectra.

1-Octanol

Visually, the 1-octanol samples all appeared to be like the original solvent. Only one sample had a different spectrum than the blank; as seen in Figure 6.

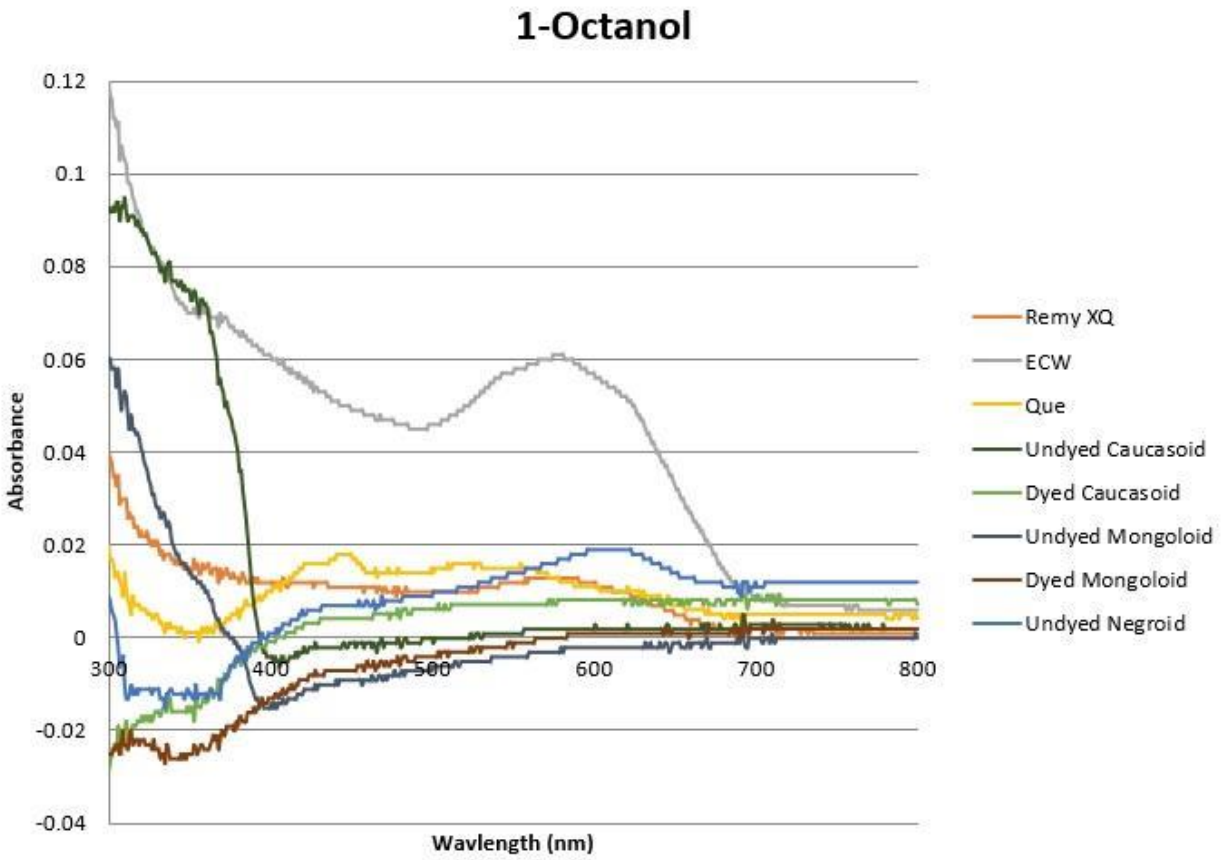

Figure 6: UV-Vis spectra from all hair samples when extracted with 1-octanol. This figure shows 0.1 gram of the eight different hair samples; three processed human hair extension samples and five natural human head hair samples, after being extracted, separately, overnight into 5 mL of 1-octanol. The extraction solvent was analyzed using UV-Vis spectroscopy from 300 to 800 nm.

The ECW sample displayed the most variation with two very distinct absorbance peaks around 350 and 575 nm. The sample from Remy XQ did have a very slight peak around 575 nm, but the absorbance was much lower than the sample from ECW. The sample from the extraction with Que hair extensions also had some slight peaks around 450 and 525 nm. These were consistent with the peaks that were observed in methanol, but were at a much weaker absorbance. Also, not all of the peaks observed in the methanol extraction UV-Vis were present in the spectra from the 1-octanol extraction. The undyed caucasoid, undyed mongoloid, and

undyed negroid samples had similar spectra that began at a high absorbance and then dropped to zero between 300 and 400 nm. All of the other samples read an absorbance around zero, meaning there was no difference between the sample and the blank.

Extraction with 1-octanol created a distinct difference between all of the samples and the dyed natural human head hair samples. All of the dyed samples began less than zero and then rose to zero as wavelength increased. They were the only samples that acted this way. The others began high and then moved toward zero as the wavelength increased. The processed human hair extensions still had some color extraction in 1-octanol, shown mainly by the ECW sample. However, since only one brand of processed human hair extension is easily differentiable, this solvent used with UV-Vis spectroscopy is not a suitable test for differentiation between processed human hair extensions and natural human head hair.

Gas Chromatography/Mass Spectrometry

All of the spectra from GC/MS data collection can be found in Appendix C, as well as the comparison to the reference spectra. Four extraction solvents were used for GC/MS analysis: hexanes, Colorist Secrets™ Hair Color Remover, methanol, and 1-octanol.

Hexanes

The samples extracted in hexanes created simple spectra that only had a few major peaks, as shown in Figure 7.

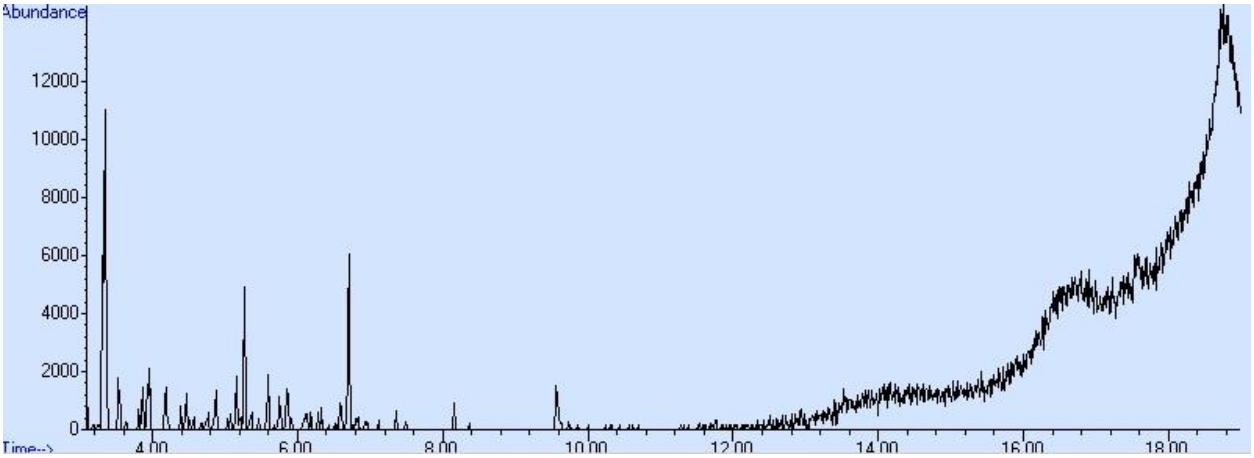

Figure 7: GC spectrum of hexanes solvent. This hexanes solvent sample was analyzed by GC/MS using a split injection method of 25:1. The temperature ramp was from 80°C to 250°C.

In all, there were not many peaks that differed from the expected solvent peaks. There was one sample that had a unique peak. The extraction of the Que hair extension had a peak at a retention time of 13.438 minutes that was not observed in any other sample, as shown in Figure 8.

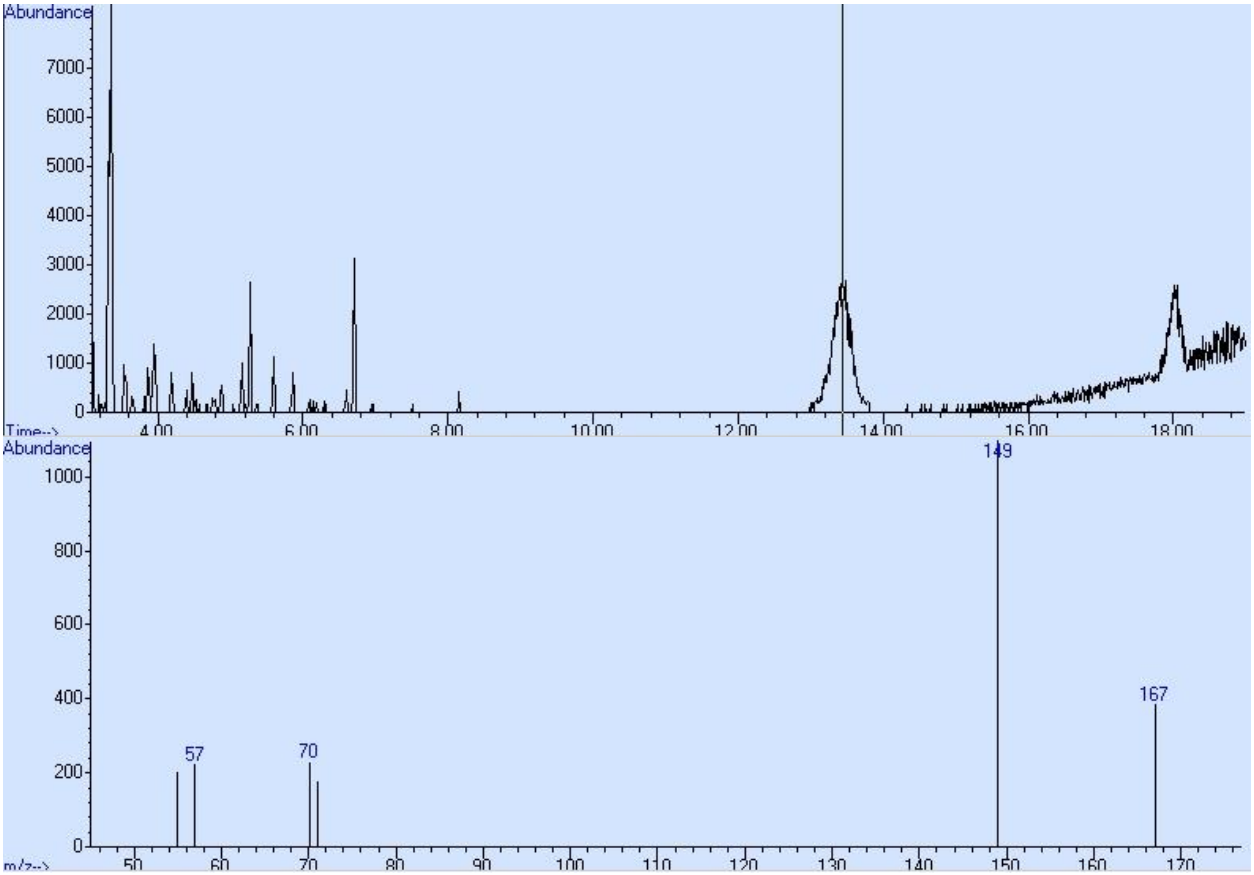

Figure 8: (a) GC spectrum of Que hair extension in hexanes. 0.1 gram of Que hair extension sample was extracted overnight into 5 mL of hexanes. This extraction solvent was analyzed by GC/MS using a split injection method of 25:1. The temperature ramp was from 80°C to 250°C. (b) MS spectrum of the Que hair extension sample in hexanes at retention time 13.438 minutes.

This sample's MS spectrum was compared to the NIST library and did not have any strong correlations to other compounds. This is most likely due to the fact that the compound added is proprietary and therefore will not be in the NIST database. It is also possible that the signal is weak and not all of the peaks in the MS spectrum are present at a high enough concentration. The peak is still unique and not even close to apparent in any of the other samples. This is

significant as GC/MS in hexanes could work as a way to differentiate processed human hair extensions from natural human head hair.

It is important to note, the hexanes solvent was not identified within the spectra because hexanes are a volatile solvent, and evaporate quickly. The spectrum does not begin collecting data until three minutes after the sample has been injected into the instrument. This is in order to prevent overloading the detector, which allows the detector to last longer. In the first three minutes the samples goes directly to waste in order to prevent overloading of the instrument. It is most likely that the hexanes, and any other volatile solvents, quickly vaporize and do not make it to the detector, within the first three minutes.

Colorist Secrets™ Hair Color Remover

Colorist Secrets™ Hair Color Remover was a difficult sample for GC/MS analysis as there were too many peaks that varied from sample to sample. While all the peaks were present, it was difficult to compare the questioned samples to the solvent sample. This is evident in Figure 9.

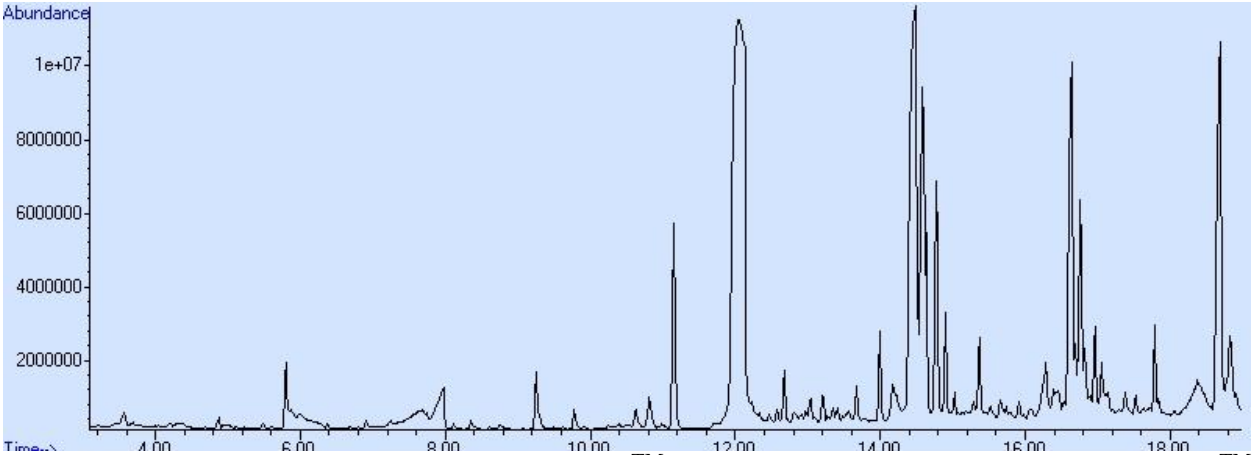

Figure 9: Solvent GC spectra of Colorist Secrets™ Hair Color Remover. This Colorist Secrets™ Hair Color Remover solvent sample was analyzed by GC/MS using a splitless injection method. The temperature ramp was from 80°C to 250°C.

The largest peaks in the solvent sample were not the largest peaks in the questioned samples. While the peaks were visible in both the solvent sample and the questioned samples, the size of the peak matters and makes a difference in the resulting MS spectrum. The multiple peaks and difficulty in analyzing this spectrum is likely due to the fact that the solvent is the only solvent that is a complex solution. All of the other solvents are single compounds. This complexity leads to a complex GC spectrum. While not impossible to analyze, the smaller peaks have weaker MS spectra, due to less of that compound being present, and therefore the MS spectrum is less reliable. All of these factors lead to this solvent not being a good choice for GC/MS analysis.

Methanol

Methanol was visually the best solvent. However, like the hexanes solvent, methanol volatilizes quickly and has a large number of chromatographic peaks, some of which are overlapping as shown in Figure 10.

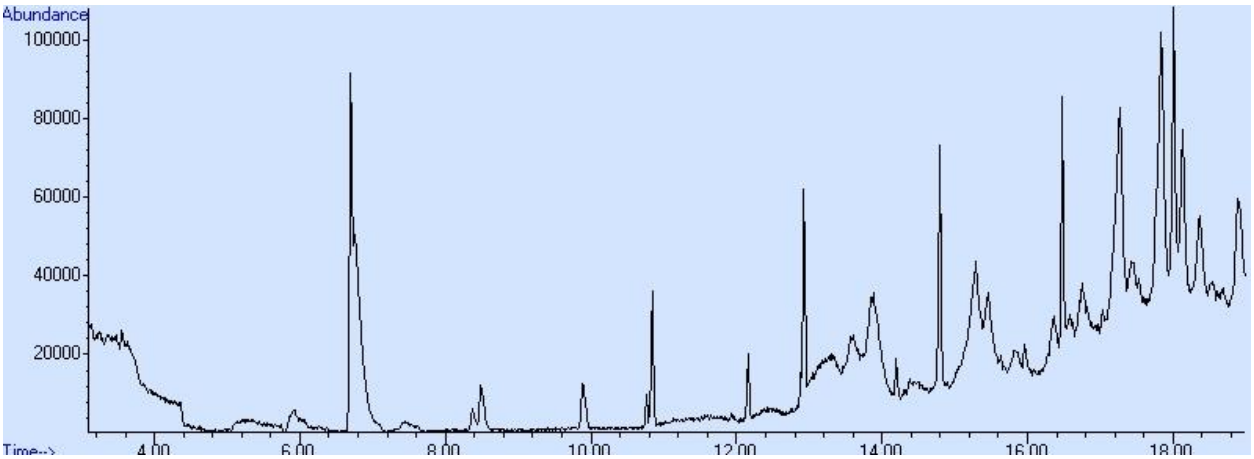

Figure 10: GC spectrum of methanol. This methanol solvent sample was analyzed by GC/MS using a splitless injection method. The temperature ramp was from 80°C to 250°C.

The large number of peaks and the variation in concentration again makes this solvent difficult to use for comparison. The peaks found in the solvent itself are not the prominent peaks in the samples, and when observing sample to sample, there are no clearly differing peaks between the samples. All the different NIST reference spectra results, were consistent with results seen in both the solvent spectra and the larger peaks in the sample spectra. While the overlapping peaks could be resolved with a longer time in the chromatography column, the number and strength of the peaks would not differ. This leads to the conclusion that methanol, while a good solvent for UV-Vis spectroscopy is not a good solvent for GC/MS.

1-Octanol

Samples that were run on GC/MS after being extracted in 1-octanol all had the same result. There were four different peaks, one large peak around 6.5 minutes which was 1-octanol, and then 3 smaller peaks toward the end of the run, as seen in Figure 12.

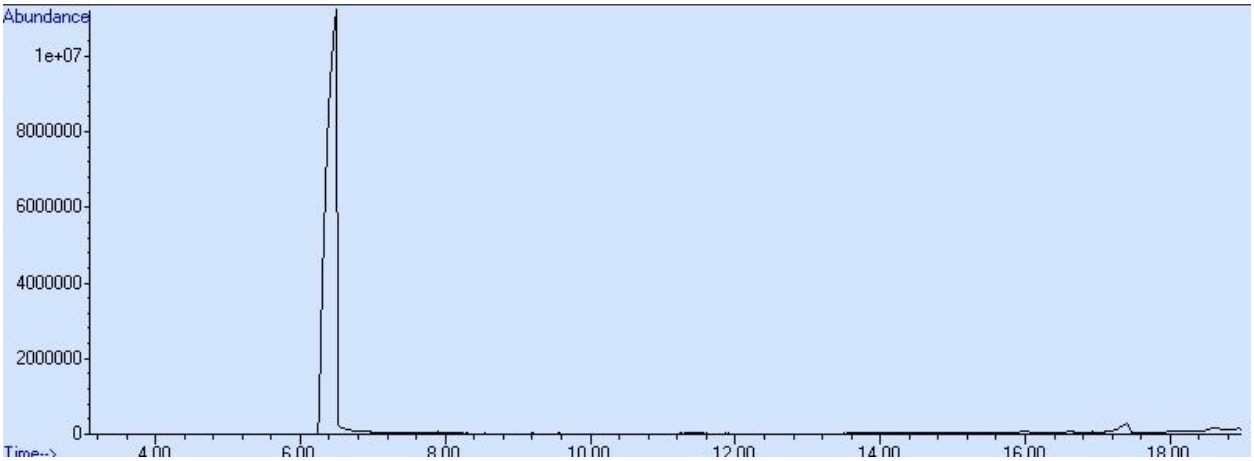

Figure 11: Solvent GC spectrum for 1-octanol. This 1-octanol solvent sample was analyzed by GC/MS using a split injection method of 25:1. The temperature ramp was from 80°C to 250°C.

1-octanol appears as a large peak, around 6 minutes, on the chromatogram because it has a high volatilization temperature and therefore takes longer to enter the column and reach the detector. While this solvent delivered the most consistent spectra, there were no differences between all of the samples. Therefore it is not a good solvent with GC/MS to distinguish chemical differences between processed human hair extensions and natural human head hair.

Nuclear Magnetic Resonance Spectroscopy

The NMR spectra for all eight samples can be found in Appendix D. All of the extractions were completed in chloroform-d. Many of the samples had a similar reaction when extracted in chloroform-d. Peaks due to chloroform-d were present at a chemical shift of approximately 7.26 and 1.54. TMS was set to a chemical shift of 0.00.

Overall, the samples all created similar spectra when extracted in chloroform-d and analyzed using NMR. The samples did have complex spectra, but they are not complex in the way that polymers are. Polymers generally have many peak groups and within those peak groups a variety of peaks that are close together. There are also typically many aromatics present, which appear around a ppm of 7. The only peaks present around 7 ppm correlates to chloroform-d However, while it is unlikely that the samples of hair contain polymers, there are two outliers who's differences are interesting and need to be discussed: the Remy XQ processed human hair extension and the undyed negroid natural hair.

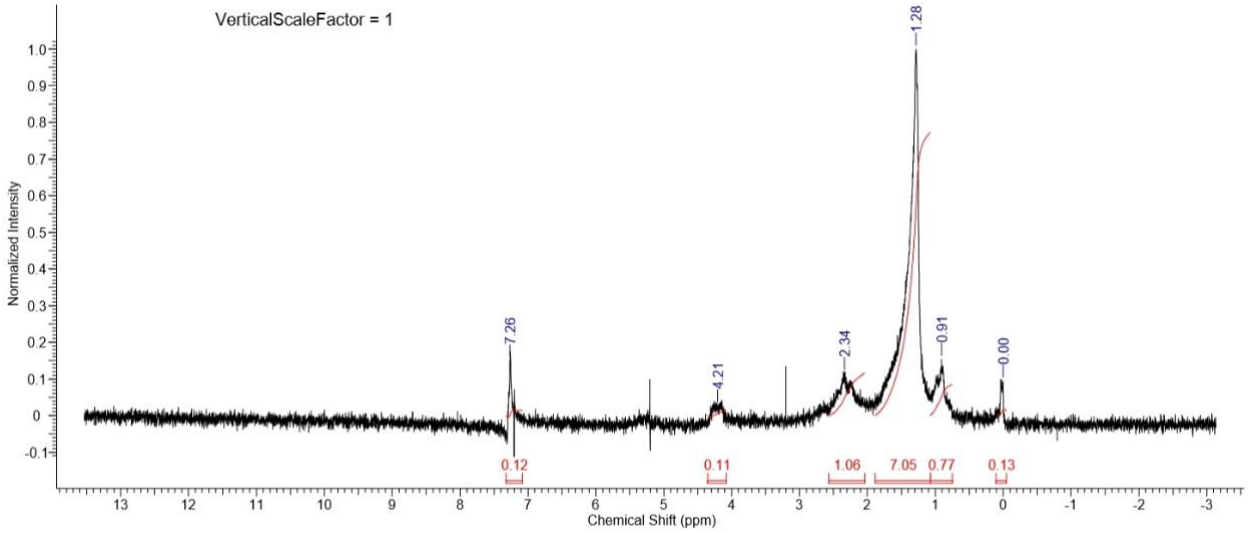

Figure 12: Proton NMR of Remy XQ sample extracted in chloroform-d. 0.02 gram Remy XQ hair extension sample was extracted into 5 mL chloroform-d and left overnight. The extraction solvent was then analyzed using proton NMR.

In Figure 12, the peaks at chemical shifts of 1.28 and 0.91 were present in all the hair samples. The larger peak at 1.28 is so wide that it masks the peak that would be seen due to chloroform-d at 1.54. The other two peaks present are weak, but apparent. They are at a chemical shift of 4.21 and 2.34. The integration information at these peaks are interesting. The peak at 4.21 has an

integration value of 0.11, which is around same integration value the TMS peak has, which is 0.13. This means that these two peaks have around the same proton concentration. The peak at 2.34 has an integration of 1.06 meaning there are ten times as many protons incorporated into this peak than TMS. While NMR spectroscopy of mixtures does not provide any information into the structure of the unknown compound, it does prove that there is something different in this sample than in any others tested.

The other sample that provided interesting results was the undyed negroid hair sample. It possesses significantly more peaks toward the lower end of the chemical shift and an extra peak at 7.18 around the expected chloroform-d peak at 7.26. This is all shown in Figure 13.

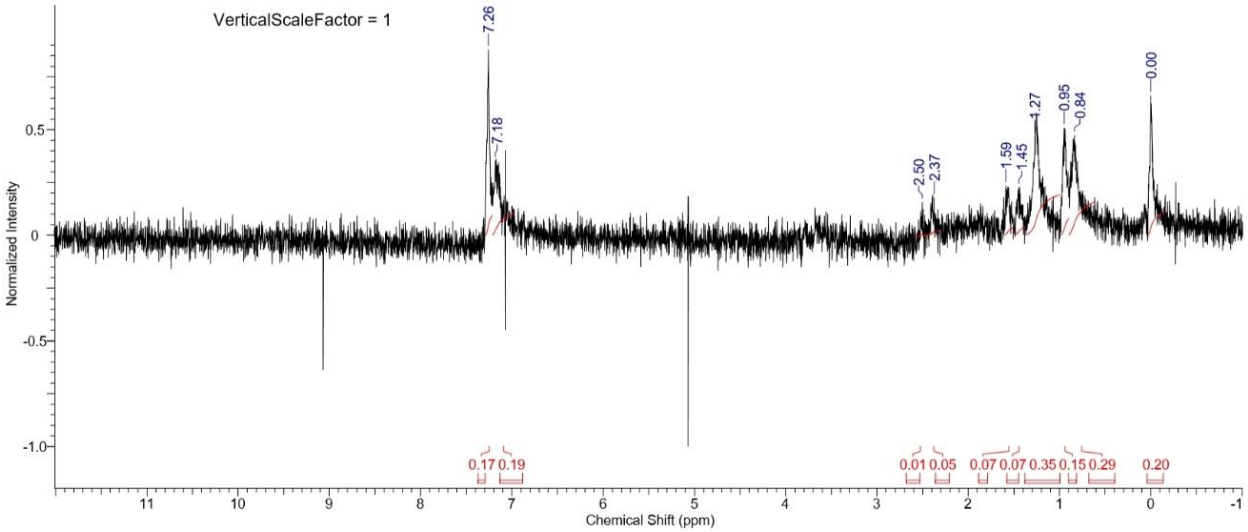

Figure 13: NMR spectrum from undyed negroid hair extracted in chloroform-d. 0.02 gram of undyed negroid natural human head hair sample was extracted into 1 mL chloroform-d and left overnight. The extraction solvent was then analyzed using proton NMR.

After some research, it is probable that these extra peaks present in the undyed negroid sample are due to the higher concentration of melanin in negroid hair. Figure 14 shows that Katritzky et al. (2002) previously had determined the proton NMR spectrum of melanin in human hair.

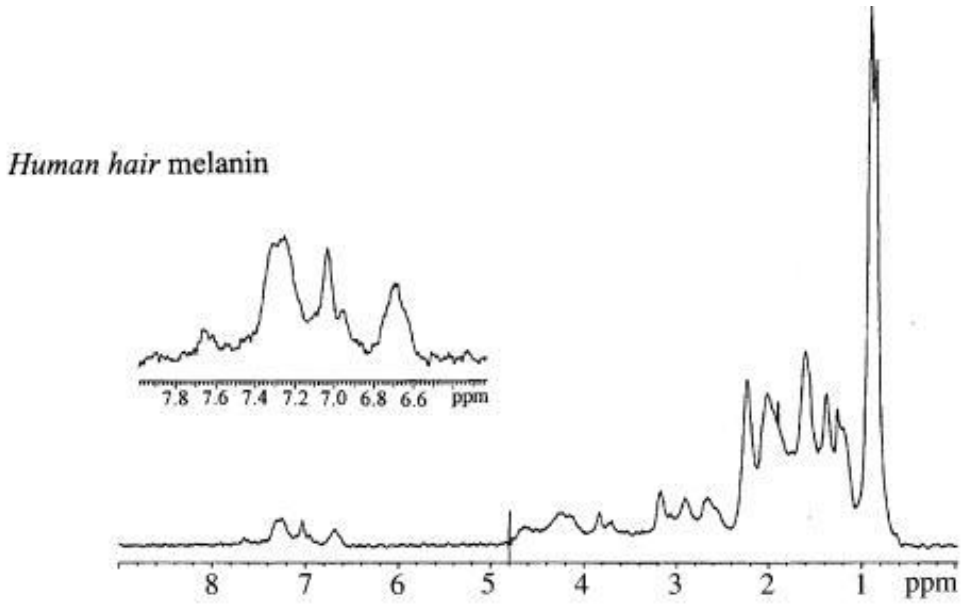

Figure 14: Proton NMR spectrum of melanin in human hair with "water suppression in D_2O/NH_4OH at pH 10" (Katritzky et al., 2002).

There is a large concentration of peaks around 7 ppm which they determined is related to the aromatic region of melanin. The lower, larger, peaks from 2.5 to TMS at 0.00, correspond to the rest of the protons in melanin (Katritzky et al., 2002). From this information, it is evident that the extra peaks present in the undyed negroid hair sample are due to increased concentrations of melanin.

NMR was able to more precisely identify unknown compounds present in the Remy XQ sample. It is impossible to determine what the compound is or what the chemical structure looks like, but NMR results show that there is a chemical difference between processed human hair extensions and natural human head hair. In addition to this, it confirms that there is more melanin present in negroid hair than caucasoid or mongoloid hair.

Conclusion

Currently, chemical analysis is not typically completed in a forensic lab for hair samples. This is because there are no documented and validated methods for performing chemical analysis on hair samples of evidentiary value. According to Guideline 12 in the Scientific Working Group on Materials Analysis Forensic Human Hair Analysis Guidelines (2005), "analyses may be performed on hair that have been chemically altered or have trace materials on the surface . . . techniques are beyond the scope of these guidelines because they are not used widely" (Scientific Working Group on Materials Analysis, 2005). This research shows that there is a chemical difference between processed human hair extensions and natural human head hair. The methods used in this research would save valuable time and money within the forensic laboratory. This chemical analysis method would only take a single day to complete and could exclude a hair prior to the expensive, and time-consuming method of mitochondrial analysis.

There were a variety of results with the solvents used for UV-Vis spectroscopy. Hexanes and Colorist Secrets™ Hair Color Remover did not have any identifiable absorption patterns that could be used to distinguish between processed human hair extensions and natural human head hair. Hexanes did have some readings, but there was nothing that was unique to processed human hair extensions. Colorist Secrets™ Hair Color Remover did not have any useful results since the readings for all of the hair samples were around zero.

The extractions completed with 1-octanol had some interesting results. The processed human hair extensions had varying results; the ECW sample did react differently enough from the other samples that it leads to the possibility of 1-octanol as a useful solvent. However, the Remy XQ and Que sample did not absorb much differently than the other samples. Undyed natural human hair samples did react differently than dyed samples from 300 to 400 nm, but then

had no change from the blank. The major issue with 1-octanol is that the absorbance values are all below 0.1. This is a weak absorbance and potentially could have reliability issues for consistently detecting minute changes.

The solvent that produced the best results using UV-Vis spectroscopy was methanol. The absorbance was consistently high enough to be reproducible and reliable. The processed human hair extensions had a wide variety of peaks that differed from the few peaks that were found in the natural human head hair. The samples of natural human head hair that did have peaks had much lower overall absorbance than the processed human hair extensions. The difference in peaks and concentration when using methanol as an extraction solvent seems to be able to differentiate between processed human hair extensions and natural human head hair.

In GC/MS analysis, the only chemical differences between the samples and the solvent occurred when a hexanes was used as the extraction solvent. Colorist Secrets™ Hair Color Remover and methanol had reproduction issues. There were far too many peaks that varied in concentration from sample to sample to make a reliable determination of what was present within the tested solution. 1-octanol had no other compounds present within the solution other than what was expected from the solvent. It is possible the GC/MS in hexanes could work as a methodology for differentiating processed human hair extensions and natural human head hair. Other methods of analysis, however, indicated the presence of additional chemical species that were not seen in the GC/MS with hexanes indicating it may not be the most reliable or specific method for identifying a hair as a processed human hair extension or a natural human head hair.

NMR confirmed the samples are mixtures of compounds, but they are not complex enough to be polymers. Once the peaks that are in the chloroform-d are taken into account, there are only two peaks that were consistent throughout both the processed human hair extensions and

natural human head hair. Only two samples presented peaks of interest: the Remy XQ processed human hair extensions sample and the undyed negroid hair sample. The extra peaks present in the undyed negroid sample are most likely due to melanin. The Remy XQ sample did have one peak that may be due to melanin, but there is one other peak that is questionable. While NMR did not identify or give insight into what this extra peak could be, it does show that there is something present on the Remy XQ hair extensions that are not present on any of the other hair samples. It is probable that since these hair extensions are higher quality than ECW or Que, that the company adds an extra substance to improve the longevity and appearance of the extension over time. While this could not identify all brands and qualities processed human hair extensions, it could lead to the exclusion of some samples prior to DNA analysis and therefore still have an effect on the hair analysis process completed in forensic laboratories.

There are chemical differences between processed human hair extensions and natural human head hair that are able to be visualized. The main issue is that the specific compounds that make-up the suite of chemicals added to processed human hair extensions are not able to be identified. Also, it is uncertain if all brands and qualities of hair extensions would produce the same types of result. All of the solvents were selected in order to extract different types of molecules: polar versus nonpolar. Many of the solvents worked in one method but not another. This proves that not all molecules react the same to different chemical analysis, but there were molecules extracted in most of the organic extractions. These molecules and reactions show differences between processed human hair extensions and natural human head hair that can ultimately be used for differentiation in forensic cases.

Each method of analysis showed different qualities of each sample. UV-Vis was able show chemical differences in all three processed human hair extensions when compared to the

natural human head hair. GC/MS only found one substance present in the Que hair extensions that was not present in any other samples. NMR was able to pick out differences in the Remy XQ hair extensions and undyed negroid hair from all the other samples. In the undyed negroid sample, the differences are associated with the melanin present in higher concentrations in negroid hair. The Remy XQ differences are unaccounted for. This research shows that there are chemical differences between processed human hair extensions and natural human head hair. Therefore, a need of recognition and adaptation of the methods shown in this research in the forensic laboratory is necessary.

Future Research

While chemical differences were identified between processed human hair extensions and natural human head hair, it is necessary to continue to complete these tests with a wider range of samples. In this study, only three different types and brands of processed human hair extensions were used for analysis. There are many more kinds of processed human hair extensions that can be researched. It is also possible that newer instrumentation might be able to zero out solvent peaks in GC/MS and therefore all of the peaks that would appear in the sample chromatogram would be from the sample extraction. After all of the analyses are completed, statistical analysis should be applied to determine the reliability of the chemical analysis method(s). One statistical test that might be used to determine the significance of variation is ANOVA. All of these additional studies may lead to a method that could be successfully added to forensic science methodologies for hair analysis, which would eliminate processed human hair extensions as probative evidence in forensic case work.

This study has proved that there are chemical differences between processed human hair extensions and natural human head hair, other methods need to be explored as more efficient means of chemical identification. One new method would be to use the physical hairs and examine them with infrared light underneath a microscope, termed infrared microspectroscopy. This is a method that is already being used for fiber analysis and has been able to reveal a variety of different chemicals present within fibers (Cho et al., 2001). This method could specifically identify which locations on the hair and hair extensions to compare the infrared spectra of and may find definitive differences in the cuticle, the cortex, and the medulla. From this, the location of substances specific to the processed human hair extensions could be identified and analysis may determine how deep into the hair the chemicals penetrate.

Another method currently used for fiber analysis that could potentially be used to differentiate natural human head hair from processed human hair extensions is infrared chemical imaging. This method is used to determine spectral differences in bicomponent fibers. When chemically imaged, the differences in the location of the different components within the fiber become obvious, as they are portrayed in different colors (Flynn et al., 2006). This could also be completed using natural human head hair and hair extensions to determine where different compounds are located within the sample.

To implement into forensic laboratories, a reliable and consistent method needs to be determined. Other brands and qualities of processed human hair extensions also need to be analyzed. In addition to this, other methods may be better suited for processed human hair differentiation and need to be attempted. This study proves this need for further research into the differentiation of processed human hair extensions and natural human head hair for use in the forensic laboratory.

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Appendix A

Tables of the Masses of Hair Samples Used for Analysis

Table 1: Hair Sample Masses Used in Hexanes Extractions

Hexanes	Sample 1 (g)	Sample 2 (g)	Sample 3 (g)	Average	Standard Deviation
Remy XQ Processed Human Hair Extention	0.1014	0.1003	0.1017	0.1011	0.0007
ECW Processed Human Hair Extension	0.1017	0.1012	0.1026	0.1018	0.0007
Que Processed Human Hair Extension	0.1022	0.1008	0.1013	0.1014	0.0007
Undyed Caucasian Natural Human Head Hair	0.1005	0.1015	0.0996	0.1005	0.0010
Dyed Caucasian Natural Human Head Hair	0.1002	0.1014	0.1017	0.1011	0.0008
Undyed Mongoloid Natural Human Head Hair	0.1002	0.1022	0.1028	0.1017	0.0014
Dyed Mongoloid Natural Human Head Hair	0.1019	0.1012	0.1022	0.1018	0.0005
Undyed Negroid Natural Human Head Hair	0.1003	0.1006	0.1008	0.1006	0.0003

Table 2: Hair Sample Masses Used in Colorist Secrets™ Hair Color Remover Extractions

Colorist Secrets™ Hair Color Remover by L'Oreal	Sample 1 (g)	Sample 2 (g)	Sample 3 (g)	Average	Standard Deviation
Remy XQ Processed Human Hair Extention	0.1064	0.1003	0.1010	0.1026	0.0033
ECW Processed Human Hair Extension	0.1016	0.1048	0.1020	0.1028	0.0017
Que Processed Human Hair Extension	0.1022	0.1005	0.1006	0.1011	0.0010
Undyed Caucasian Natural Human Head Hair	0.1040	0.1013	0.1017	0.1023	0.0015
Dyed Caucasian Natural Human Head Hair	0.1008	0.1035	0.1026	0.1023	0.0014
Undyed Mongoloid Natural Human Head Hair	0.1038	0.1001	0.1018	0.1019	0.0019
Dyed Mongoloid Natural Human Head Hair	0.1008	0.1024	0.1035	0.1022	0.0014
Undyed Negroid Natural Human Head Hair	0.1019	0.1033	0.1006	0.1019	0.0014

Table 3: Hair Sample Masses Used in Methanol Extractions

Methanol	Sample 1 (g)	Sample 2 (g)	Sample 3 (g)	Average	Standard Deviation
Remy XQ Processed Human Hair Extention	0.1030	0.1034	0.1040	0.1035	0.0005
ECW Processed Human Hair Extension	0.1012	0.1033	0.1012	0.1019	0.0012
Que Processed Human Hair Extension	0.1035	0.1030	0.1004	0.1023	0.0017
Undyed Caucasian Natural Human Head Hair	0.1026	0.1020	0.1003	0.1016	0.0012
Dyed Caucasian Natural Human Head Hair	0.1003	0.1031	0.1031	0.1022	0.0016
Undyed Mongoloid Natural Human Head Hair	0.1039	0.1039	0.1011	0.1030	0.0016
Dyed Mongoloid Natural Human Head Hair	0.1035	0.1015	0.1048	0.1033	0.0017
Undyed Negroid Natural Human Head Hair	0.1020	0.1029	0.1035	0.1028	0.0008

Table 4: Hair Sample Masses Used in 1-Octanol Extractions

1-Octanol	Sample 1 (g)	Sample 2 (g)	Sample 3 (g)	Average	Standard Deviation
Remy XQ Processed Human Hair Extention	0.1003	0.1016	0.1002	0.1007	0.0008
ECW Processed Human Hair Extension	0.1001	0.1019	0.1009	0.1010	0.0009
Que Processed Human Hair Extension	0.1010	0.1008	0.1013	0.1010	0.0003
Undyed Caucasian Natural Human Head Hair	0.1017	0.1002	0.1012	0.1010	0.0008
Dyed Caucasian Natural Human Head Hair	0.1025	0.1013	0.1004	0.1014	0.0011
Undyed Mongoloid Natural Human Head Hair	0.1018	0.1017	0.1018	0.1018	0.0001
Dyed Mongoloid Natural Human Head Hair	0.0999	0.1017	0.1008	0.1008	0.0009
Undyed Negroid Natural Human Head Hair	0.1022	0.1022	0.1023	0.1022	0.0001

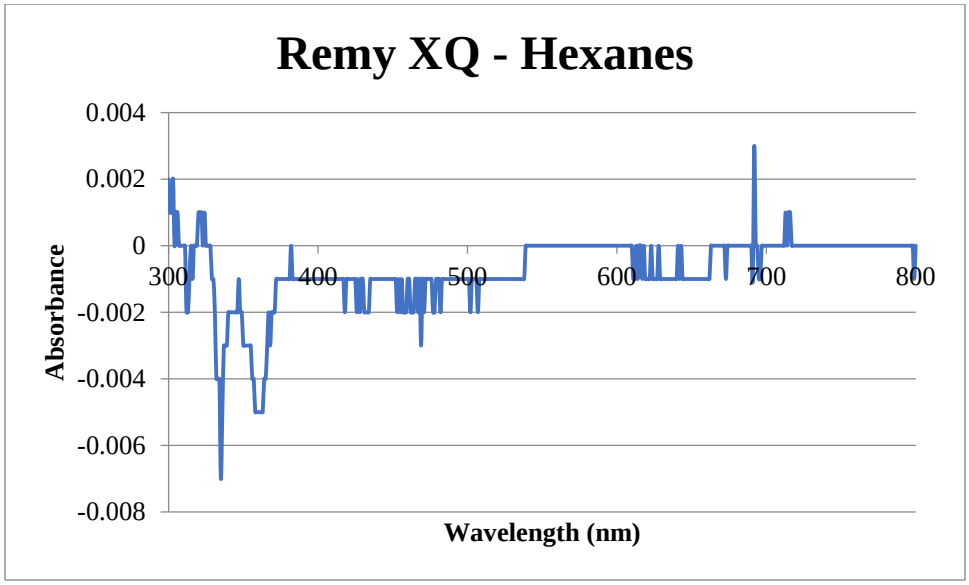
Table 5: Hair Sample Masses Used in Chloroform-D Extractions

Chloroform-D	Sample (g)
Remy XQ Processed Human Hair Extention	0.0198
ECW Processed Human Hair Extension	0.0201
Que Processed Human Hair Extension	0.0209
Undyed Caucasian Natural Human Head Hair	0.0212
Dyed Caucasian Natural Human Head Hair	0.0198
Undyed Mongoloid Natural Human Head Hair	0.0207
Dyed Mongoloid Natural Human Head Hair	0.0216
Undyed Negroid Natural Human Head Hair	0.0227

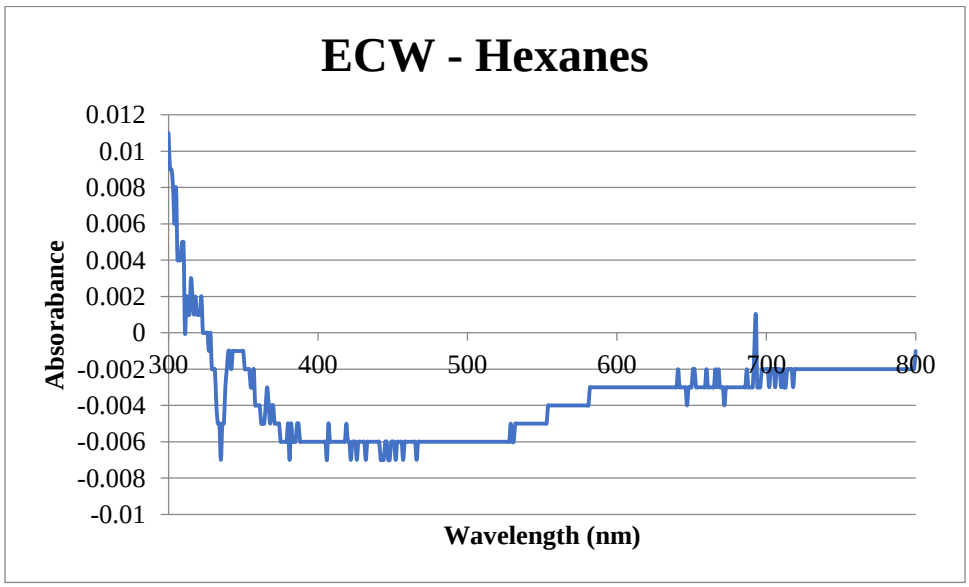
Appendix B

Ultraviolet-Visible Spectroscopy Spectra

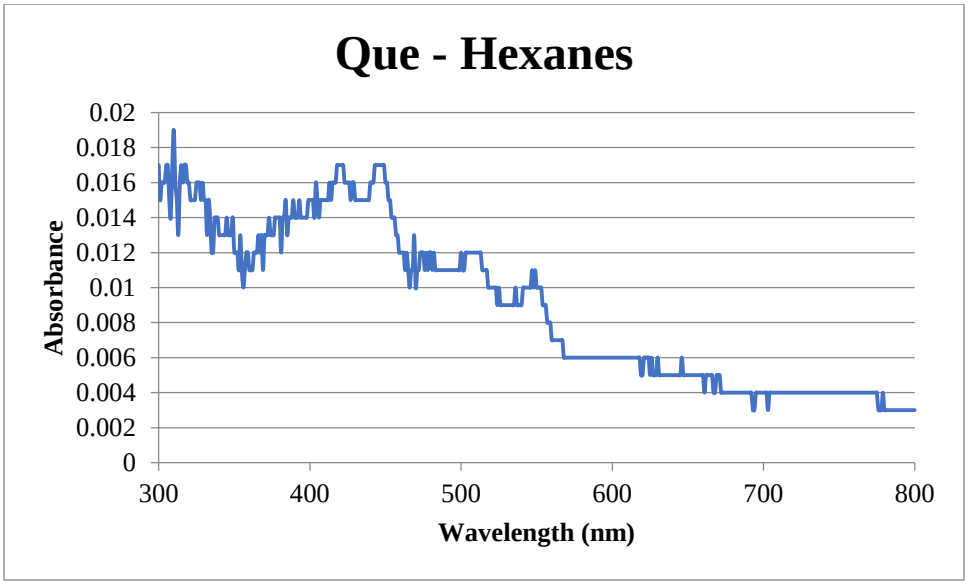
Spectrum 1: Remy XQ Extracted in Hexanes



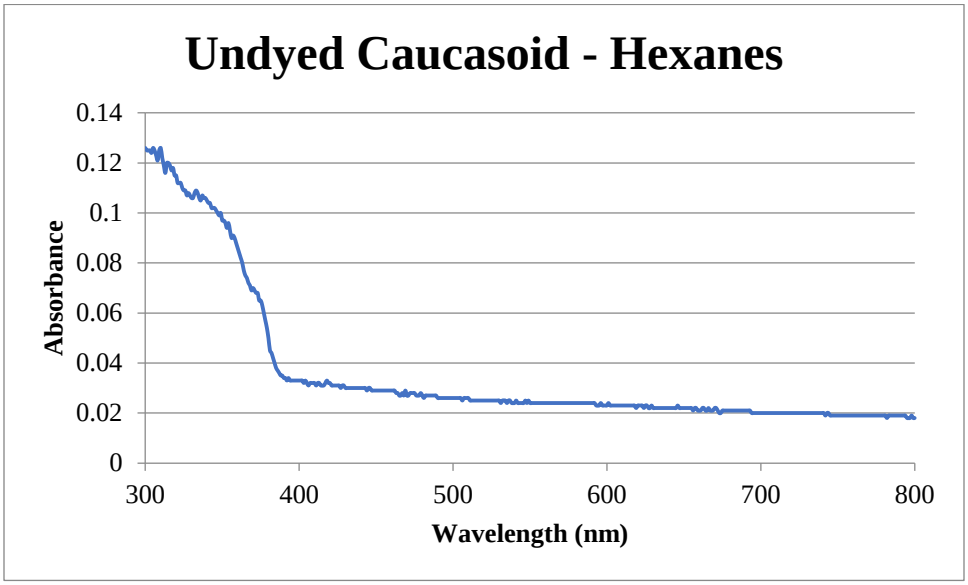
Spectrum 2: ECW Extracted in Hexanes



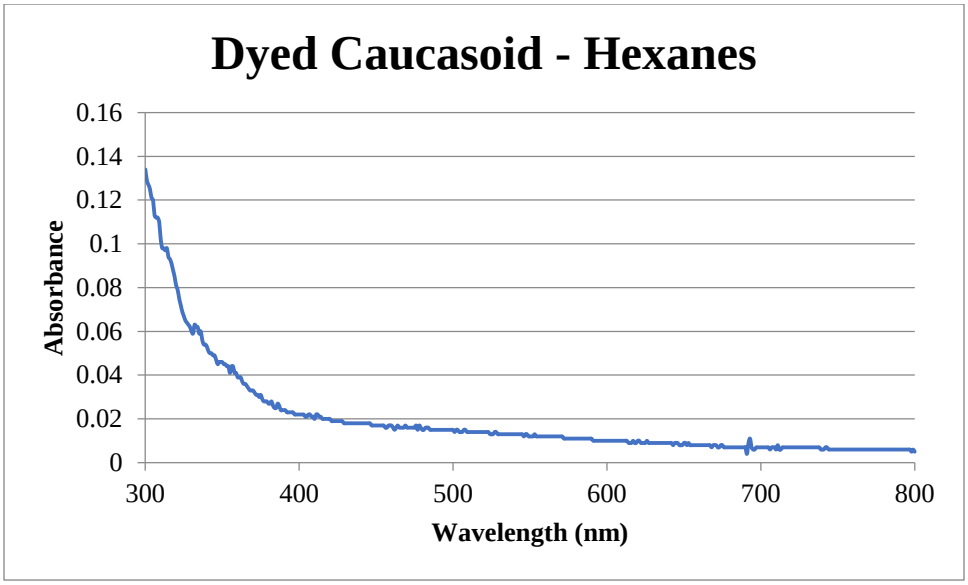
Spectrum 3: Que Extracted in Hexanes



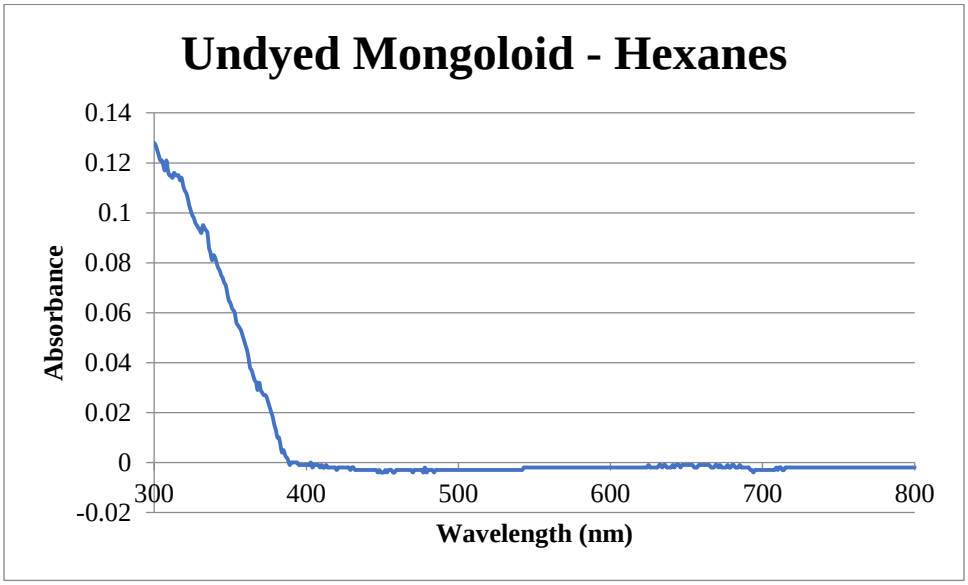
Spectrum 4: Undyed Caucasoid Extracted in Hexanes



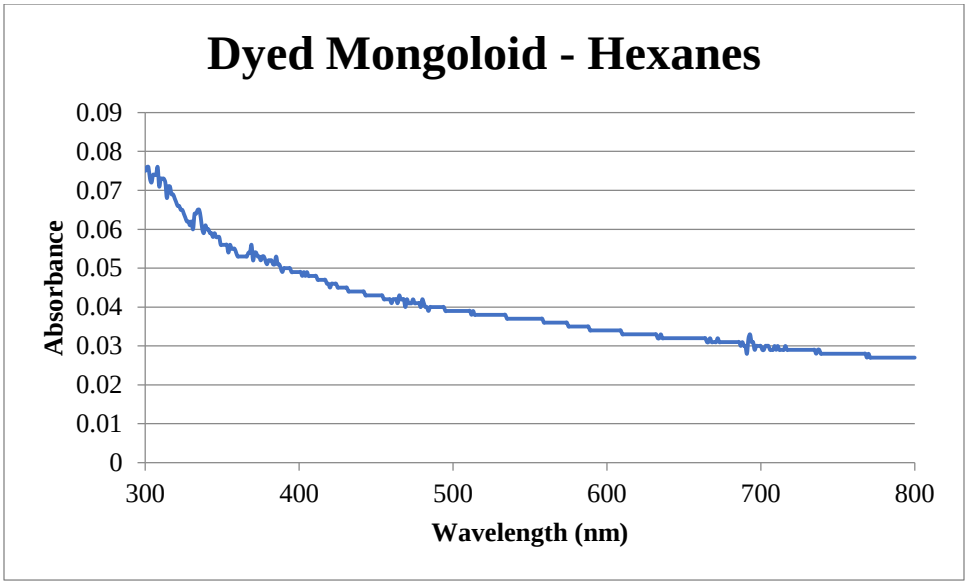
Spectrum 5: Dyed Caucasoid Extracted in Hexanes



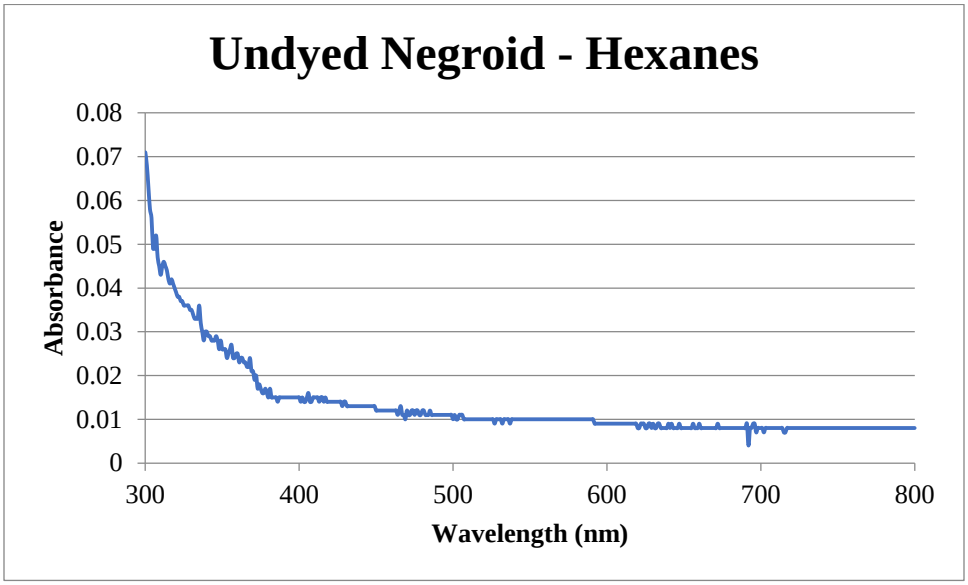
Spectrum 6: Undyed Mongoloid Extracted in Hexanes



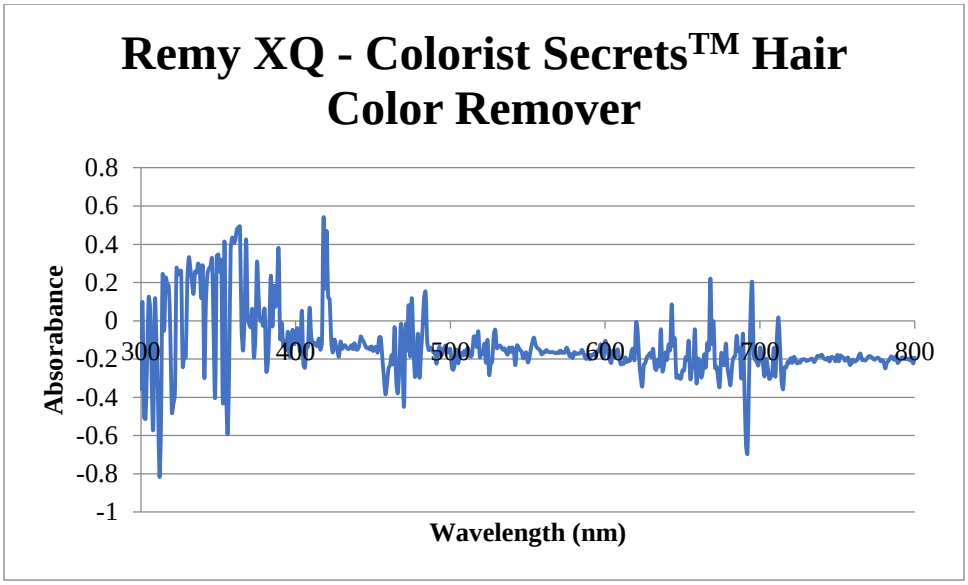
Spectrum 7: Dyed Mongoloid Extracted in Hexanes



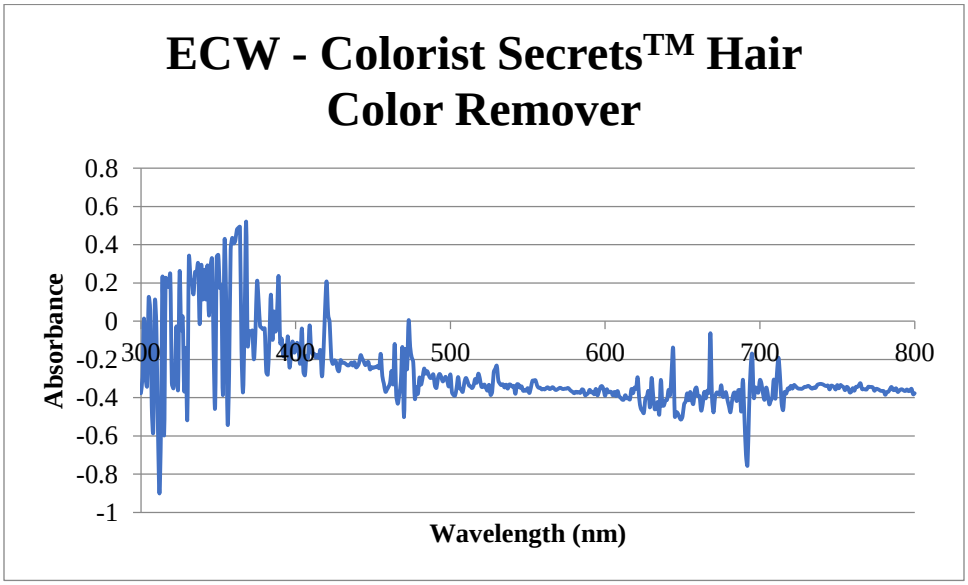
Spectrum 8: Undyed Negroid Extracted in Hexanes



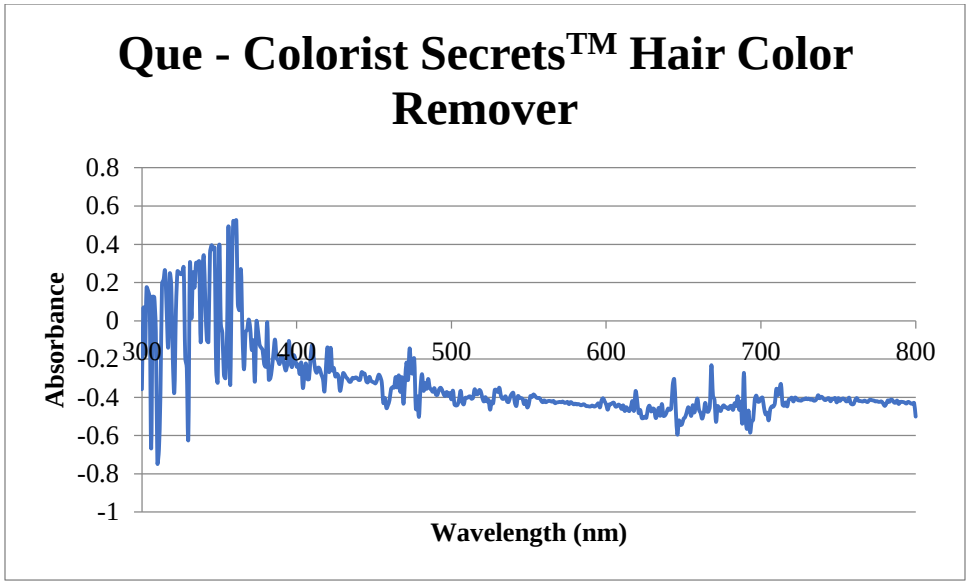
Spectrum 9: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover



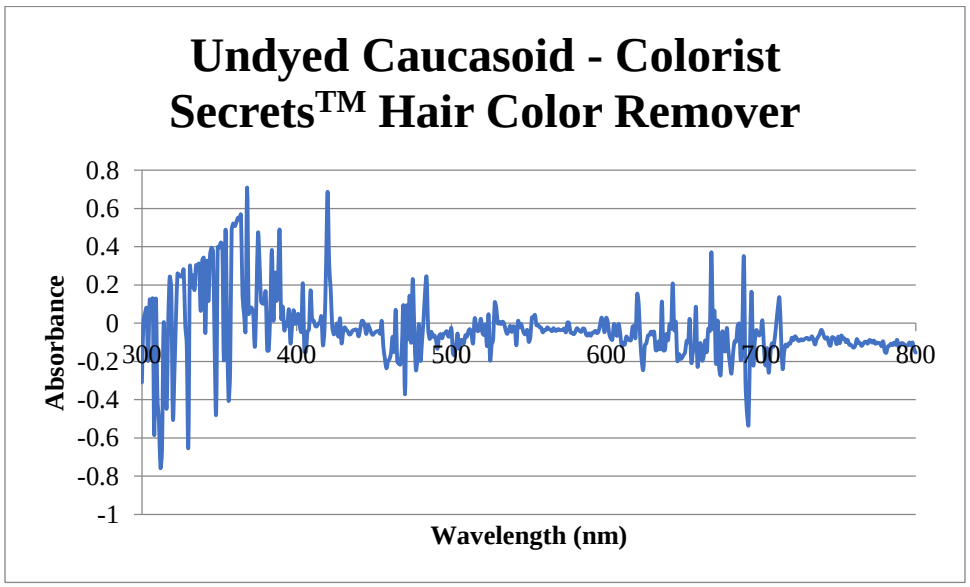
Spectrum 10: ECW Extracted in Colorist Secrets™ Hair Color Remover



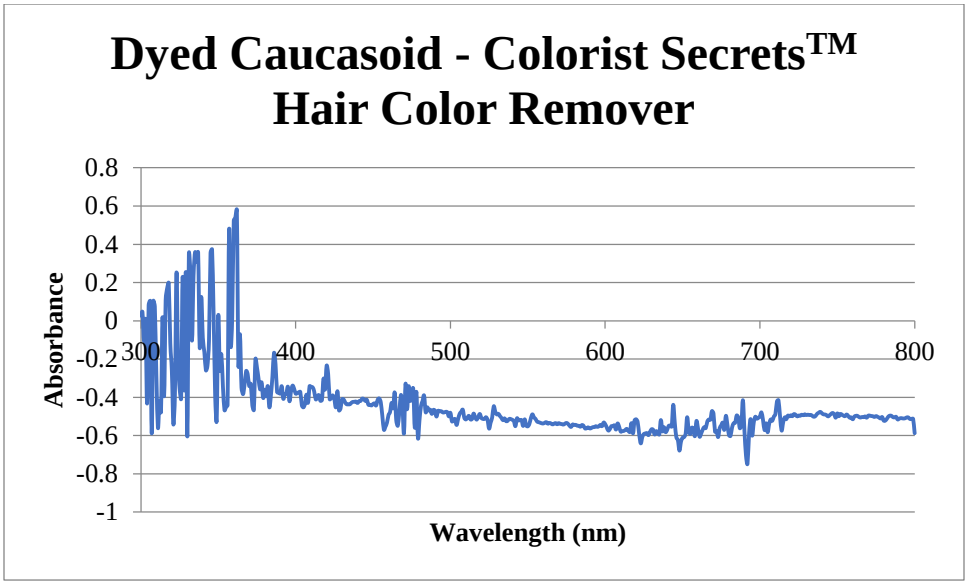
Spectrum 11: Que Extracted in Colorist Secrets™ Hair Color Remover



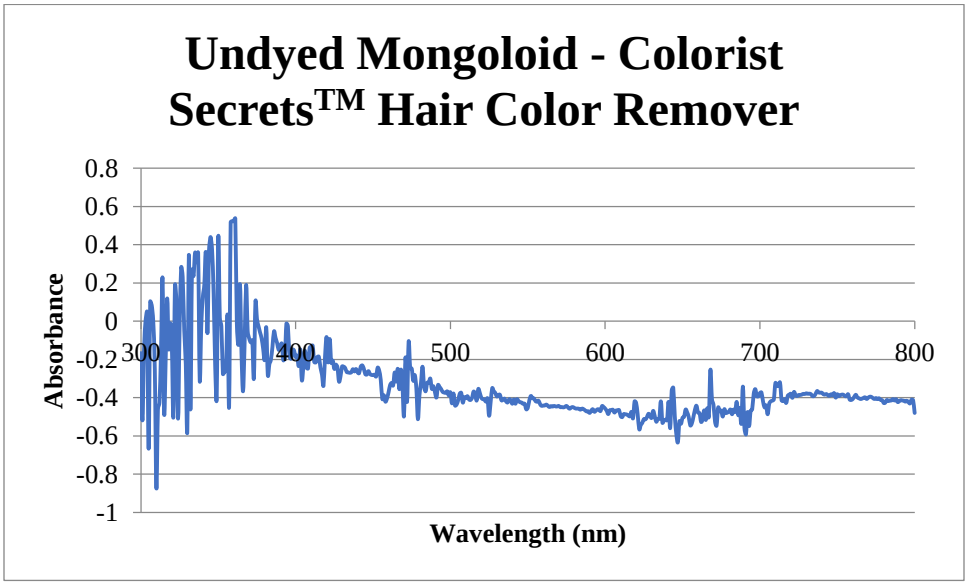
Spectrum 12: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover



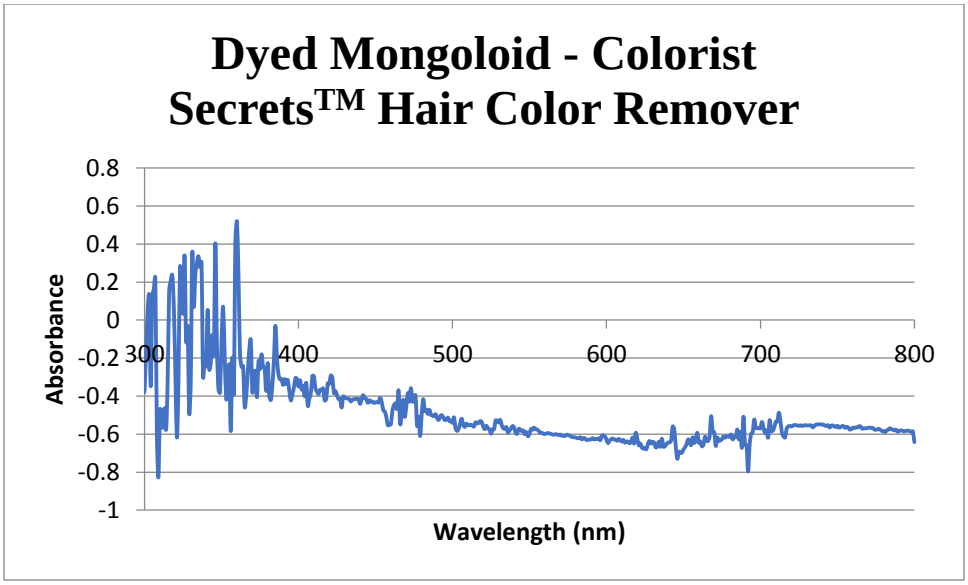
Spectrum 13: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover



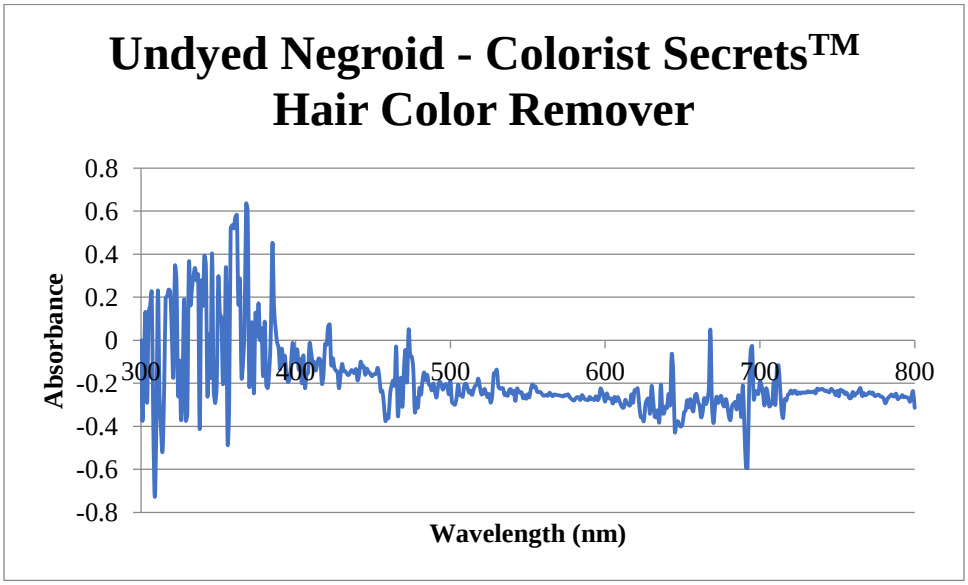
Spectrum 14: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover



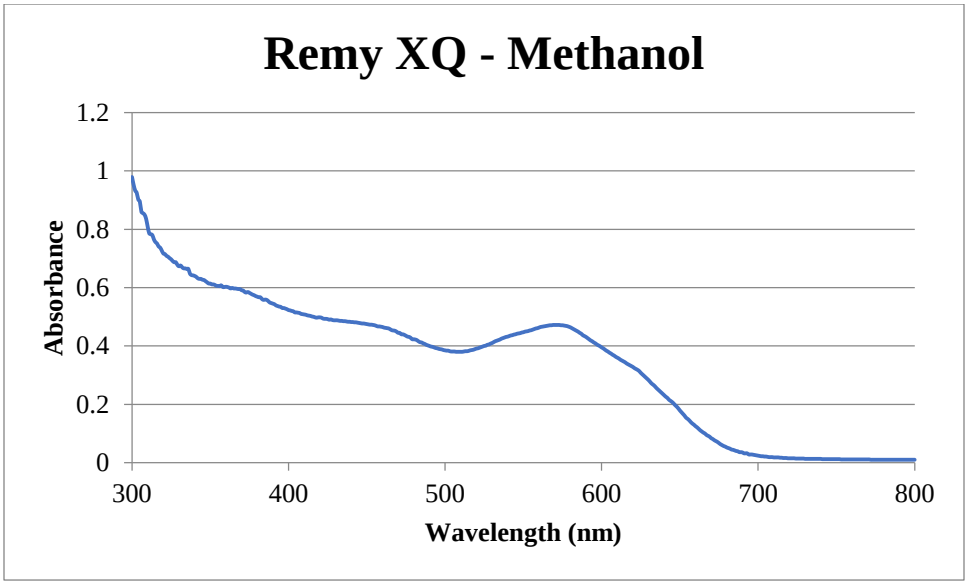
Spectrum 15: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover



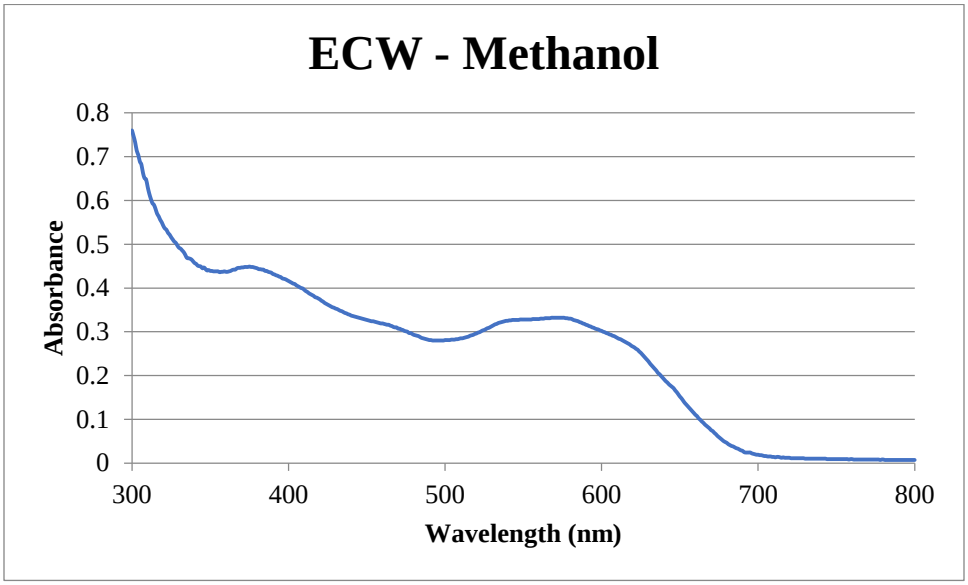
Spectrum 16: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover



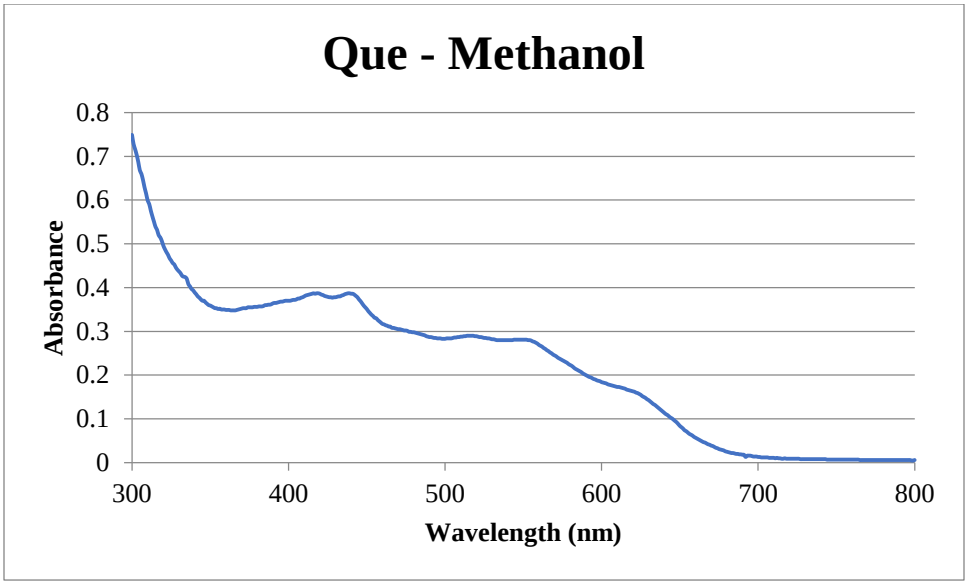
Spectrum 17: Remy XQ Extracted in Methanol



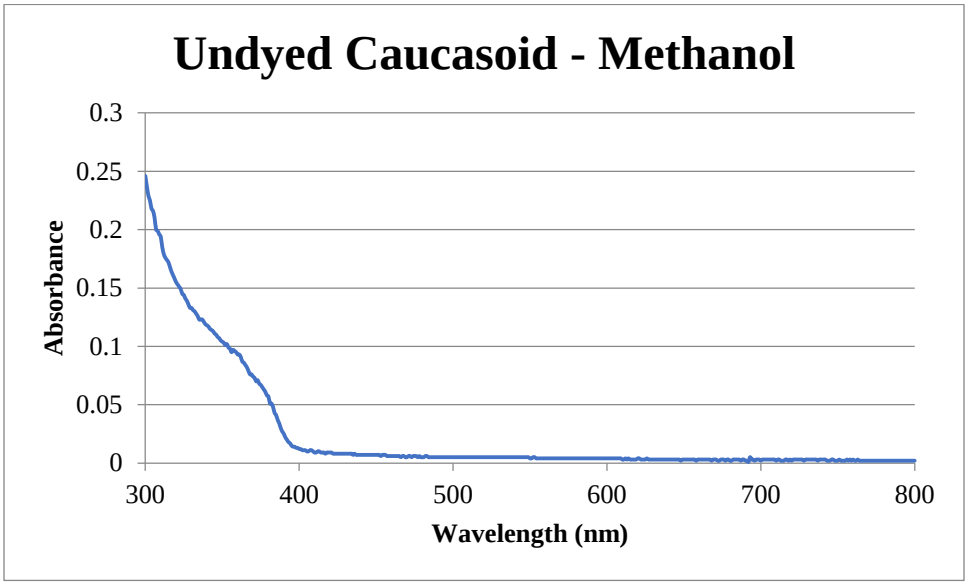
Spectrum 18: ECW Extracted in Methanol



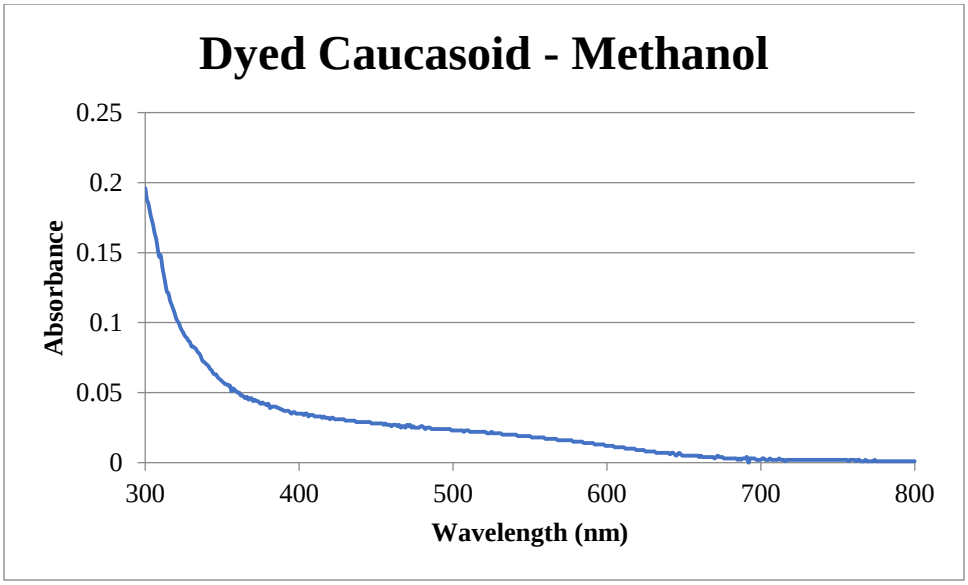
Spectrum 19: Que Extracted in Methanol



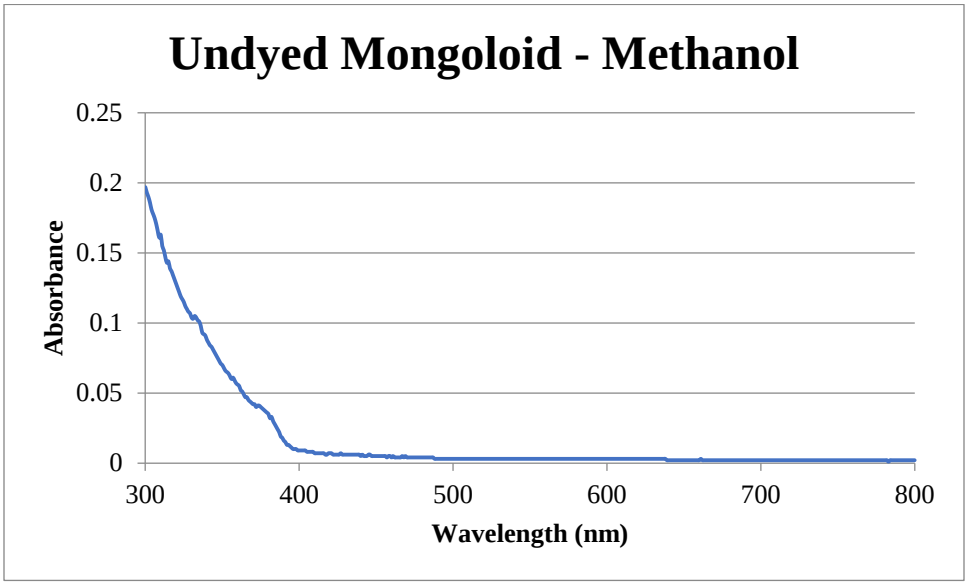
Spectrum 20: Undyed Caucasoid Extracted in Methanol



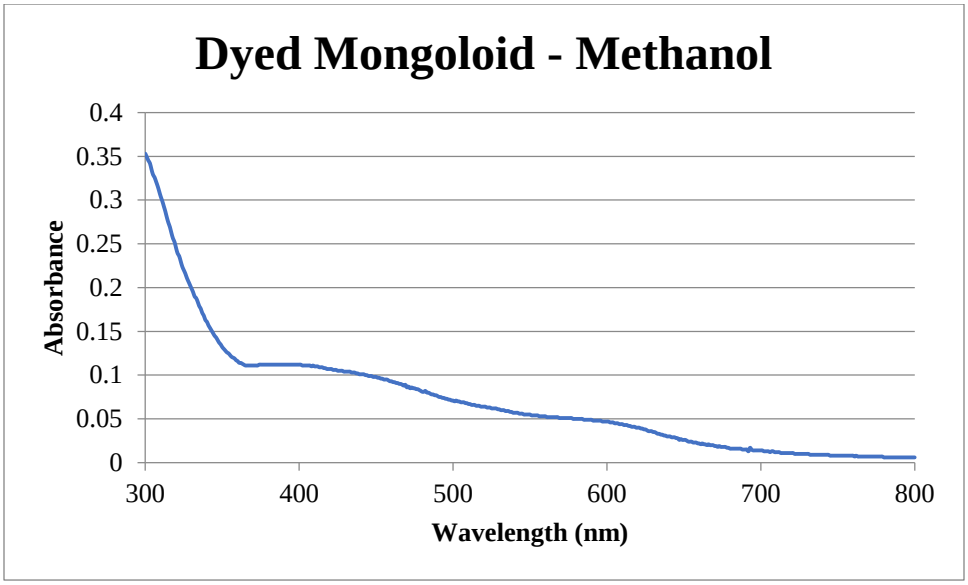
Spectrum 21: Dyed Caucasoid Extracted in Methanol



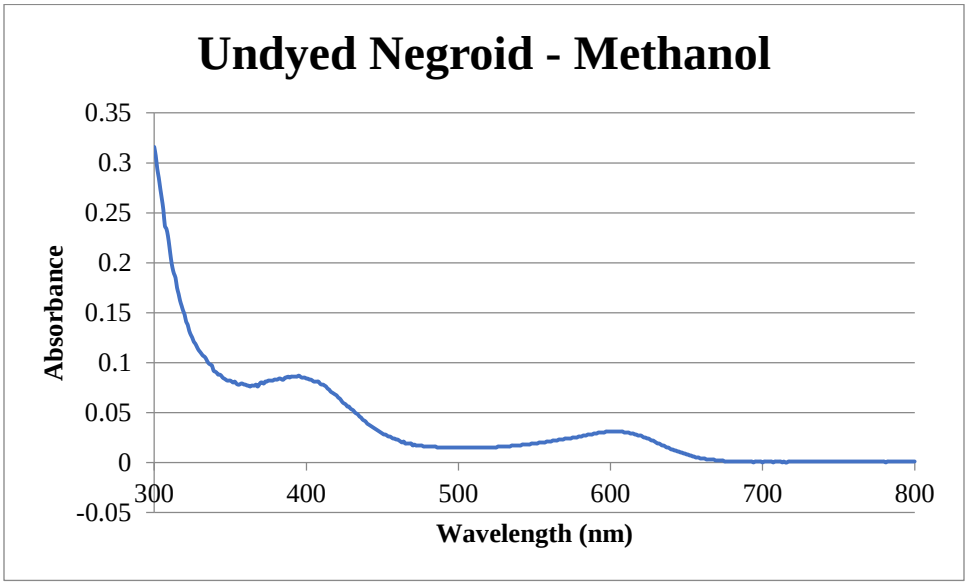
Spectrum 22: Undyed Mongoloid Extracted in Methanol



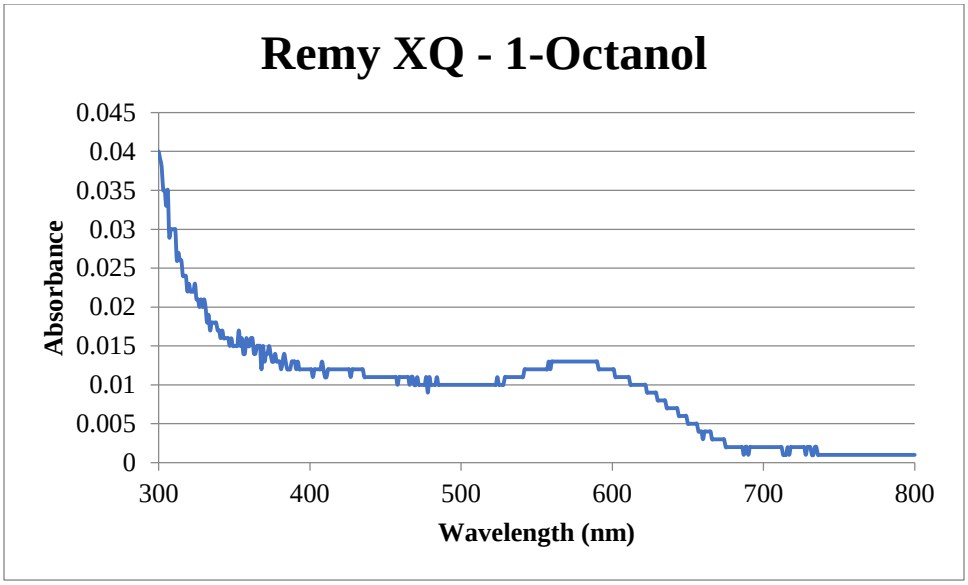
Spectrum 23: Dyed Mongoloid Extracted in Methanol



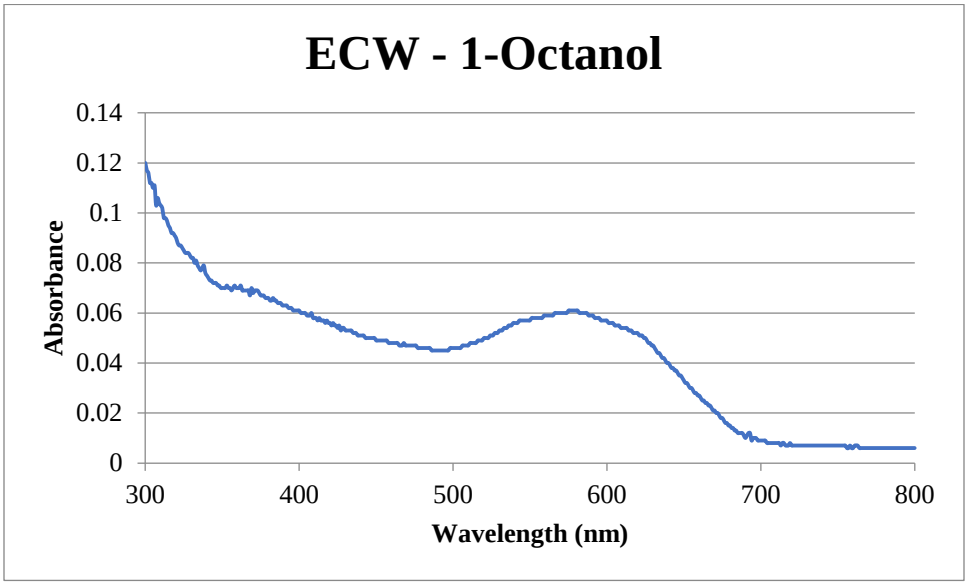
Spectrum 24: Undyed Negroid Extracted in Methanol



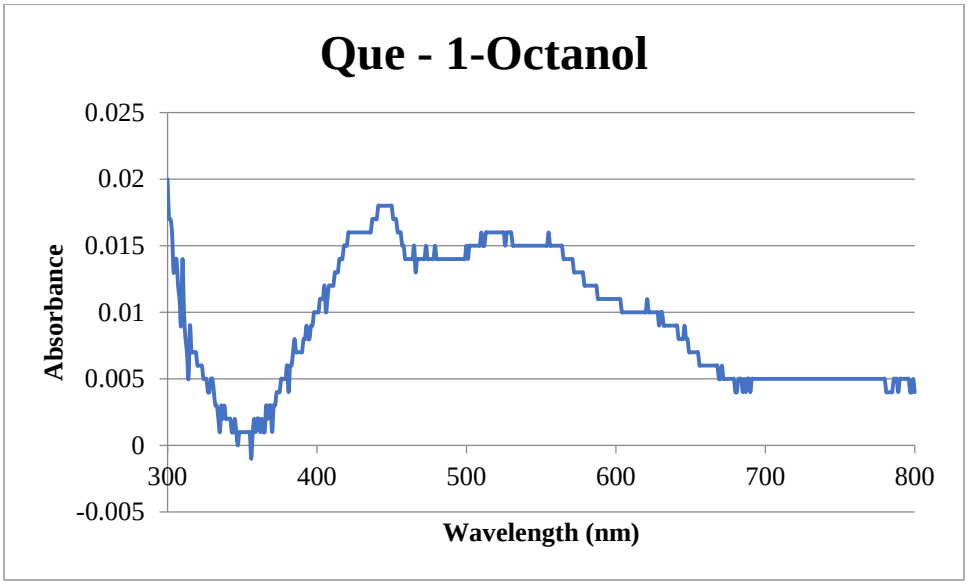
Spectrum 25: Remy XQ Extracted in 1-Octanol



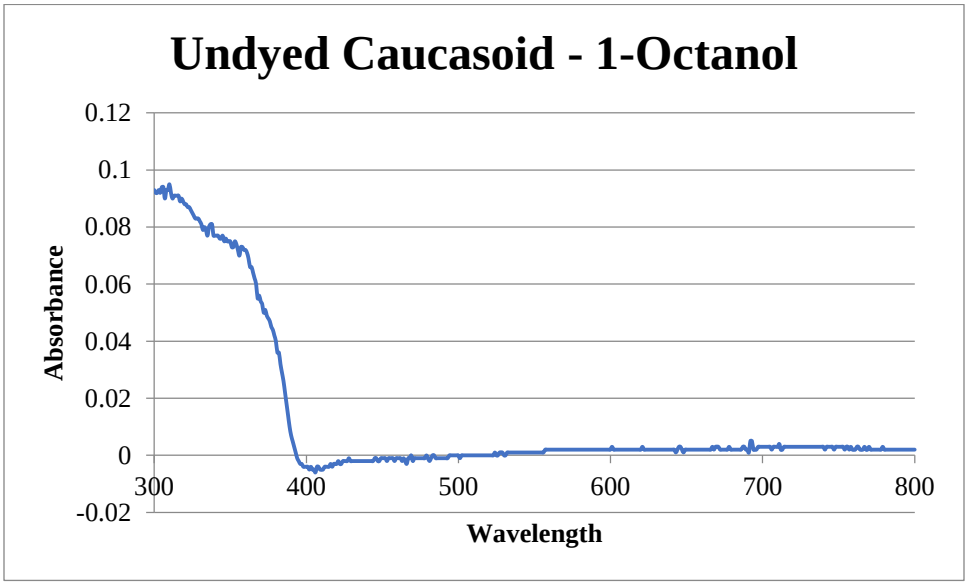
Spectrum 26: ECW Extracted in 1-Octanol



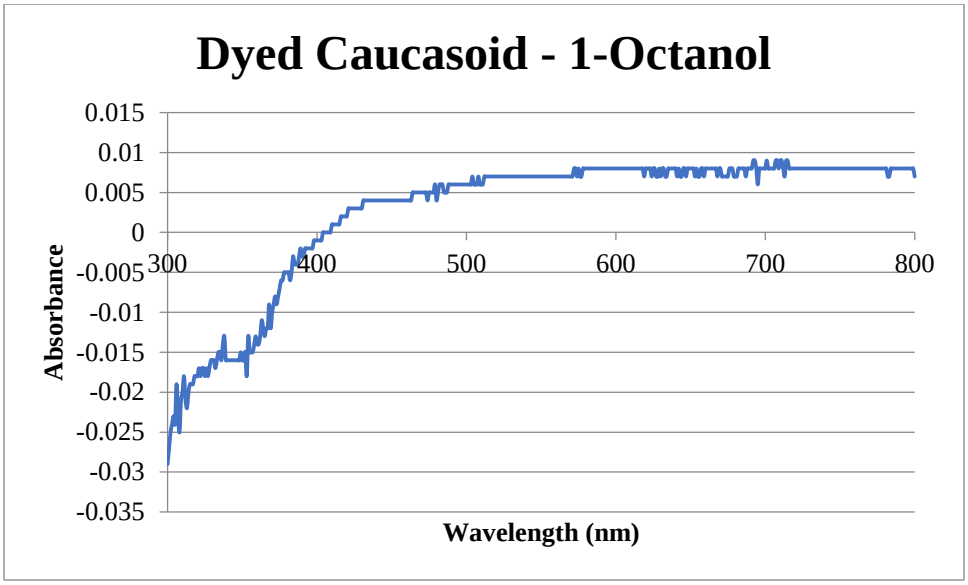
Spectrum 27: Que Extracted in 1-Octanol



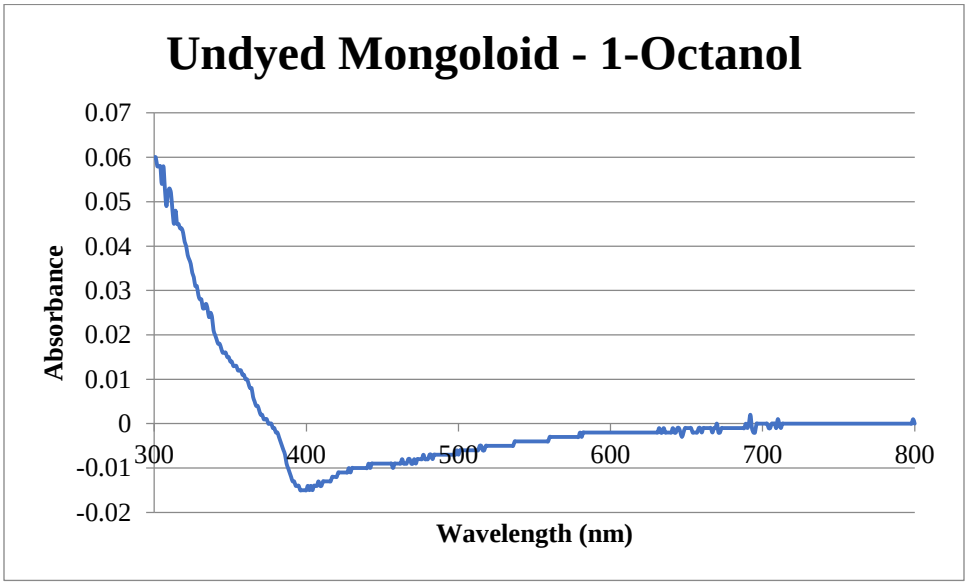
Spectrum 28: Undyed Caucasoid Extracted in 1-Octanol



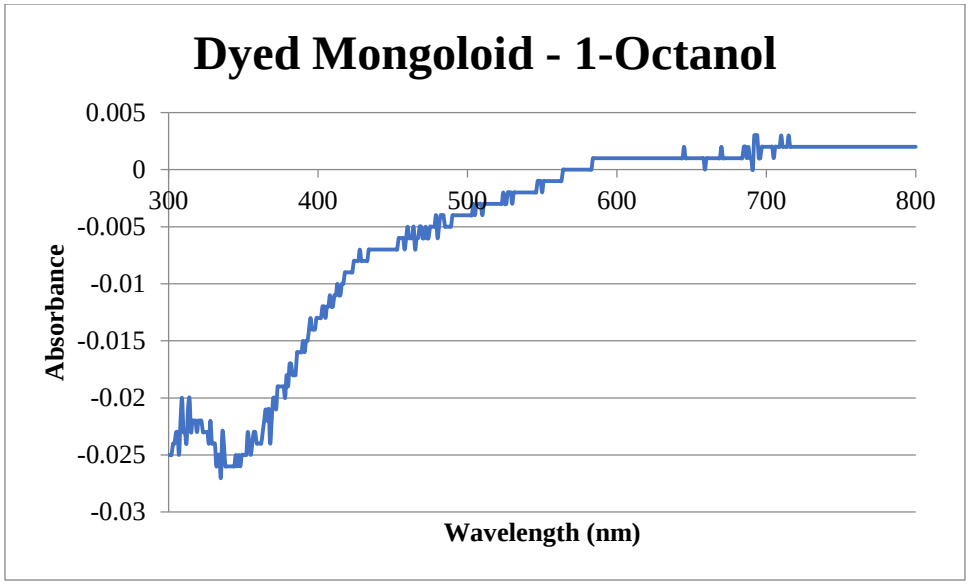
Spectrum 29: Dyed Caucasoid Extracted in 1-Octanol



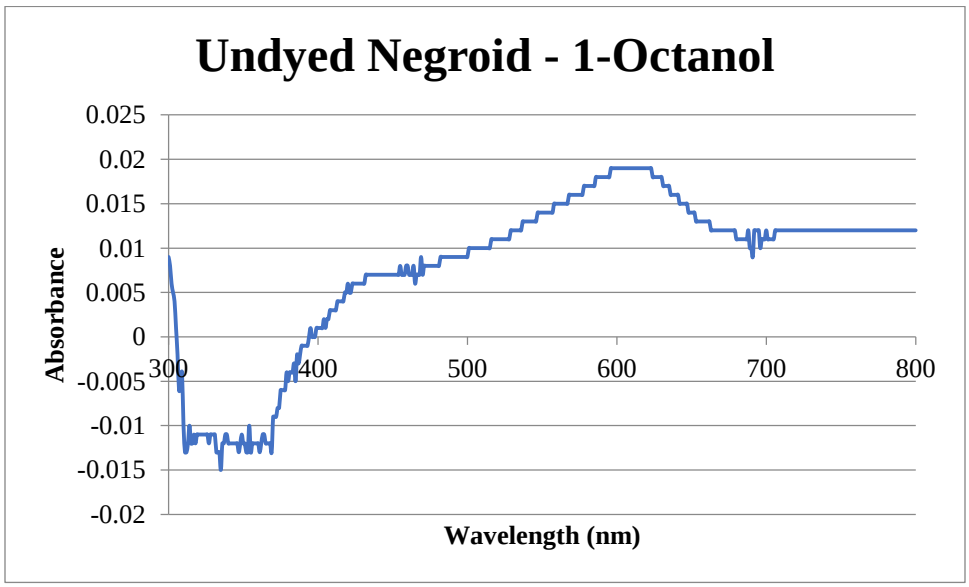
Spectrum 30: Undyed Mongoloid Extracted in 1-Octanol



Spectrum 31: Dyed Mongoloid Extracted in 1-Octanol



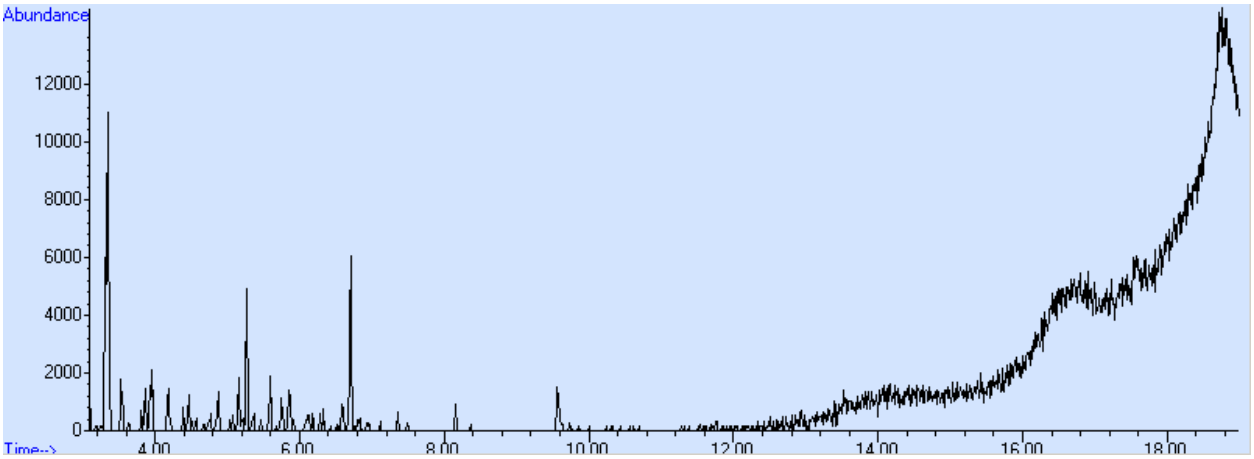
Spectrum 32: Undyed Negroid Extracted in 1-Octanol



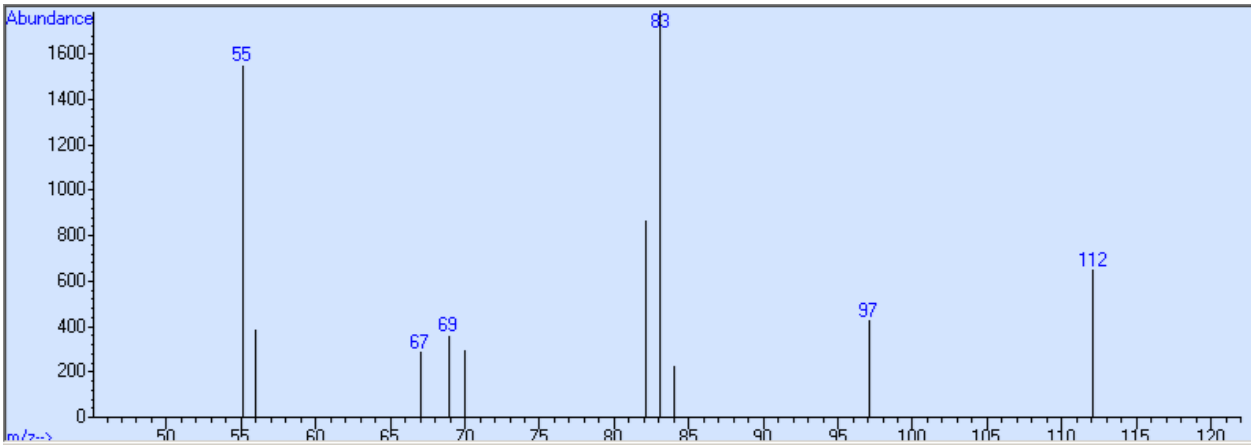
Appendix C

Gas Chromatography/Mass Spectrometry Spectra

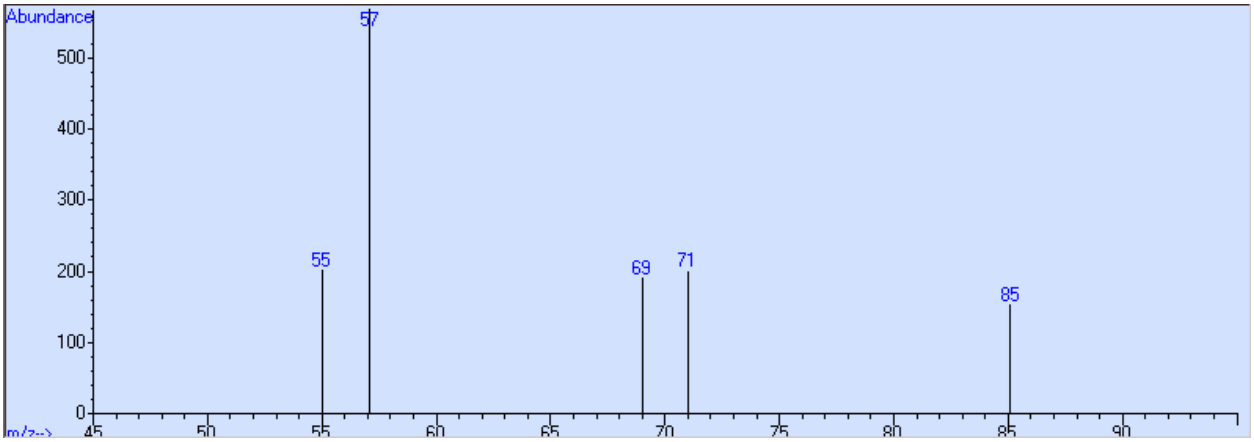
GC Spectrum 1: Solvent Hexanes



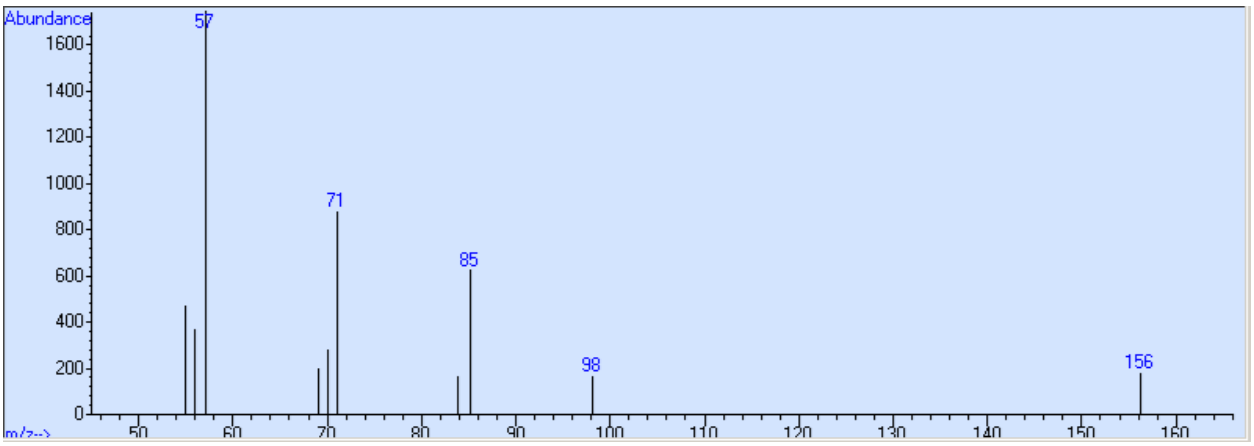
MS Spectrum 1: Solvent Hexanes – RT 3.333



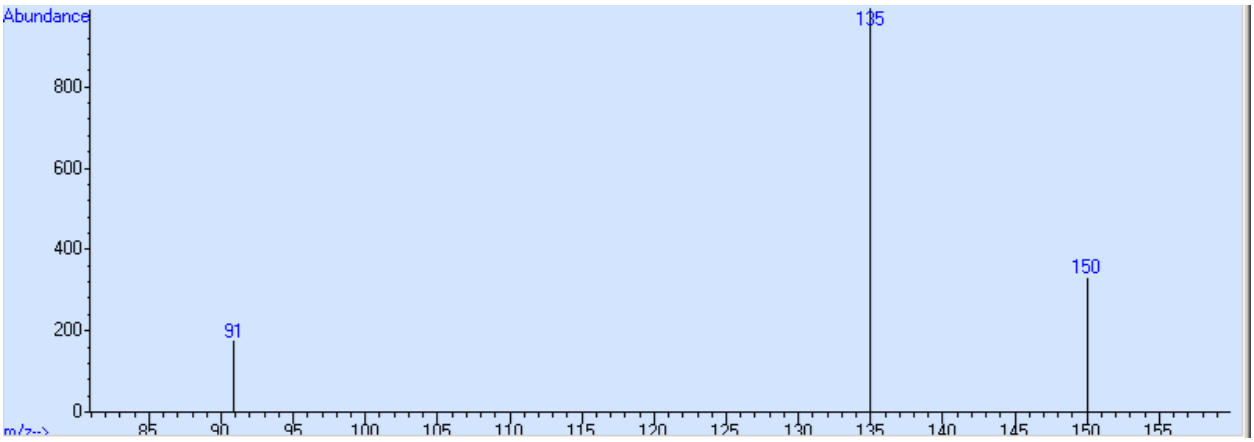
MS Spectrum 2: Solvent Hexanes – RT 5.250



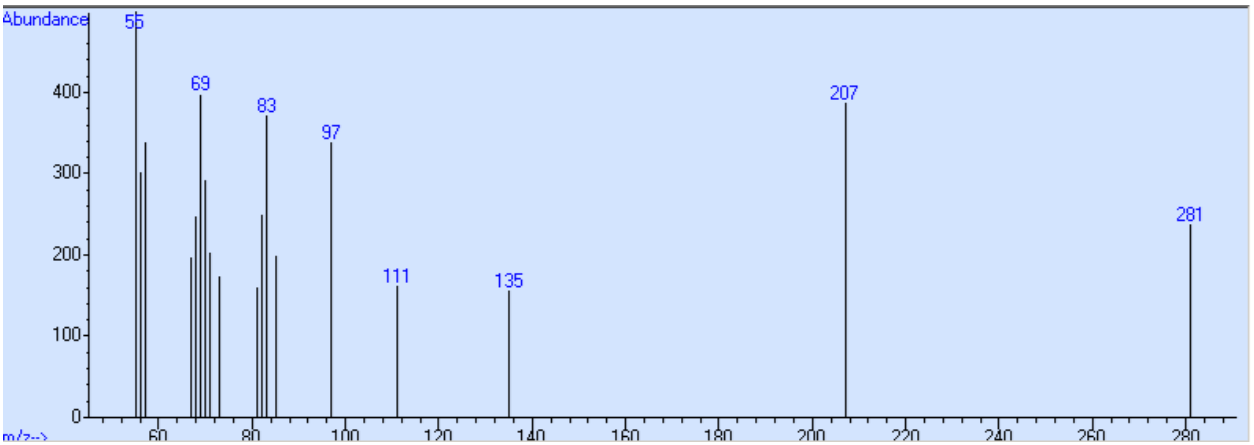
MS Spectrum 3: Solvent Hexanes – RT 6.709



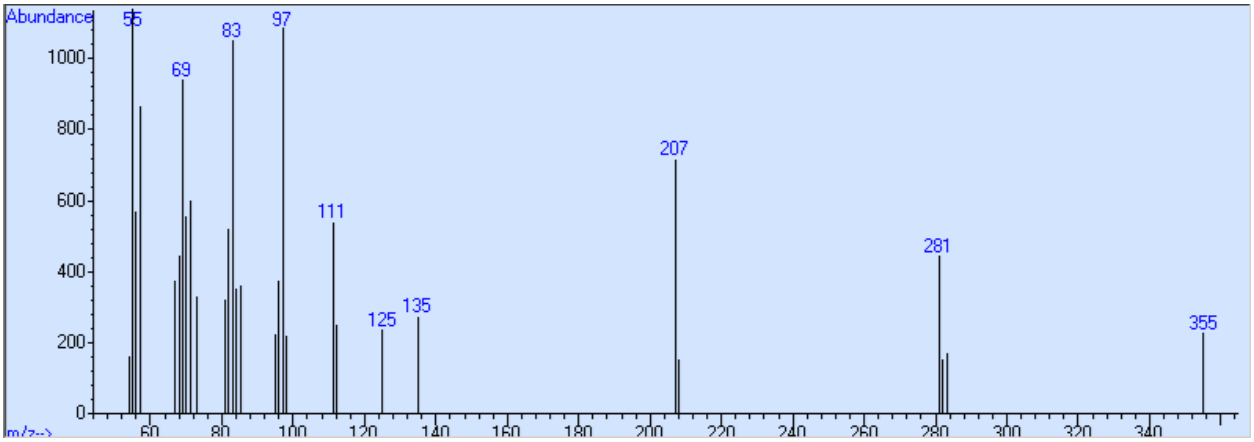
MS Spectrum 4: Solvent Hexanes – RT 9.564



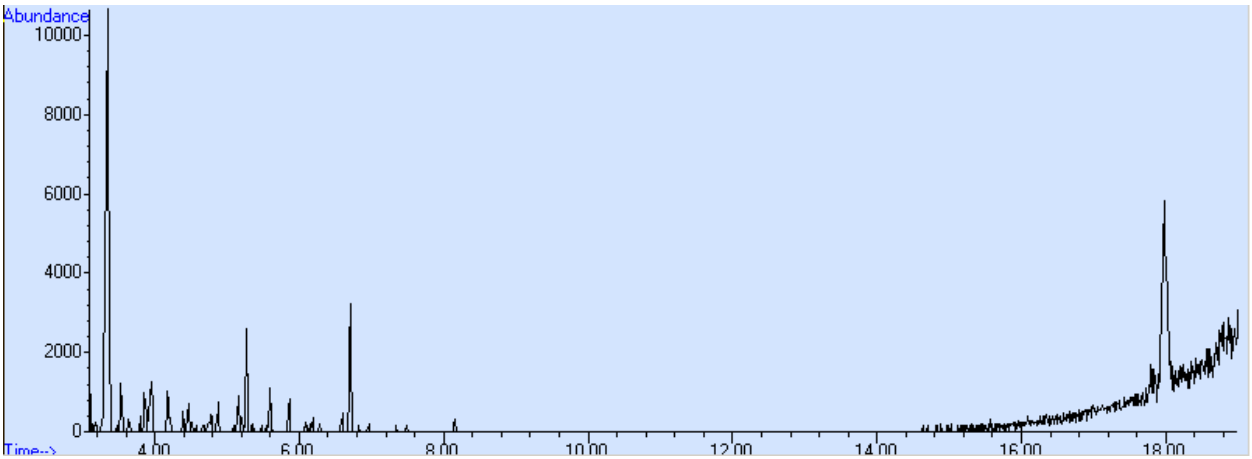
MS Spectrum 5: Solvent Hexanes – RT 16.751



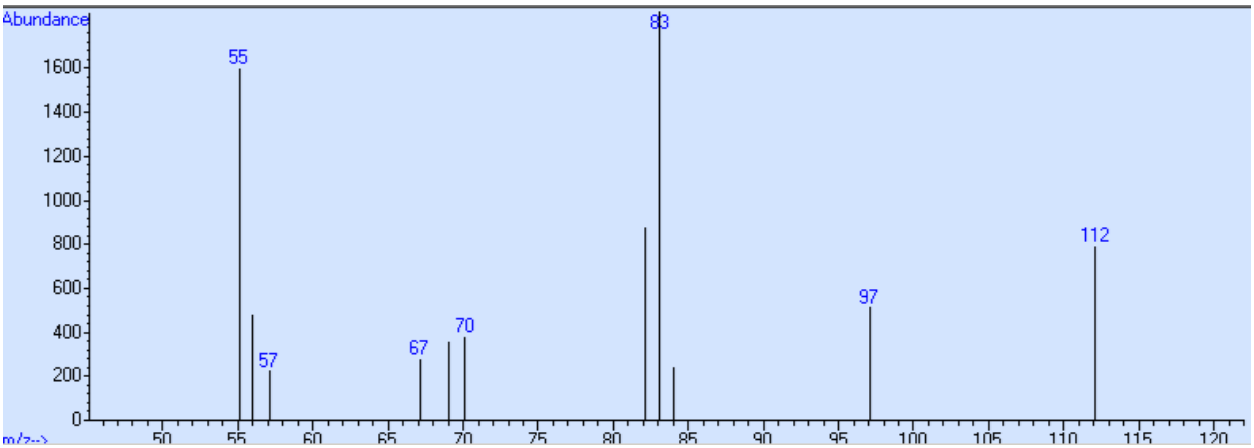
MS Spectrum 6: Solvent Hexanes – RT 18.822



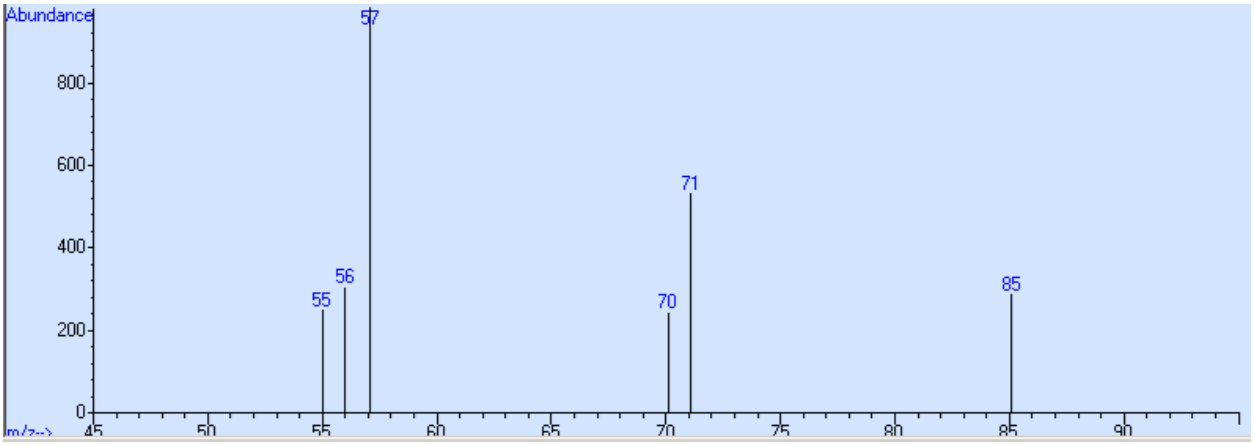
GC Spectrum 2: Remy XQ Extracted in Hexanes



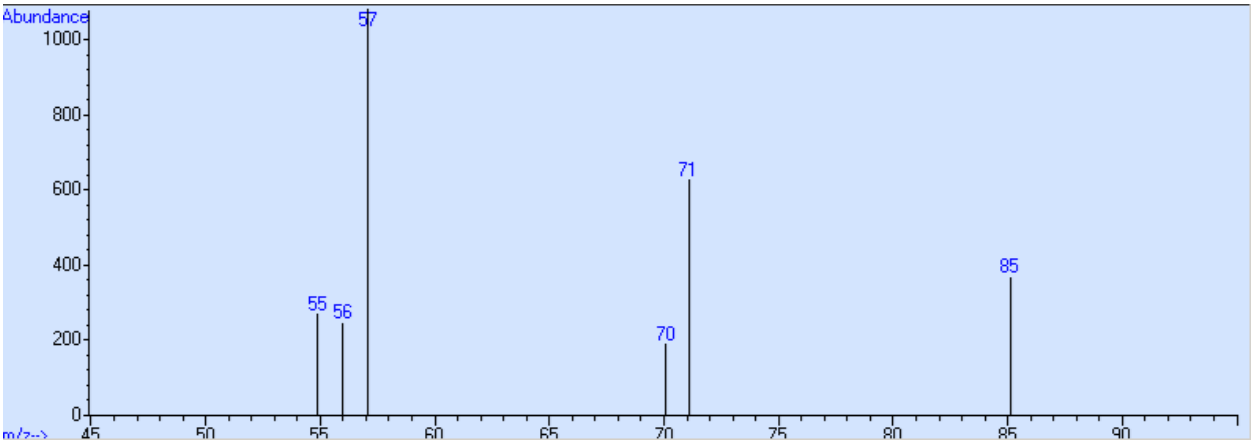
MS Spectrum 7: Remy XQ Extracted in Hexanes – RT 3.333



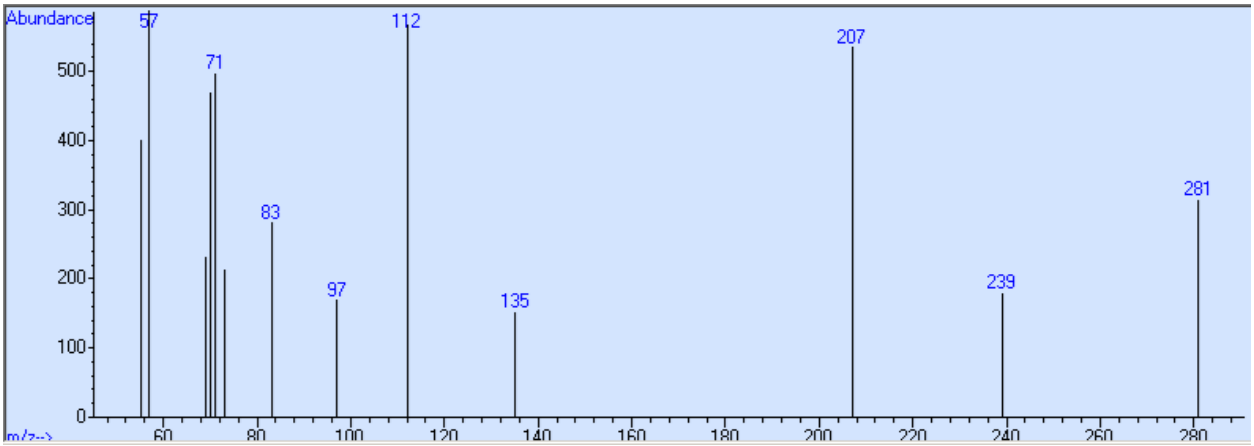
MS Spectrum 8: Remy XQ Extracted in Hexanes – RT 5.272



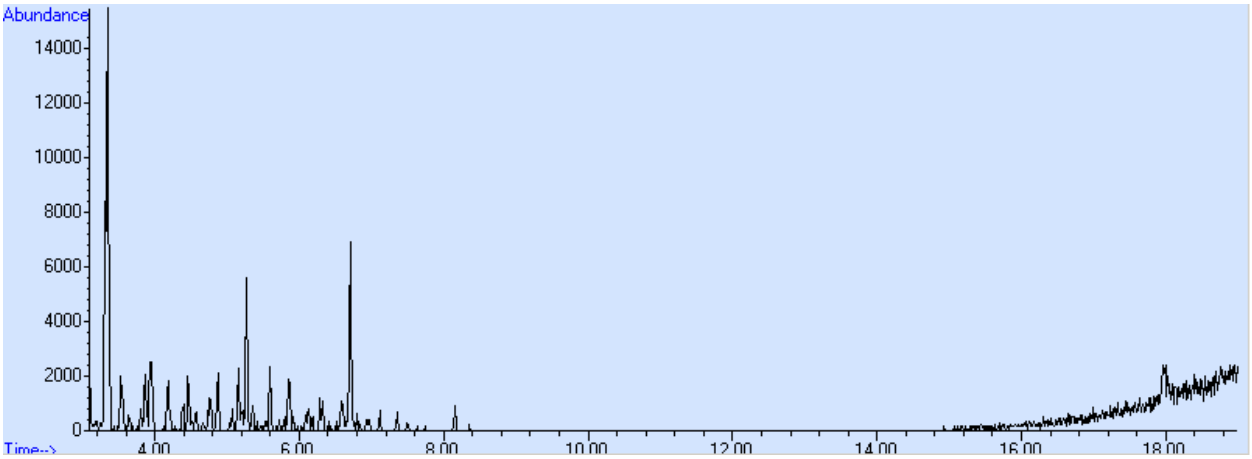
MS Spectrum 9: Remy XQ Extracted in Hexanes – RT 6.709



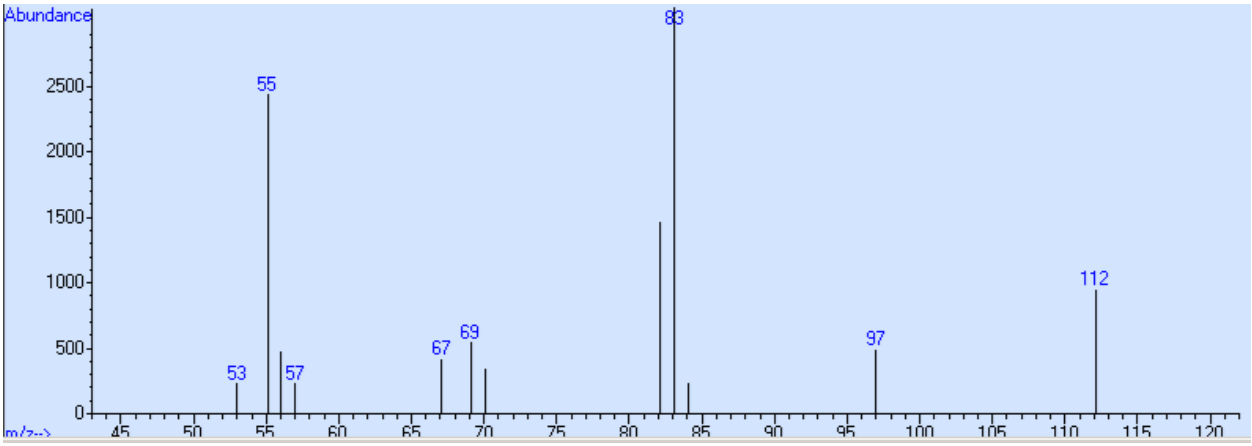
MS Spectrum 10: Remy XQ Extracted in Hexanes – RT 17.992



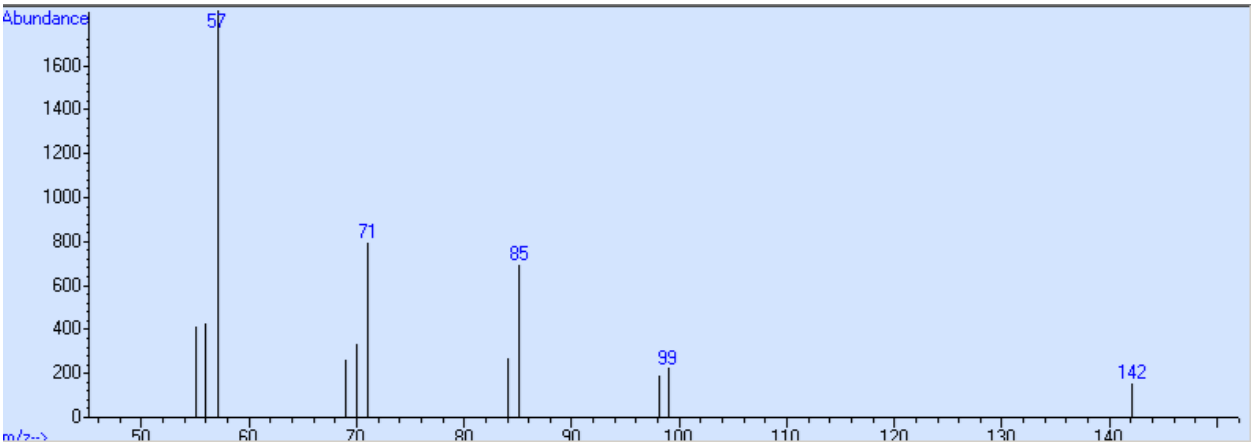
GC Spectrum 3: ECW Extracted in Hexanes



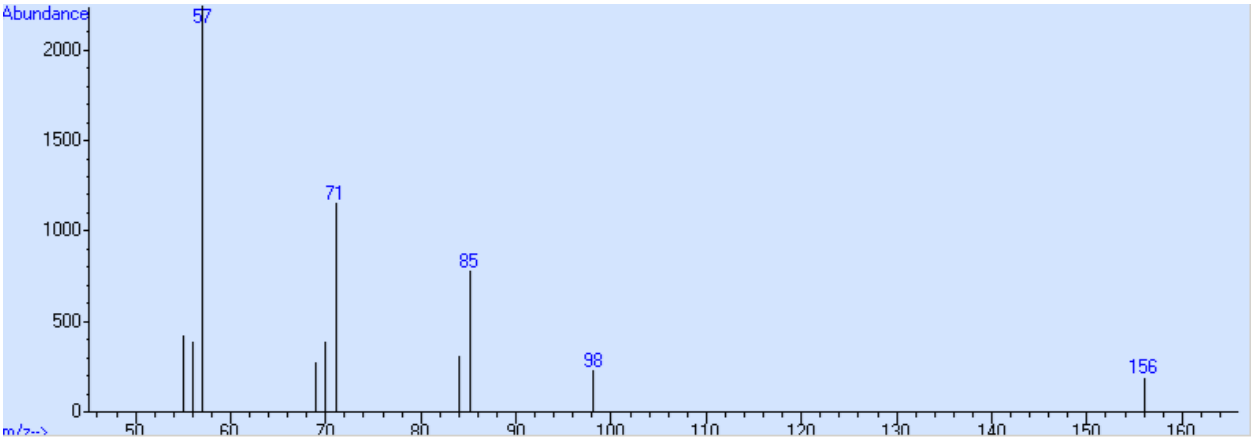
MS Spectrum 11: ECW Extracted in Hexanes – RT 3.333



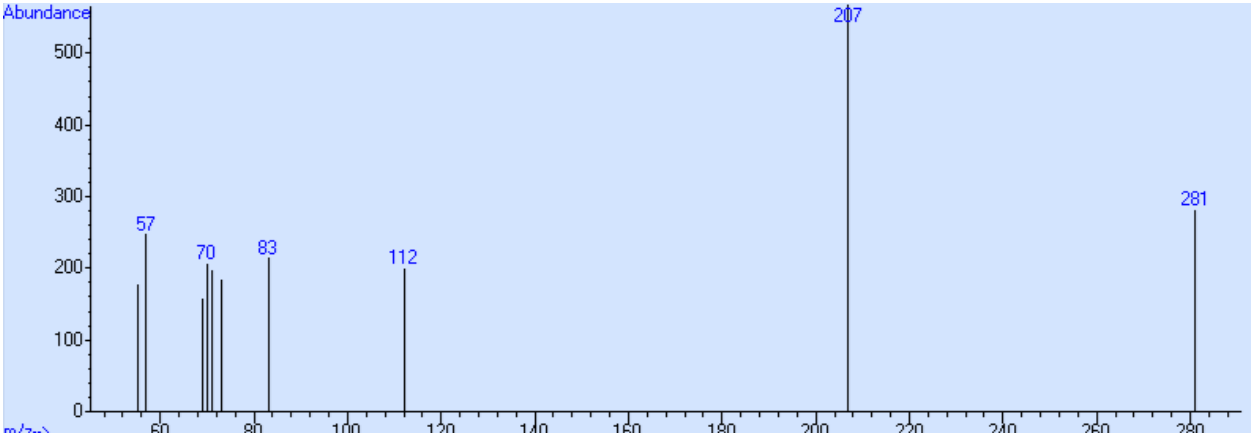
MS Spectrum 12: ECW Extracted in Hexanes – RT 5.273



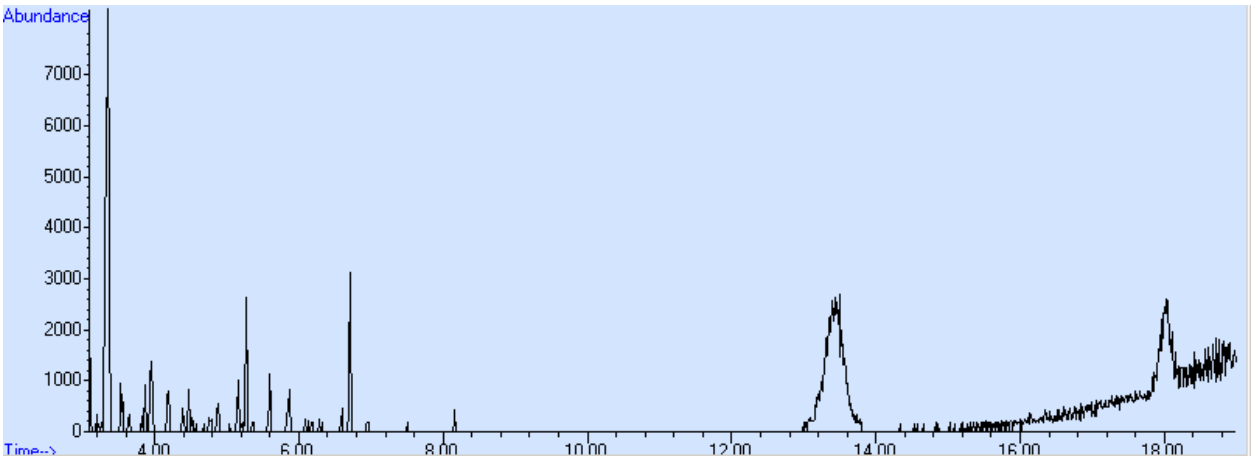
MS Spectrum 13: ECW Extracted in Hexanes – RT 6.709



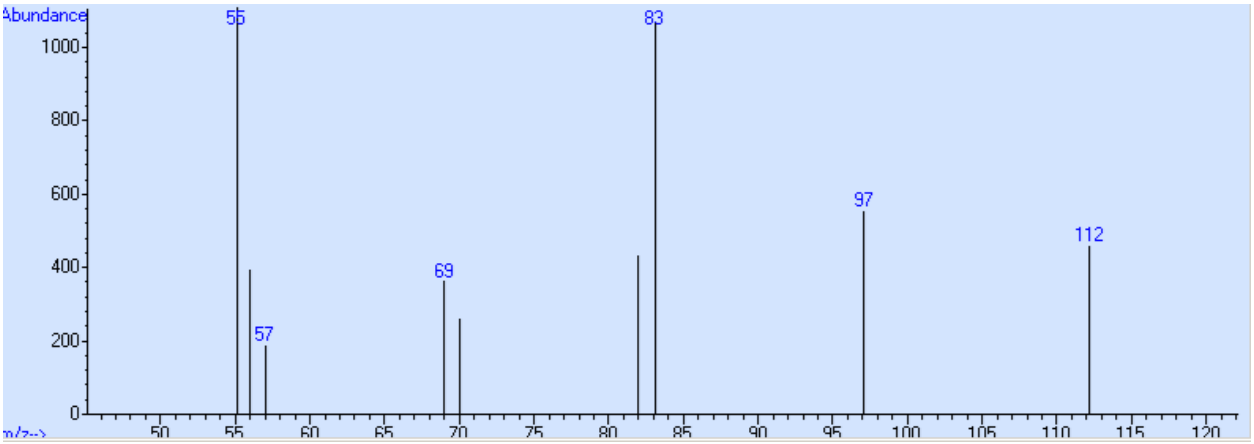
MS Spectrum 14: ECW Extracted in Hexanes – RT 17.970



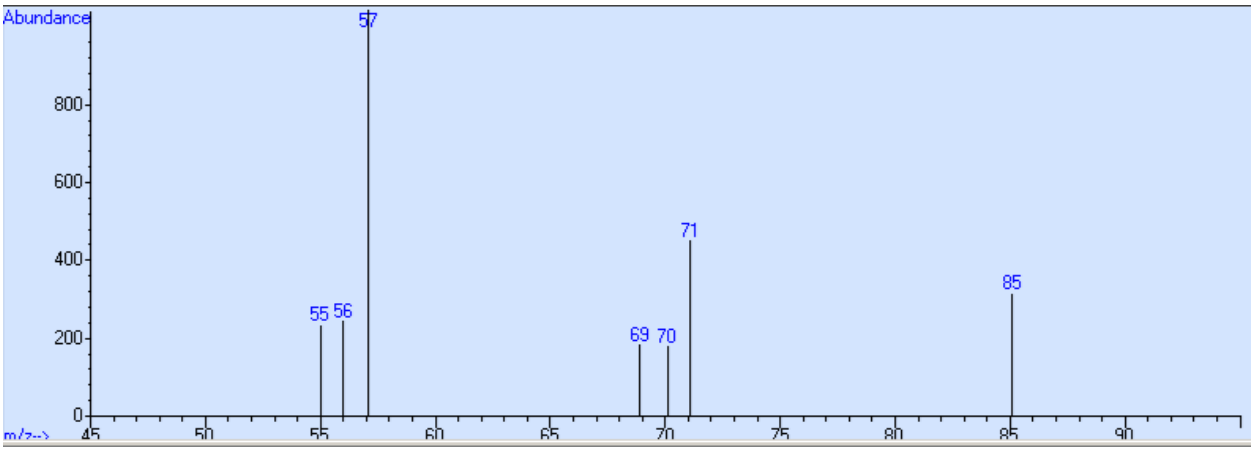
GC Spectrum 4: Que Extracted in Hexanes



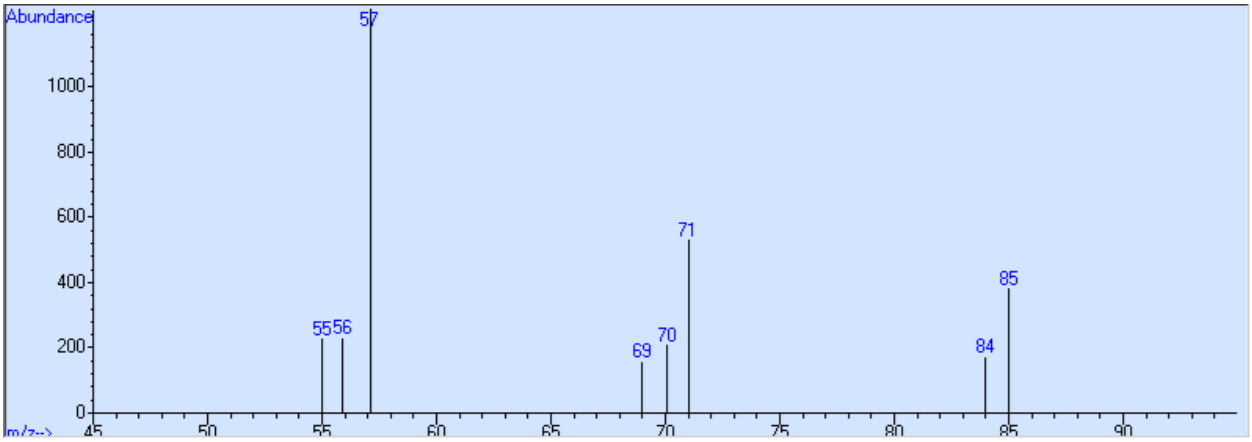
MS Spectrum 15: Que Extracted in Hexanes – RT 3.333



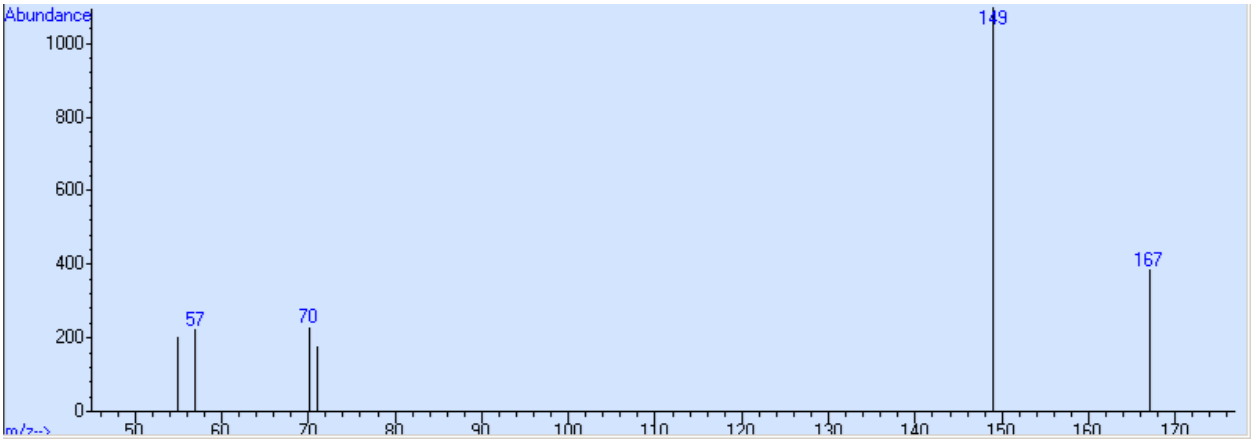
MS Spectrum 16: Que Extracted in Hexanes – RT 5.273



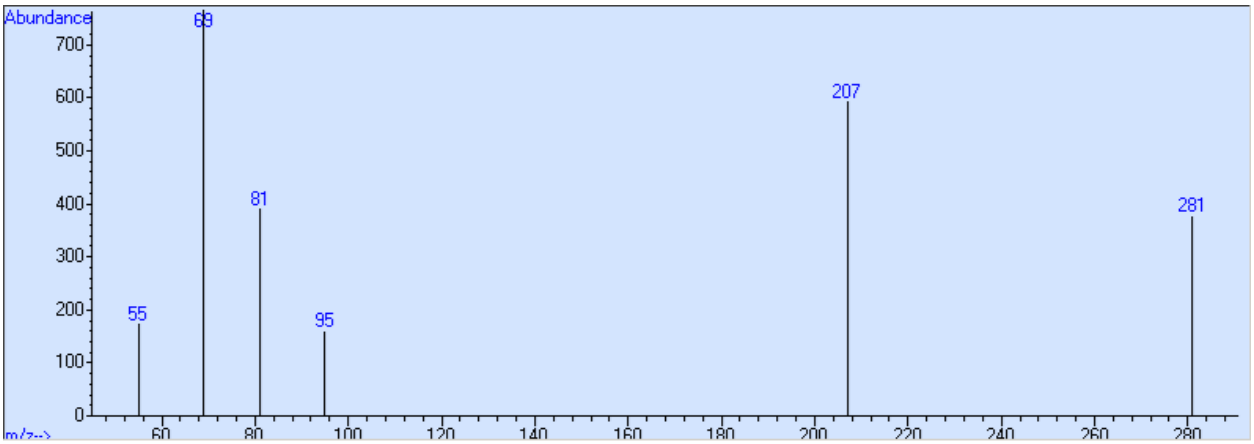
MS Spectrum 17: Que Extracted in Hexanes – RT 6.709



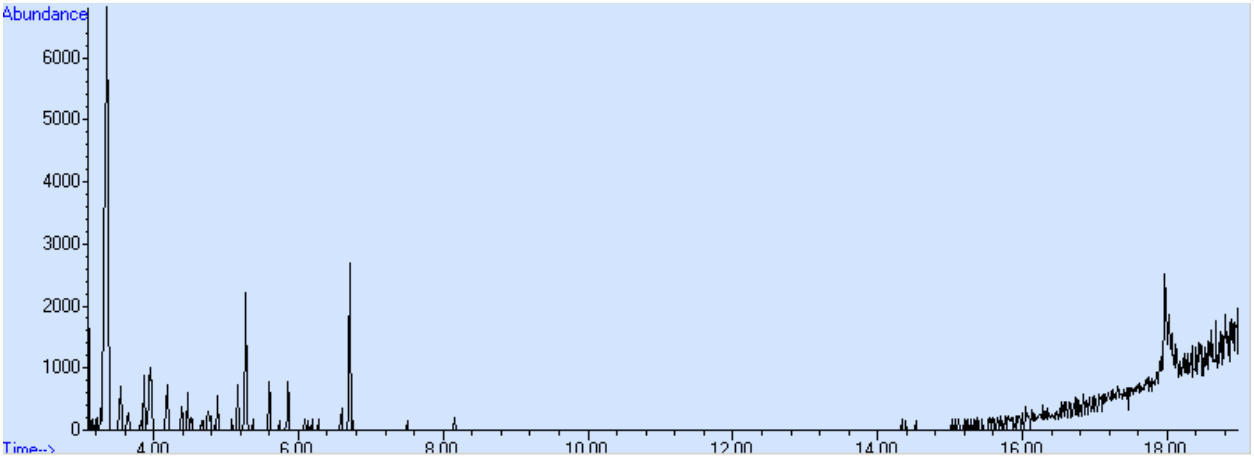
MS Spectrum 18: Que Extracted in Hexanes – RT 13.438



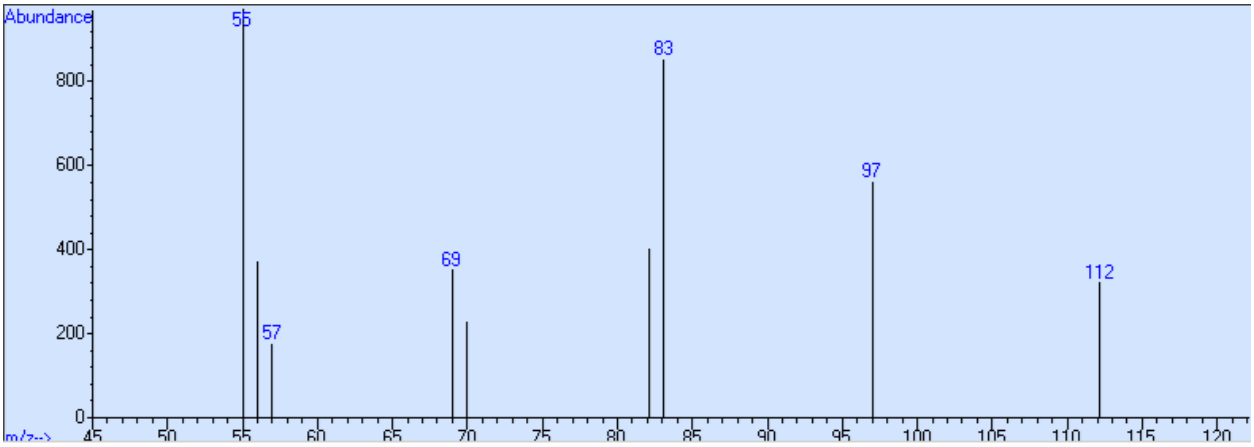
MS Spectrum 19: Que Extracted in Hexanes – RT 18.015



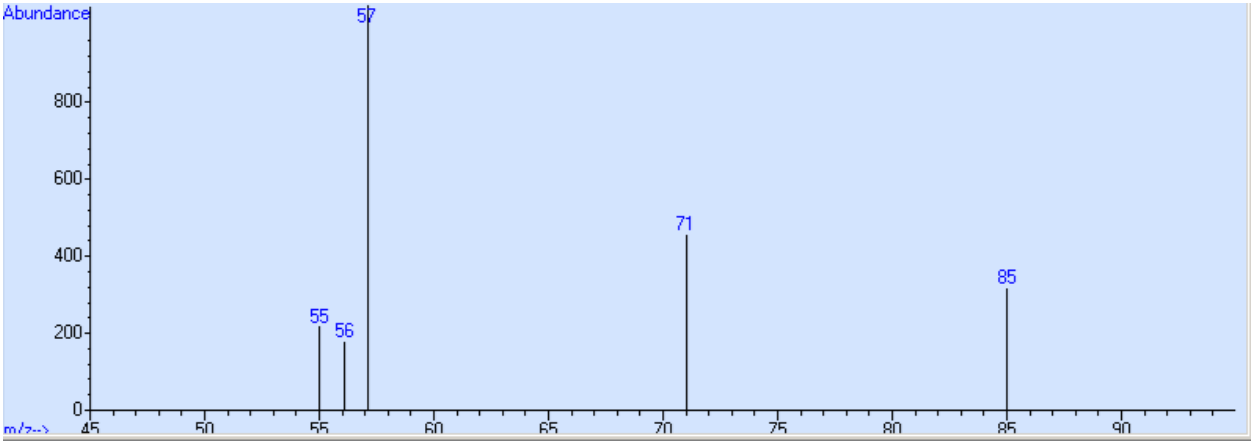
GC Spectrum 5: Undyed Caucasoid Extracted in Hexanes



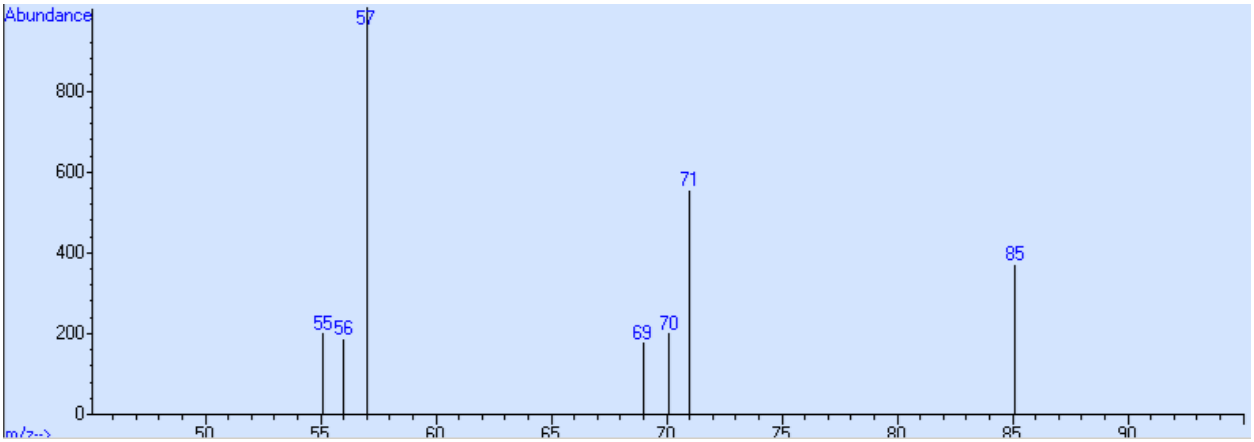
MS Spectrum 20: Undyed Caucasoid Extracted in Hexanes – RT 3.333



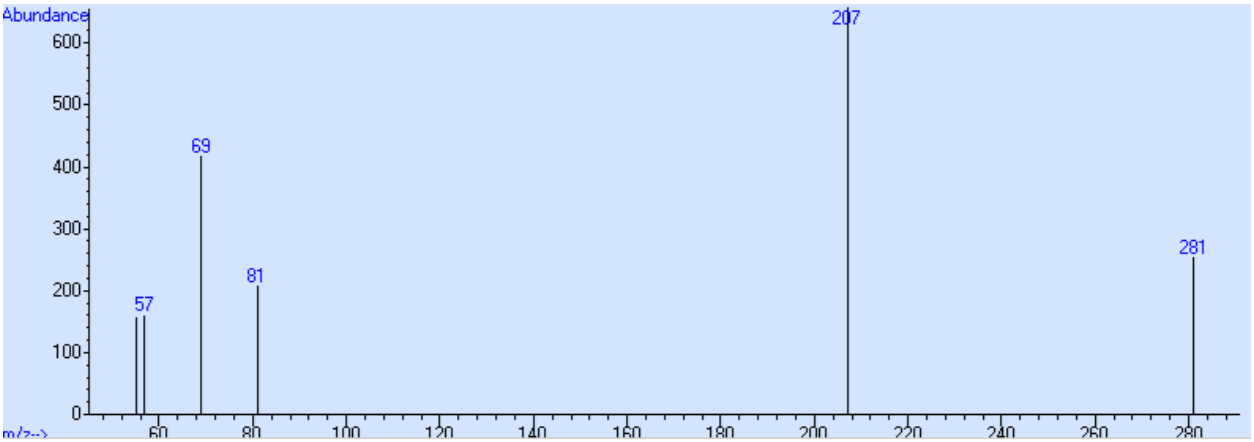
MS Spectrum 21: Undyed Caucasoid Extracted in Hexanes – RT 5.273



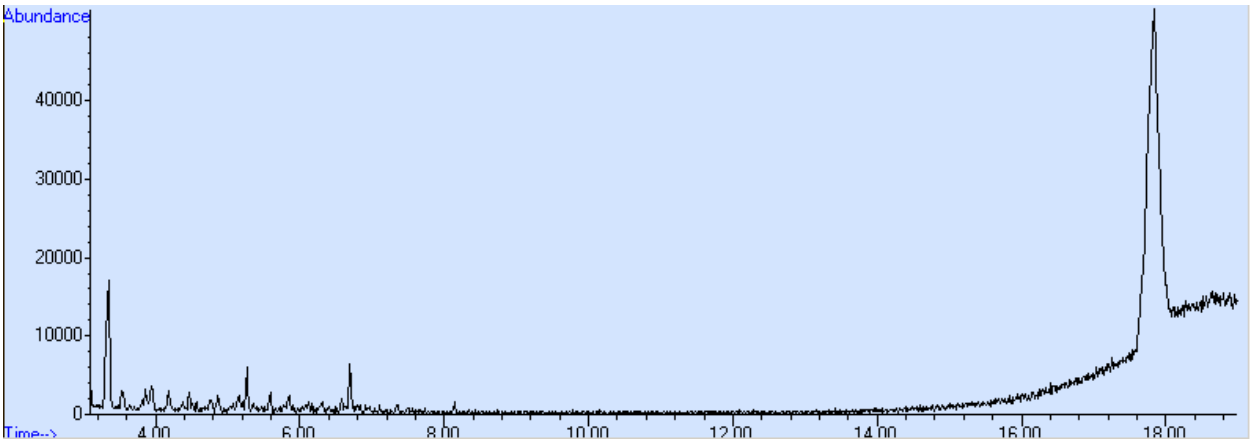
MS Spectrum 22: Undyed Caucasoid Extracted in Hexanes – RT 6.709



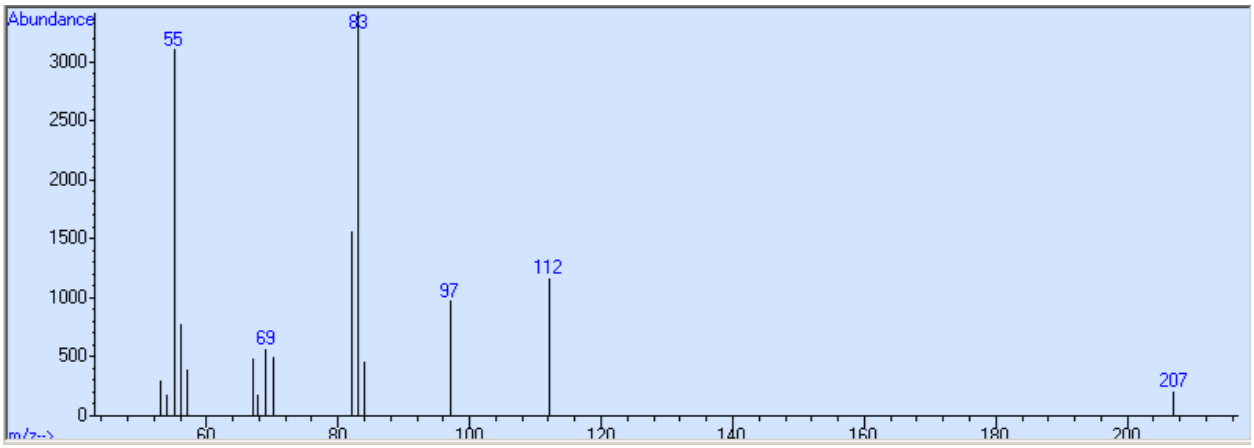
MS Spectrum 23: Undyed Caucasoid Extracted in Hexanes – RT 18.015



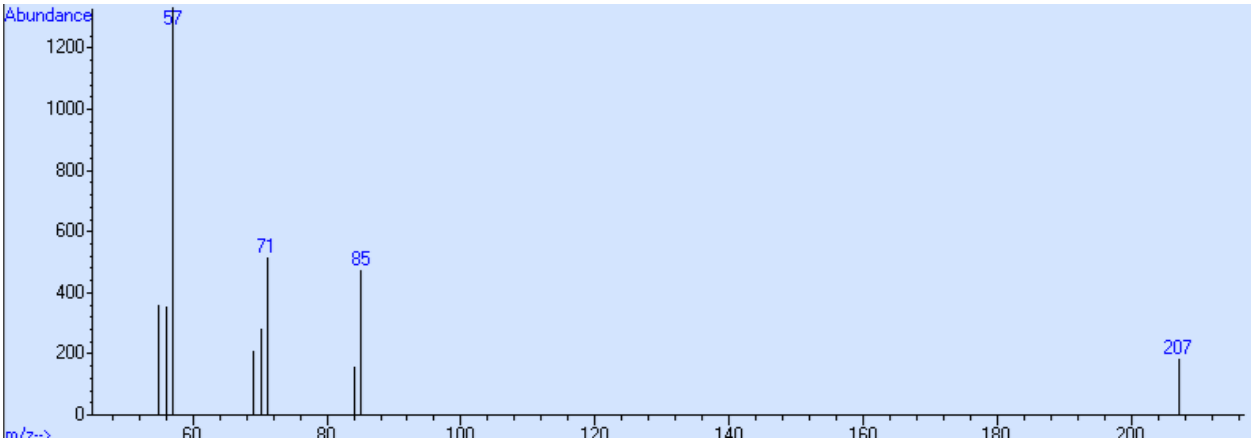
GC Spectrum 6: Dyed Caucasoid Extracted in Hexanes



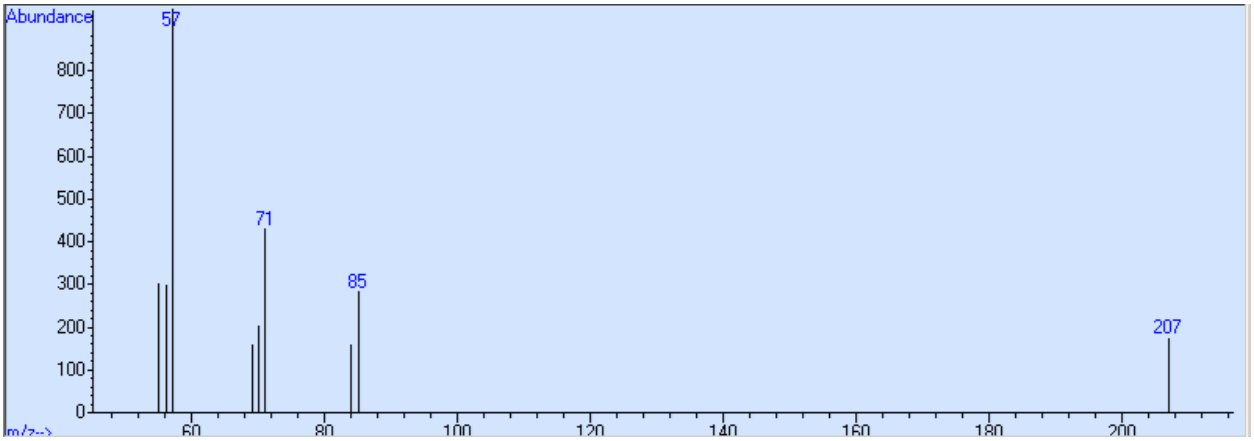
MS Spectrum 24: Dyed Caucasoid Extracted in Hexanes – RT 3.333



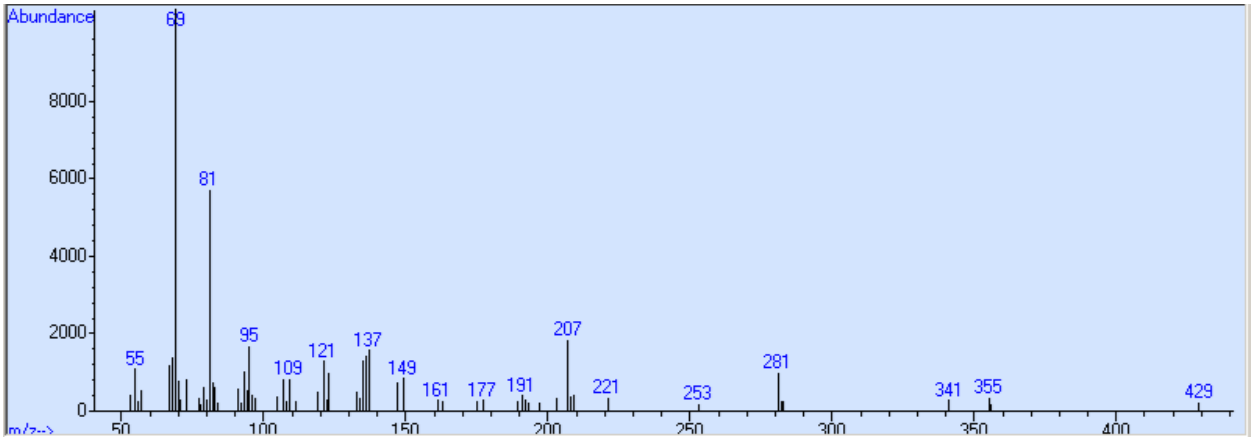
MS Spectrum 25: Dyed Caucasoid Extracted in Hexanes – RT 5.273



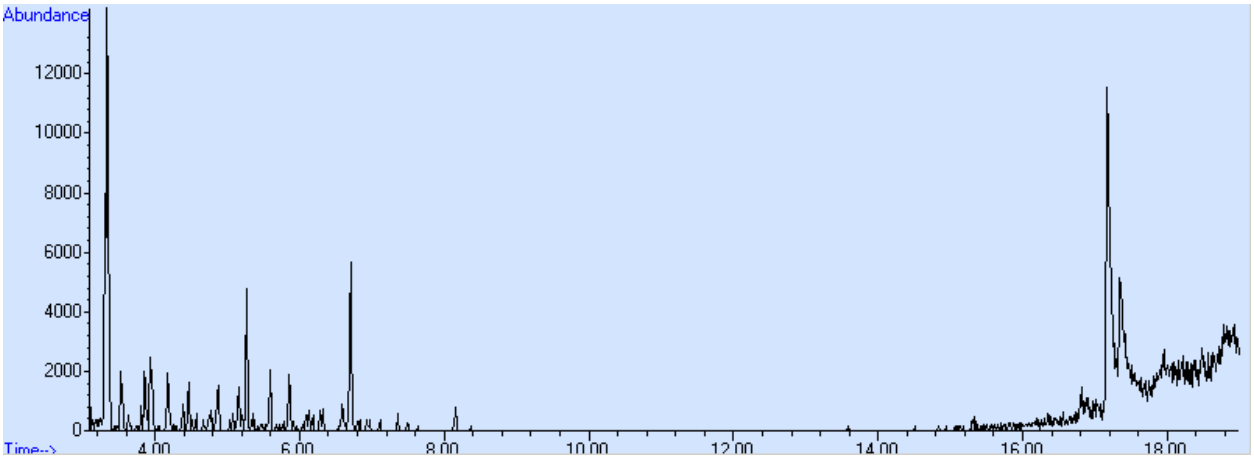
MS Spectrum 26: Dyed Caucasoid Extracted in Hexanes – RT 6.709



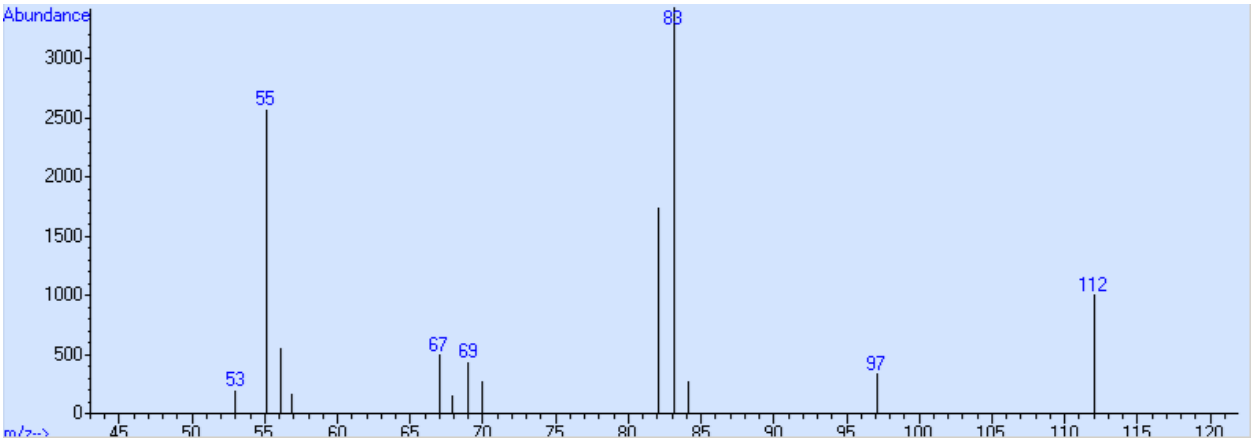
MS Spectrum 27: Dyed Caucasoid Extracted in Hexanes – RT 17.838



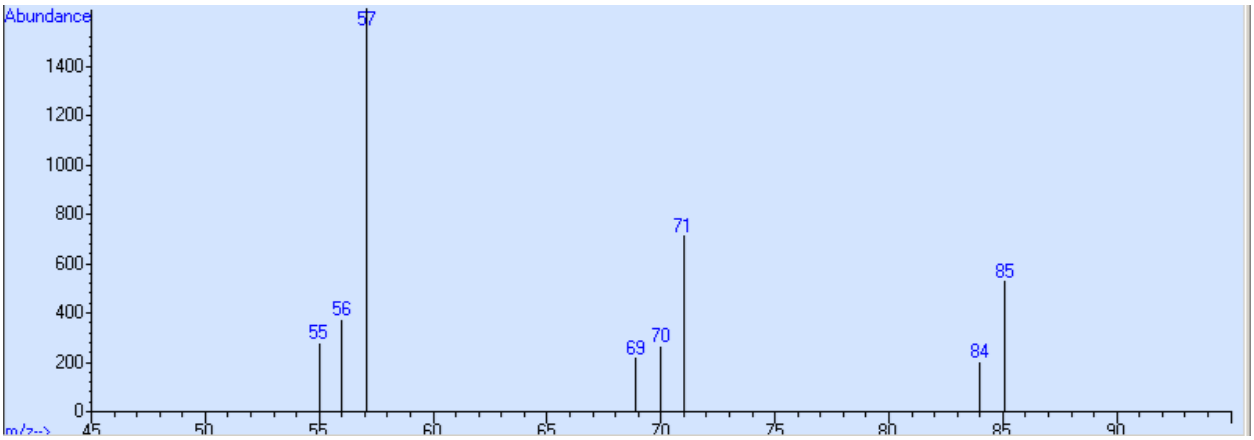
GC Spectrum 7: Undyed Mongoloid Extracted in Hexanes



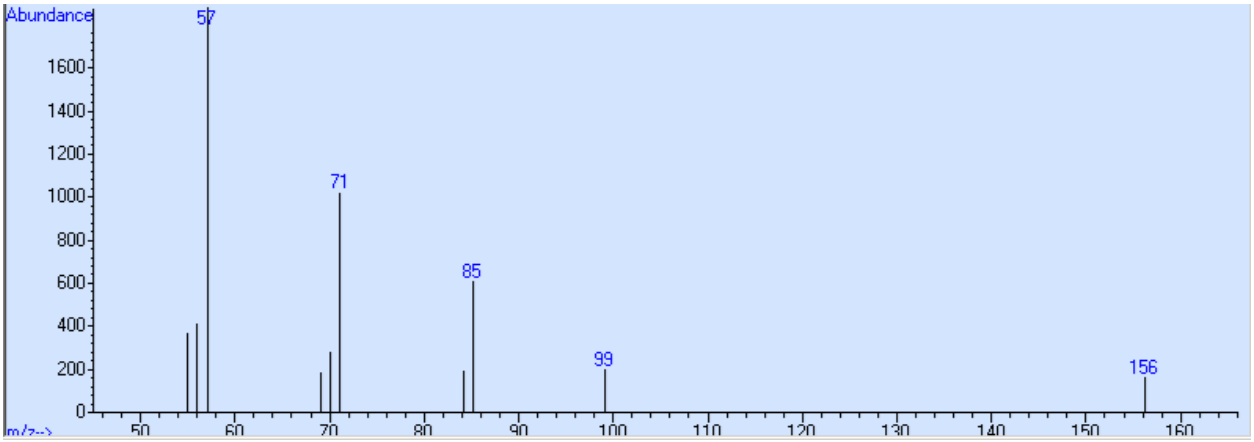
MS Spectrum 28: Undyed Mongoloid Extracted in Hexanes – RT 3.333



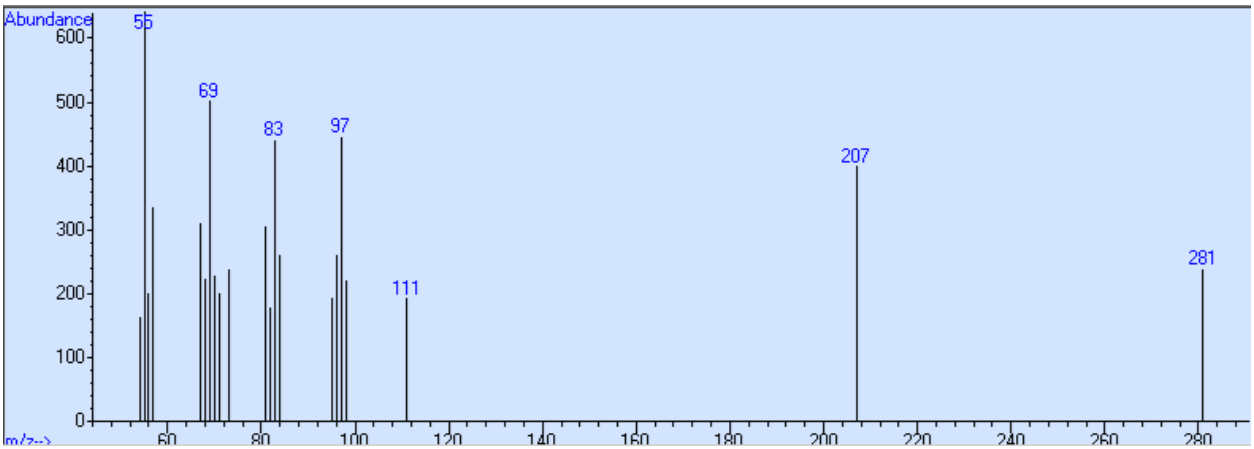
MS Spectrum 29: Undyed Mongoloid Extracted in Hexanes – RT 5.273



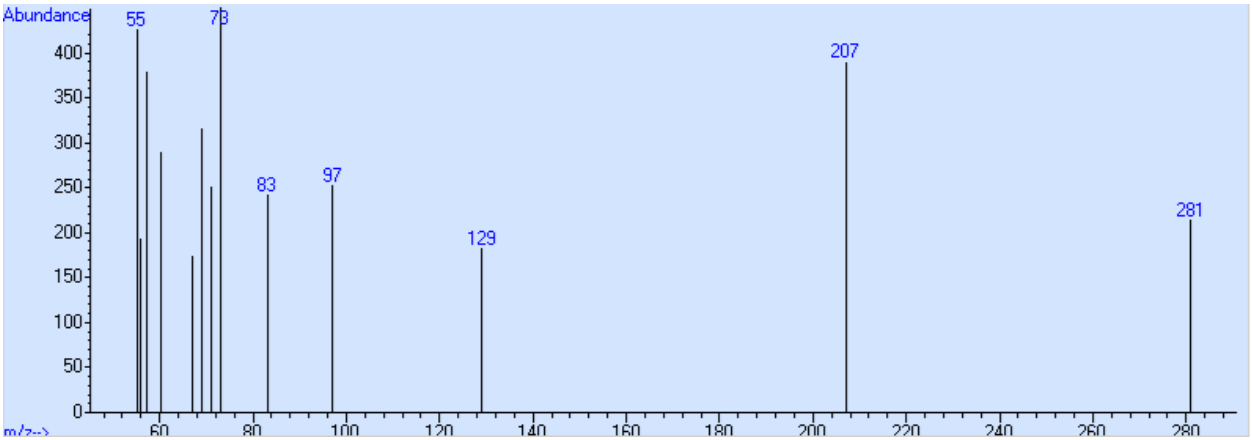
MS Spectrum 30: Undyed Mongoloid Extracted in Hexanes – RT 6.709



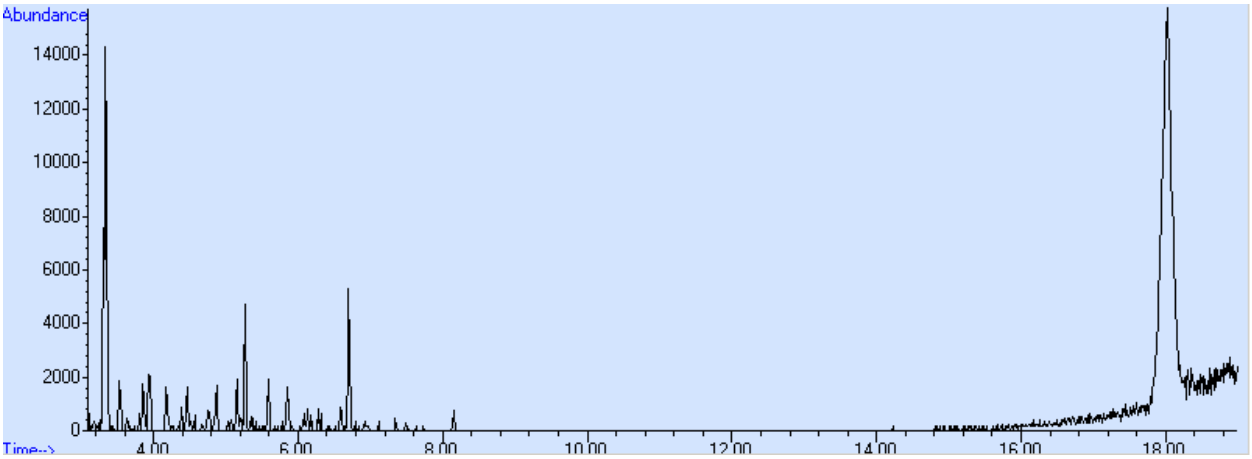
MS Spectrum 31: Undyed Mongoloid Extracted in Hexanes – RT 17.209



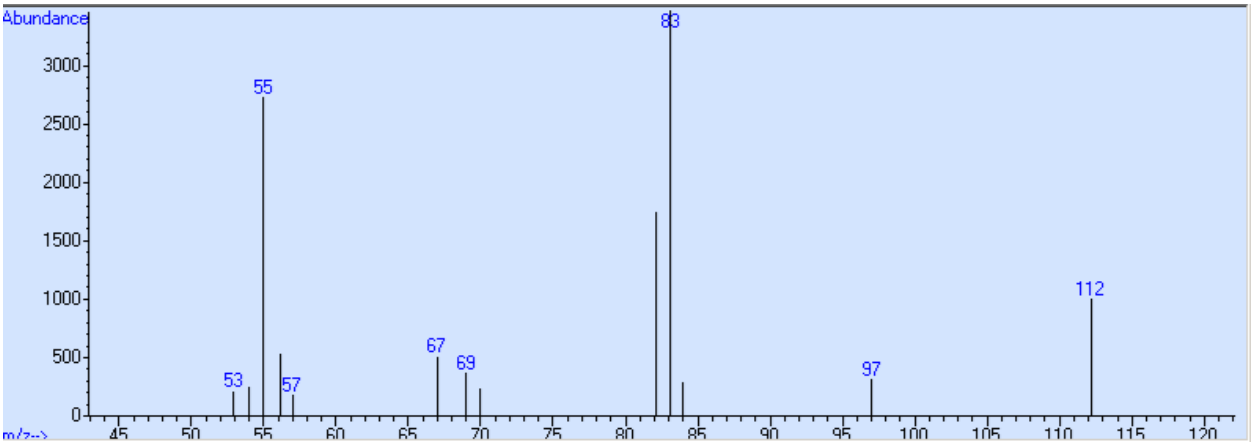
MS Spectrum 32: Undyed Mongoloid Extracted in Hexanes – RT 17.380



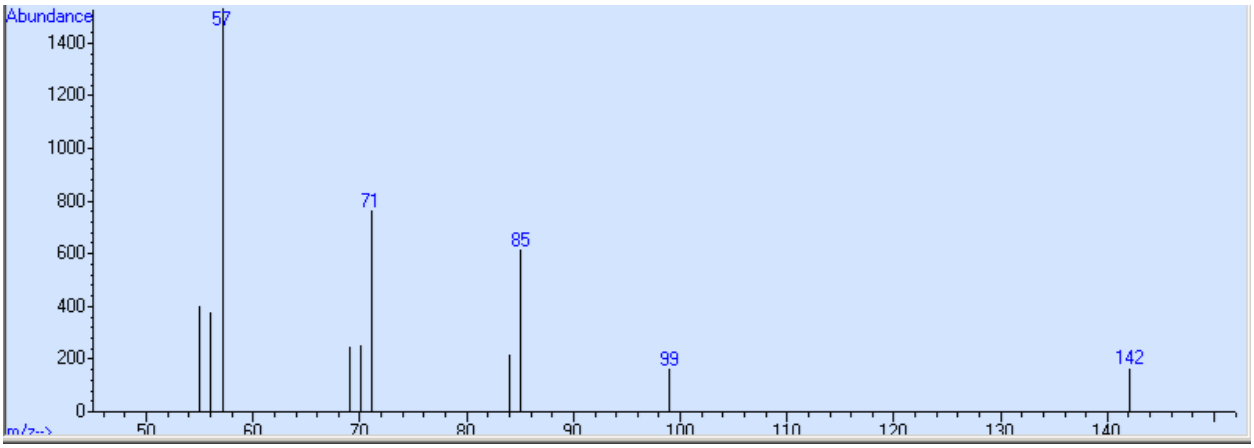
GC Spectrum 8: Dyed Mongoloid Extracted in Hexanes



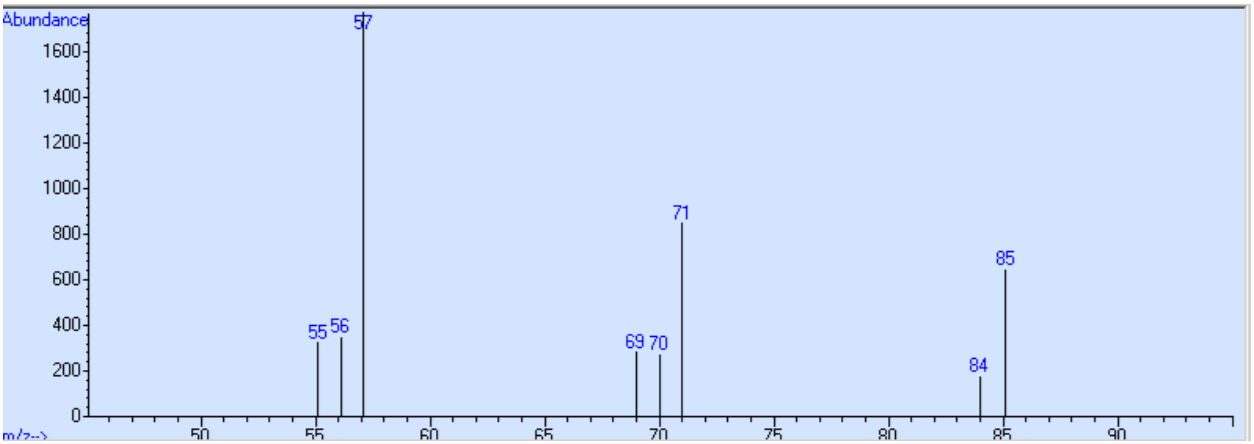
MS Spectrum 33: Dyed Mongoloid Extracted in Hexanes – RT 3.333



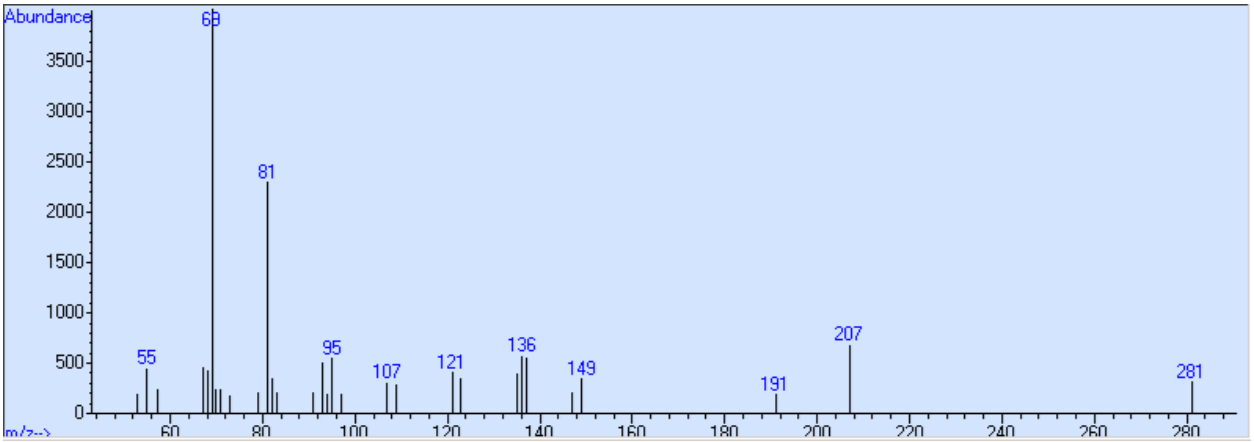
MS Spectrum 34: Dyed Mongoloid Extracted in Hexanes – RT 5.273



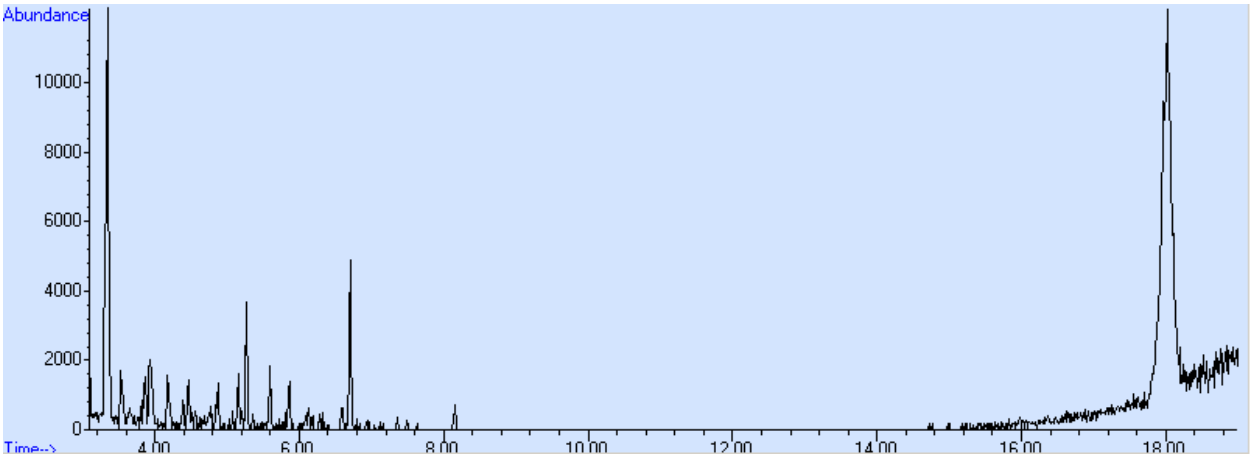
MS Spectrum 35: Dyed Mongoloid Extracted in Hexanes – RT 6.709



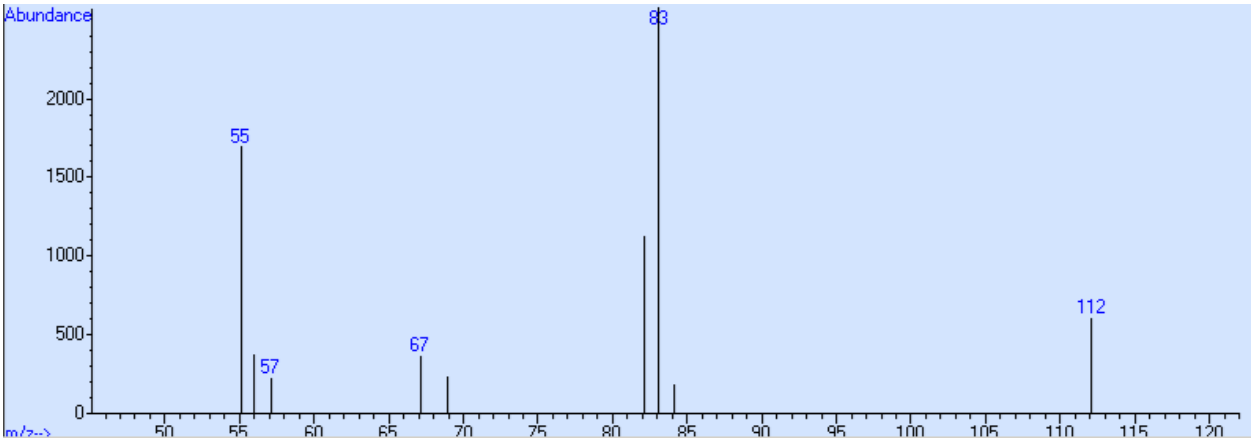
MS Spectrum 36: Dyed Mongoloid Extracted in Hexanes – RT 18.016



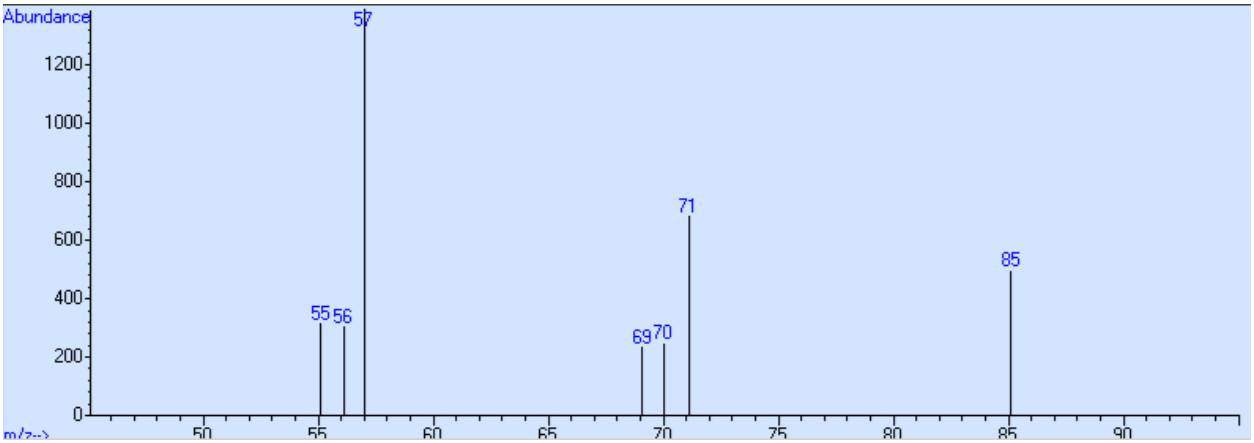
GC Spectrum 9: Undyed Negroid Extracted in Hexanes



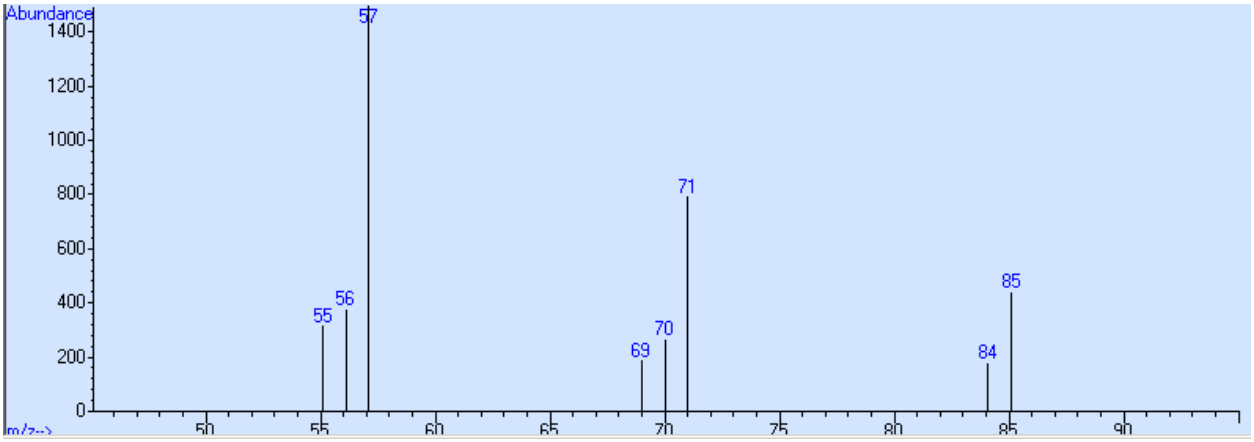
MS Spectrum 37: Undyed Negroid Extracted in Hexanes – RT 3.356



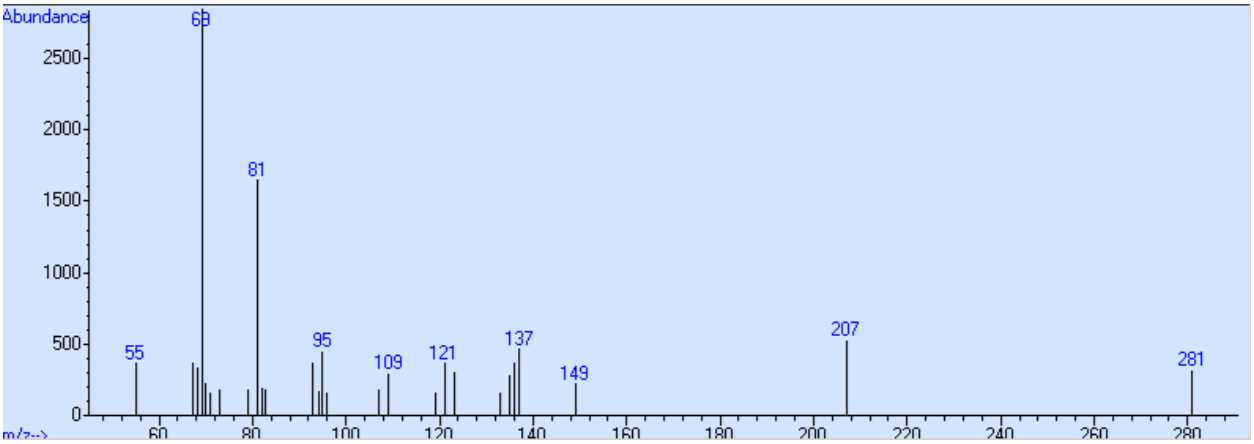
MS Spectrum 38: Undyed Negroid Extracted in Hexanes – RT 5.273



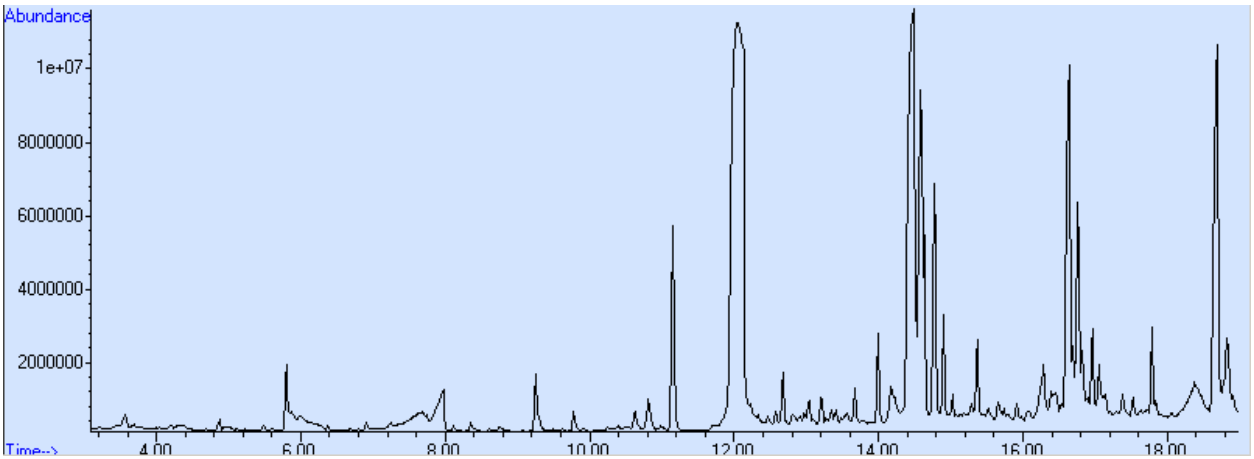
MS Spectrum 39: Undyed Negroid Extracted in Hexanes – RT 6.709



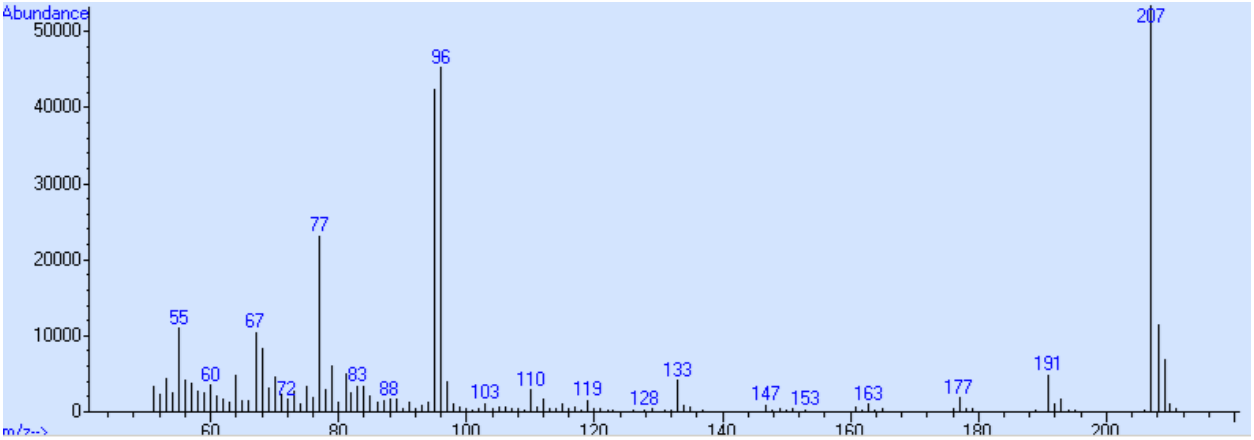
MS Spectrum 40: Undyed Negroid Extracted in Hexanes – RT 18.015



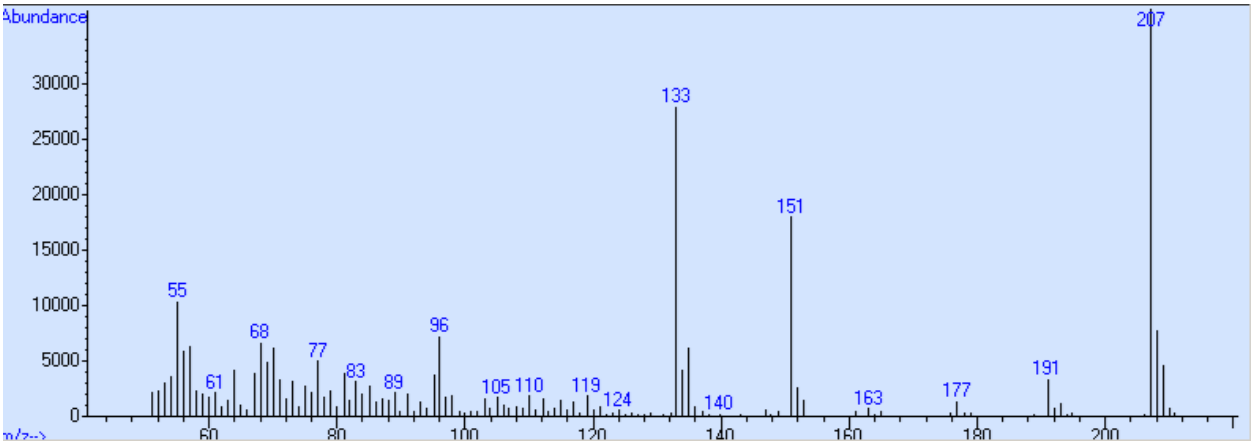
GC Spectrum 10: Solvent Colorist Secrets™ Hair Color Remover



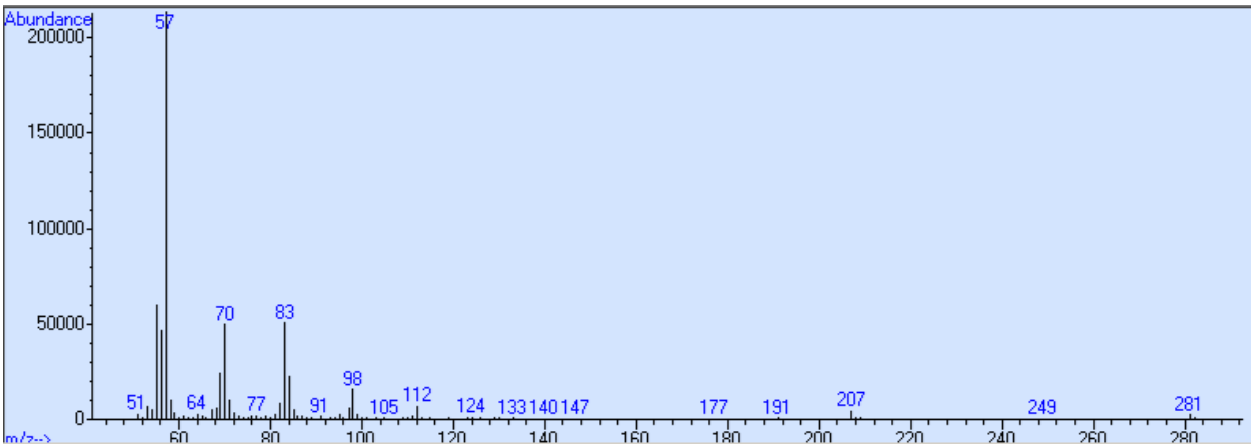
MS Spectrum 41: Solvent Colorist Secrets™ Hair Color Remover – RT 3.527



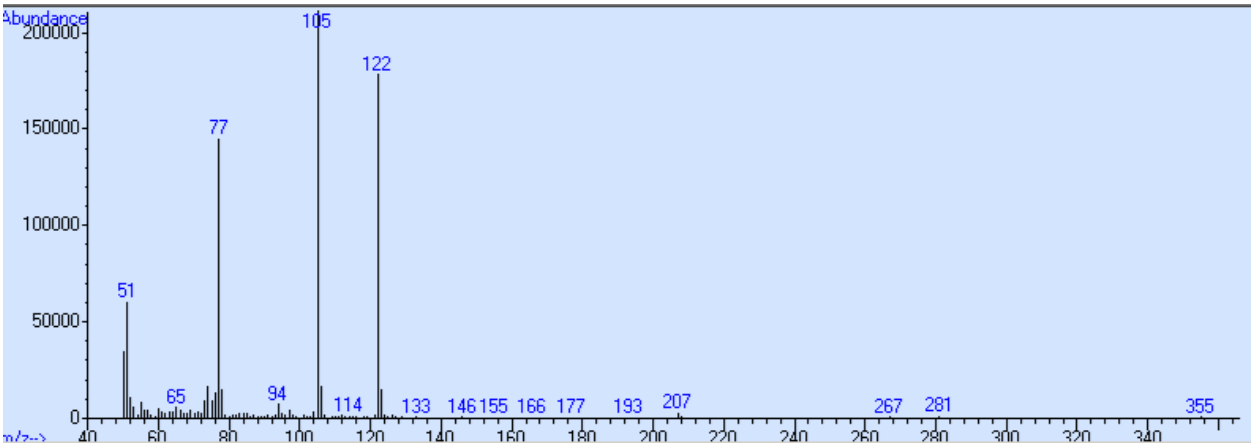
MS Spectrum 42: Solvent Colorist Secrets™ Hair Color Remover – RT 4.311



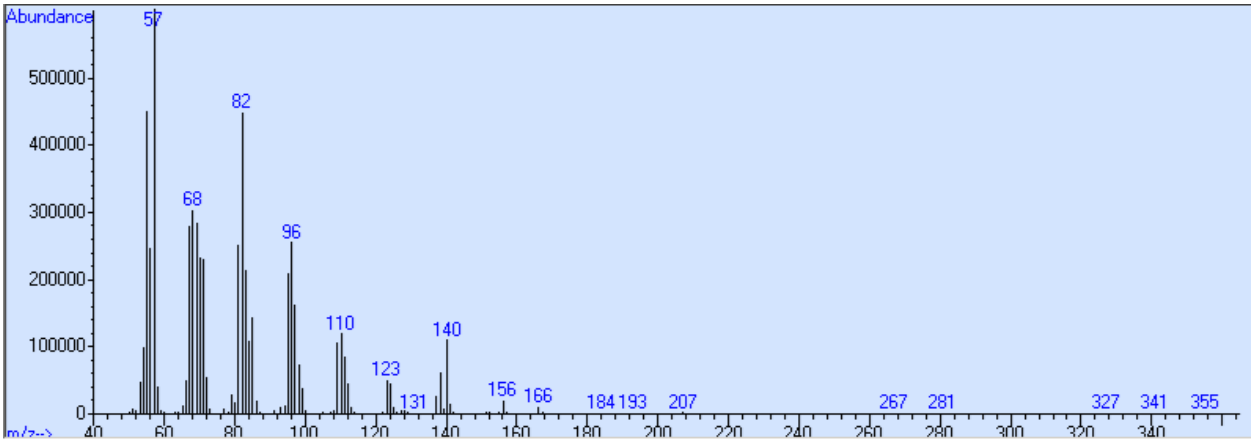
MS Spectrum 43: Solvent Colorist Secrets™ Hair Color Remover – RT 5.839



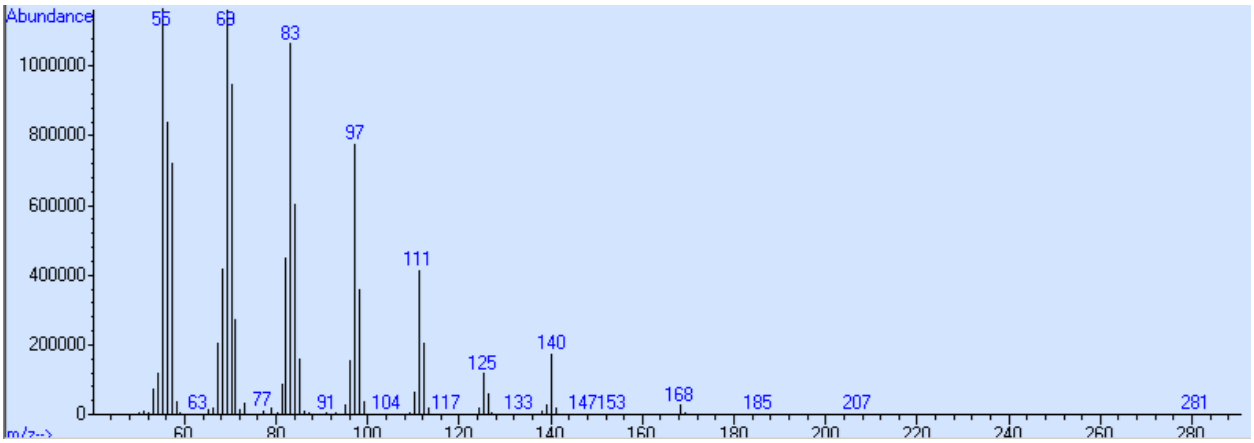
MS Spectrum 44: Solvent Colorist Secrets™ Hair Color Remover – RT 7.905



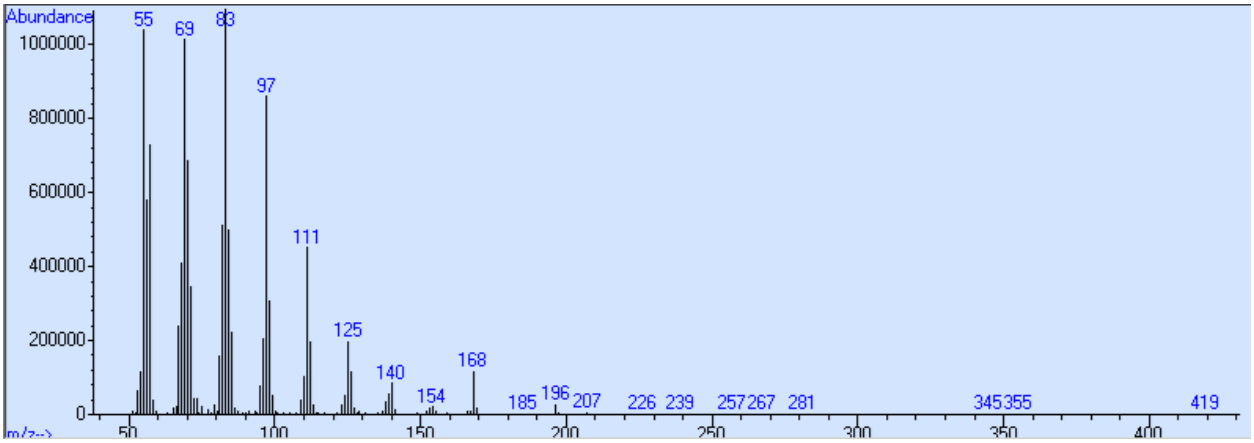
MS Spectrum 45: Solvent Colorist Secrets™ Hair Color Remover – RT 11.155



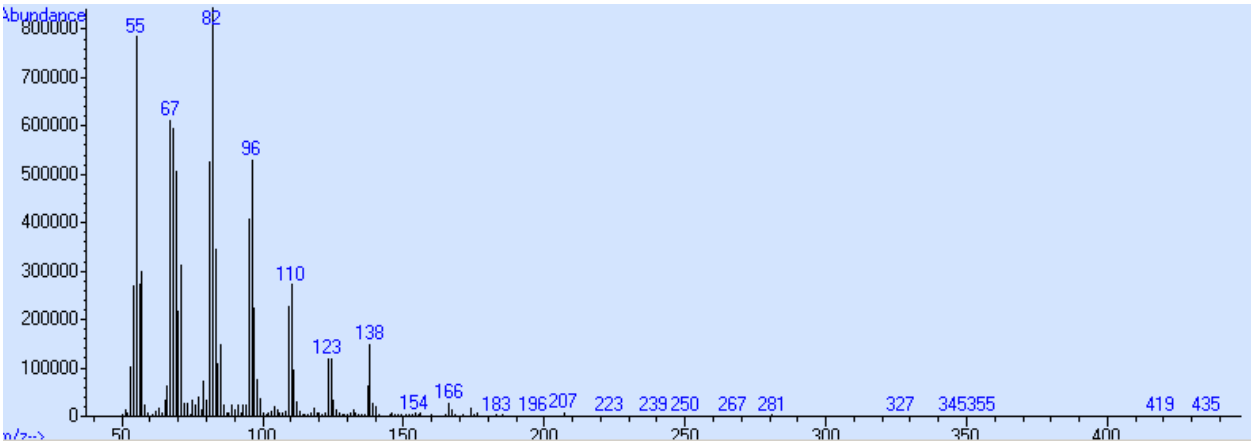
MS Spectrum 46: Solvent Colorist Secrets™ Hair Color Remover – RT 12.088



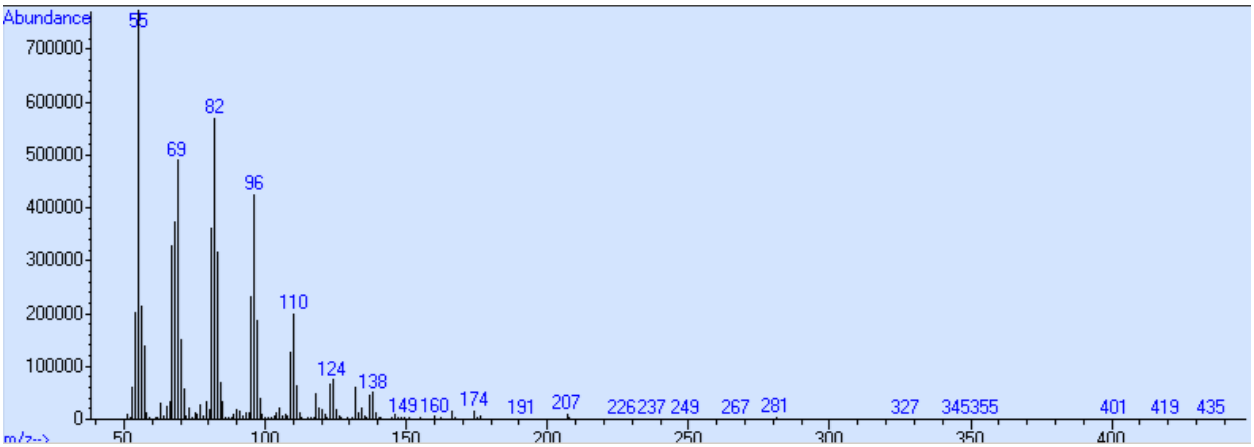
MS Spectrum 47: Solvent Colorist Secrets™ Hair Color Remover – RT 14.462



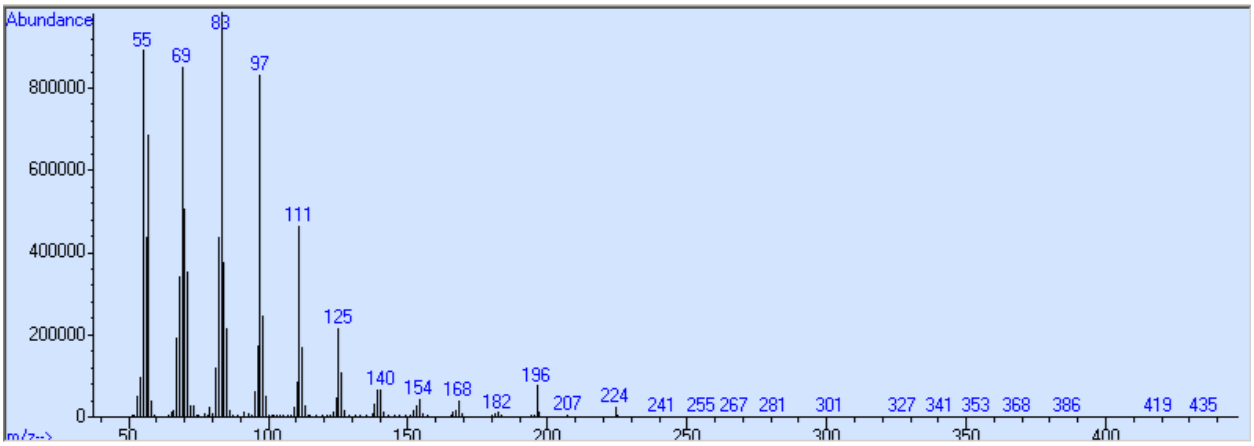
MS Spectrum 48: Solvent Colorist Secrets™ Hair Color Remover – RT 14.594



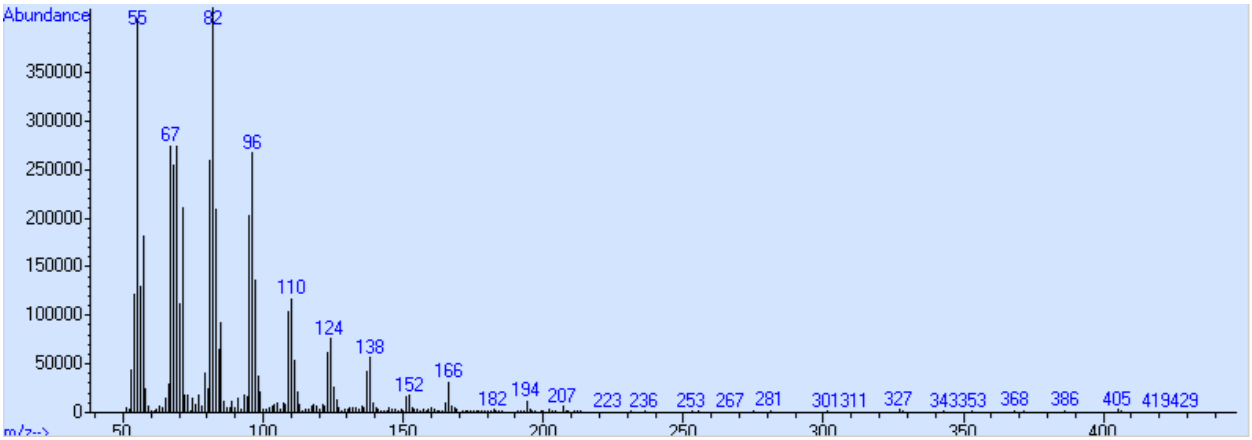
MS Spectrum 49: Solvent Colorist Secrets™ Hair Color Remover – RT 14.771



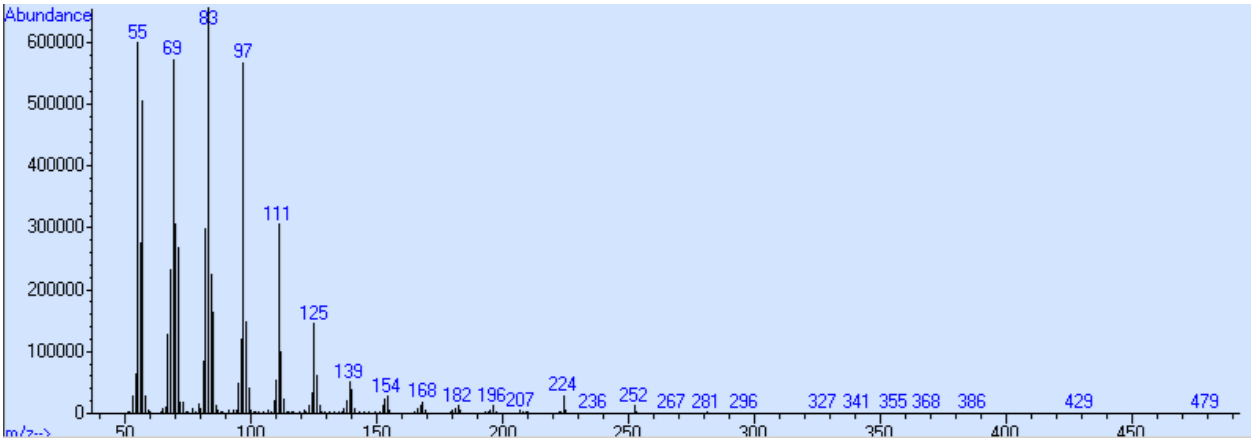
MS Spectrum 50: Solvent Colorist Secrets™ Hair Color Remover – RT 16.642



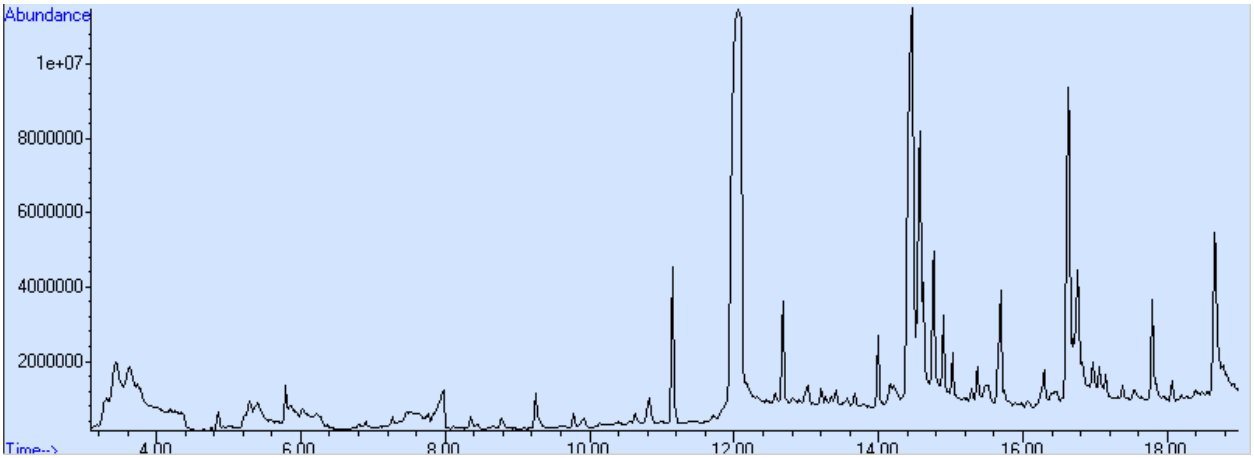
MS Spectrum 51: Solvent Colorist Secrets™ Hair Color Remover – RT 16.774



MS Spectrum 52: Solvent Colorist Secrets™ Hair Color Remover – RT 18.645

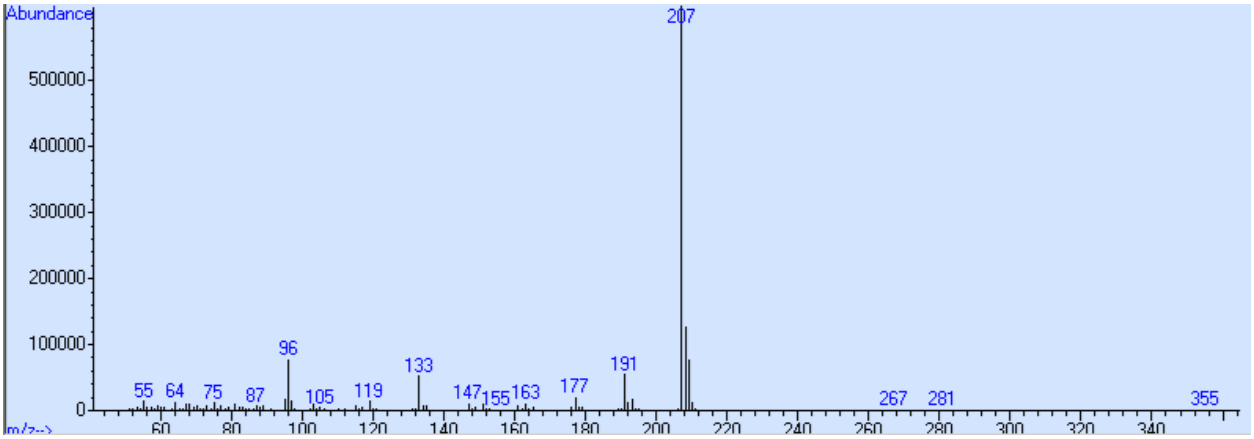


GC Spectrum 11: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover



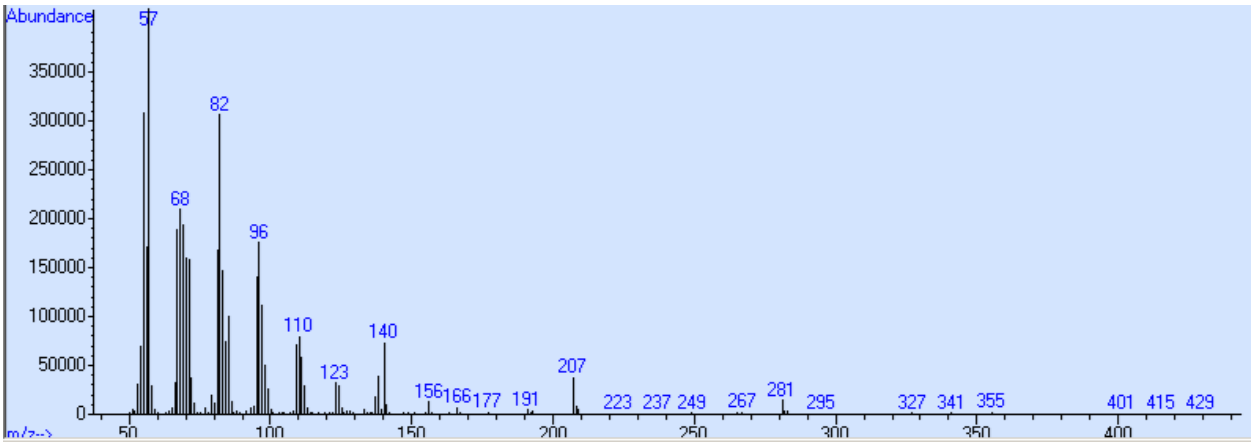
MS Spectrum 53: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

RT 3.573



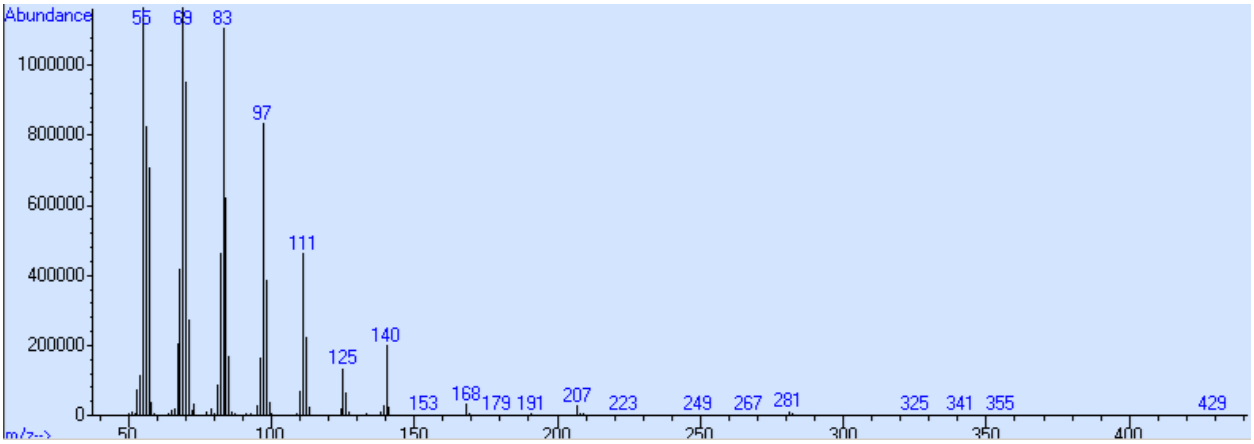
MS Spectrum 54: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

RT 11.155



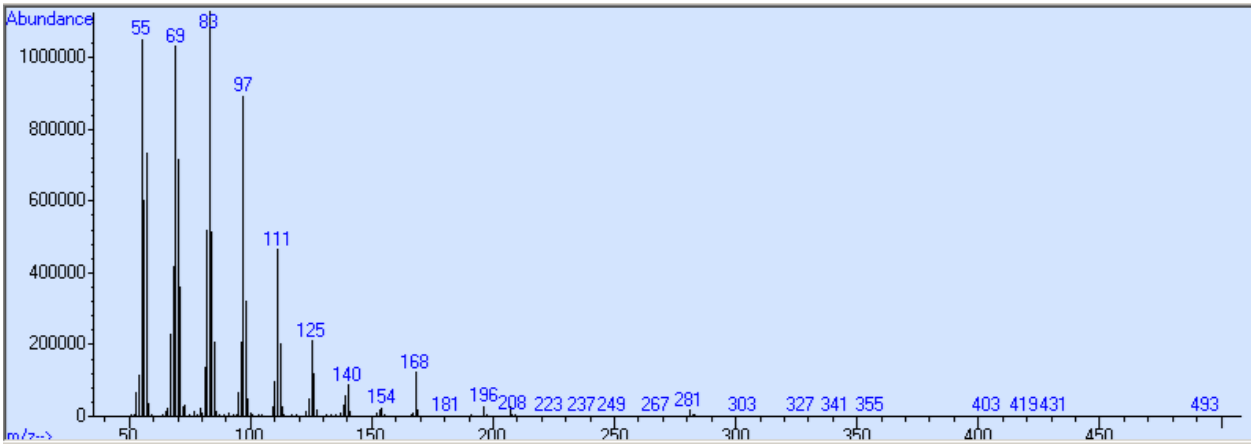
MS Spectrum 55: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

RT 12.047



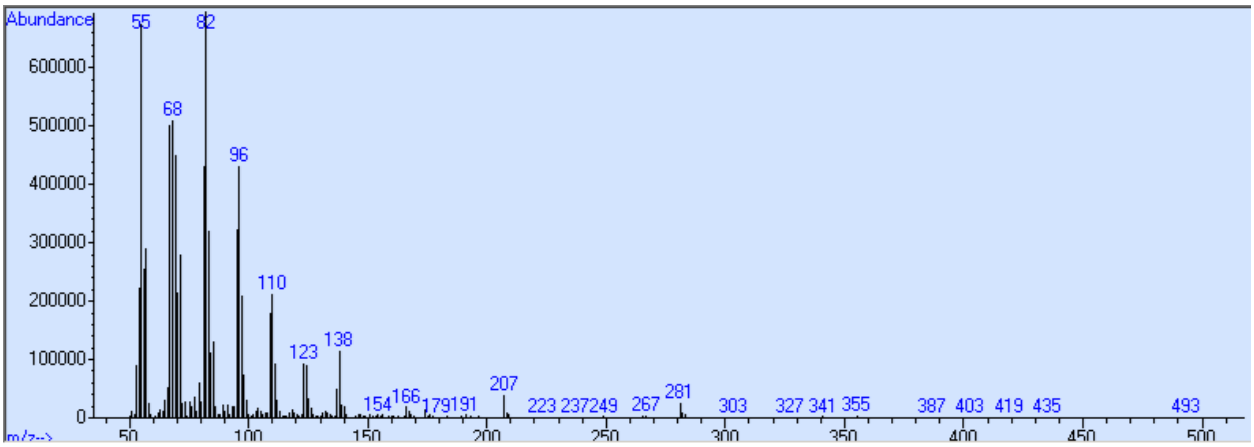
MS Spectrum 56: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

RT 14.462



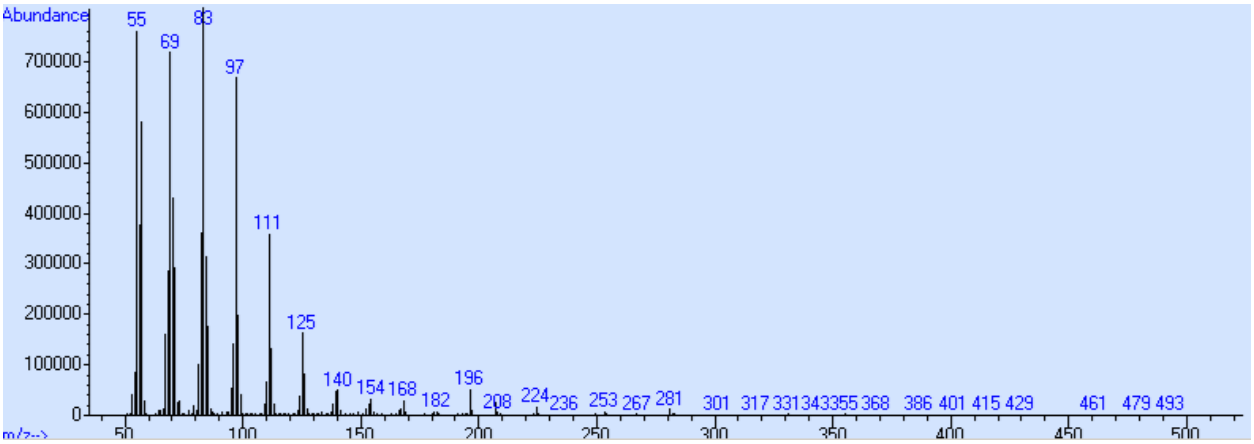
MS Spectrum 57: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

RT 14.571



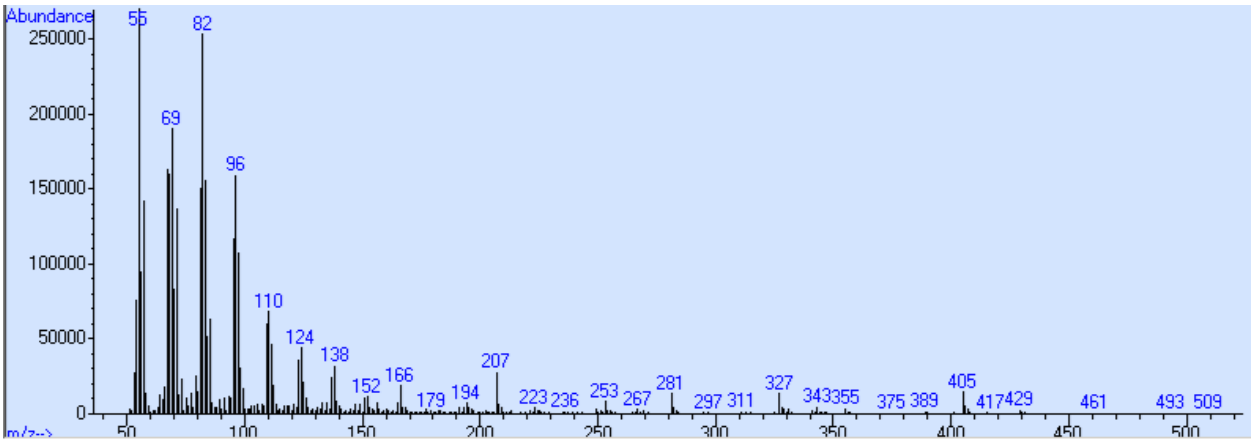
MS Spectrum 58: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

RT 16.619



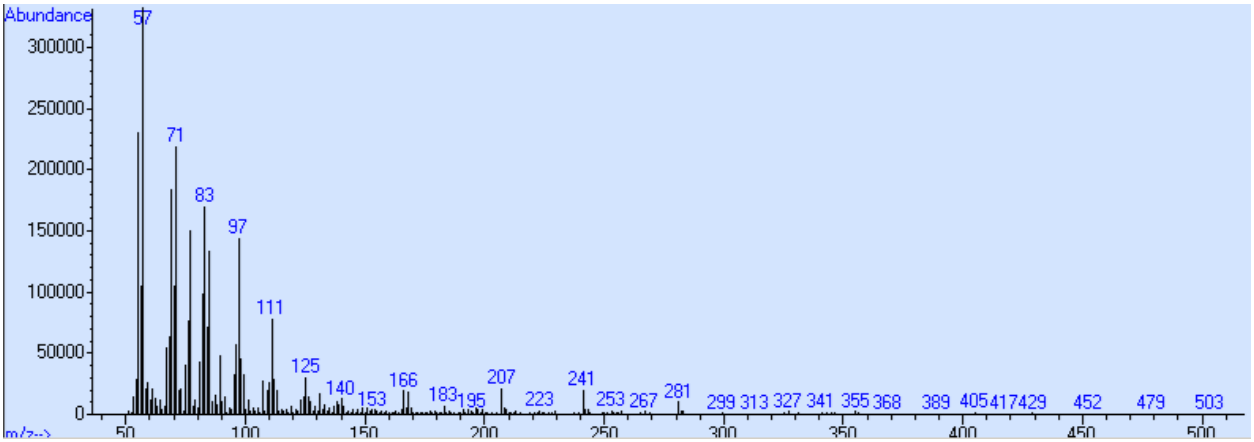
MS Spectrum 59: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

RT 16.774



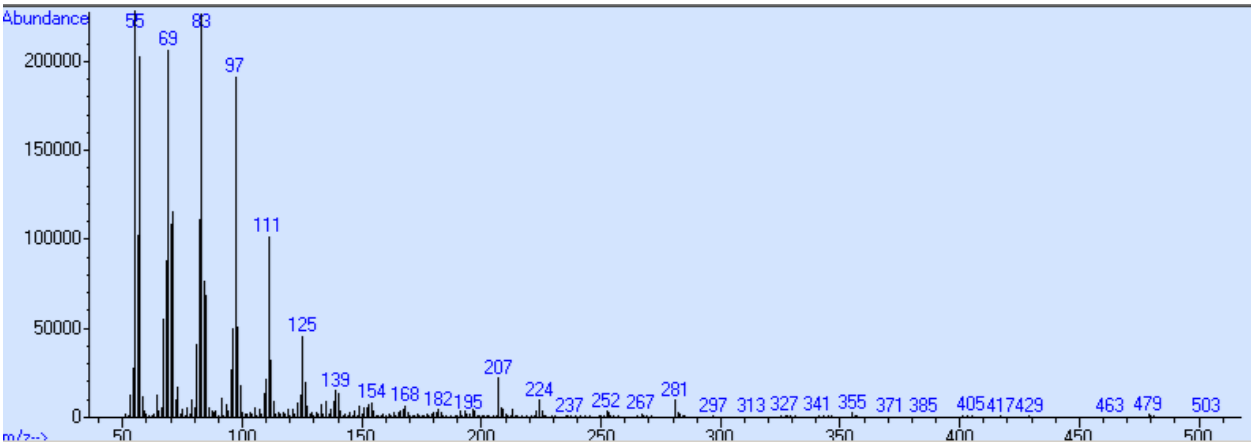
MS Spectrum 60: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

RT 17.798

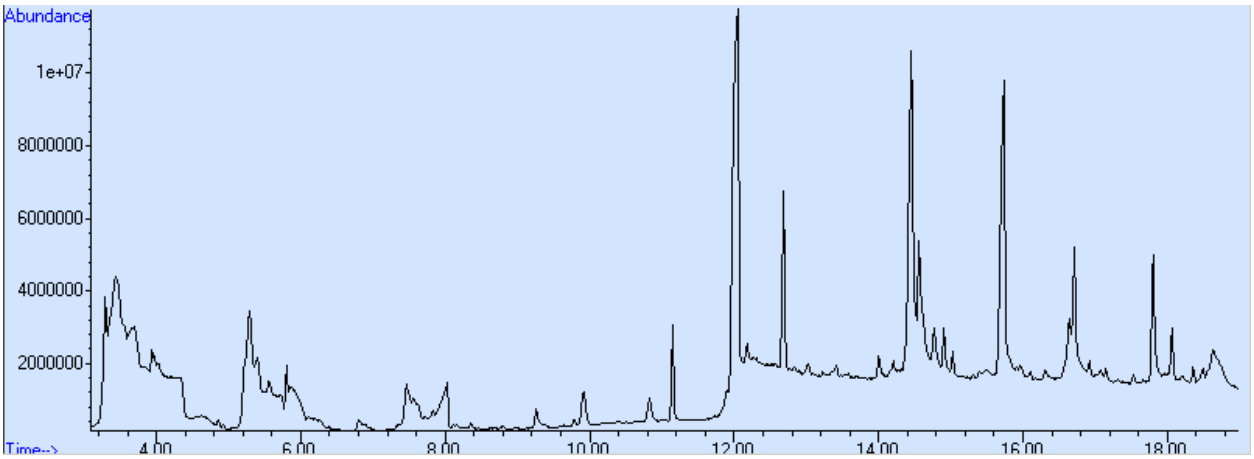


MS Spectrum 61: Remy XQ Extracted in Colorist Secrets™ Hair Color Remover

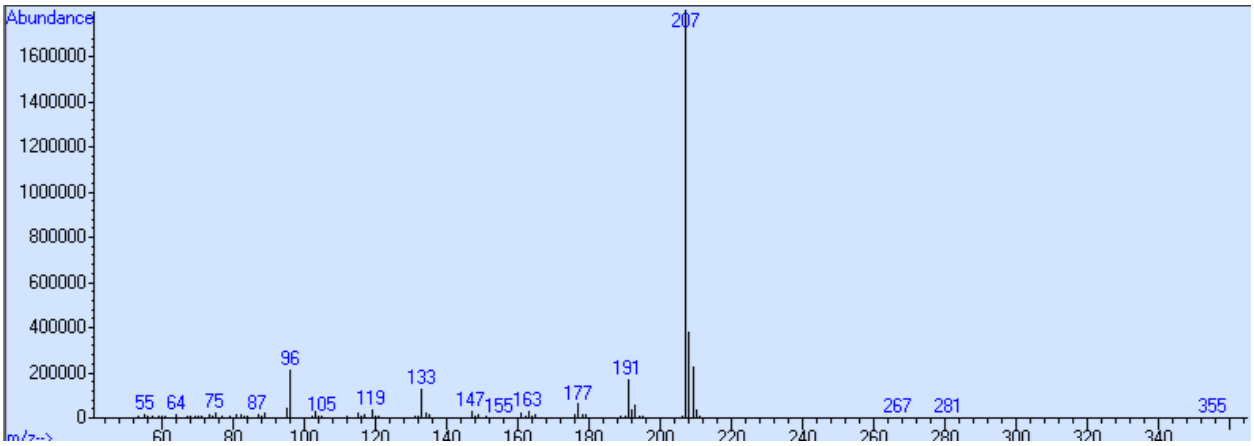
RT 18.691



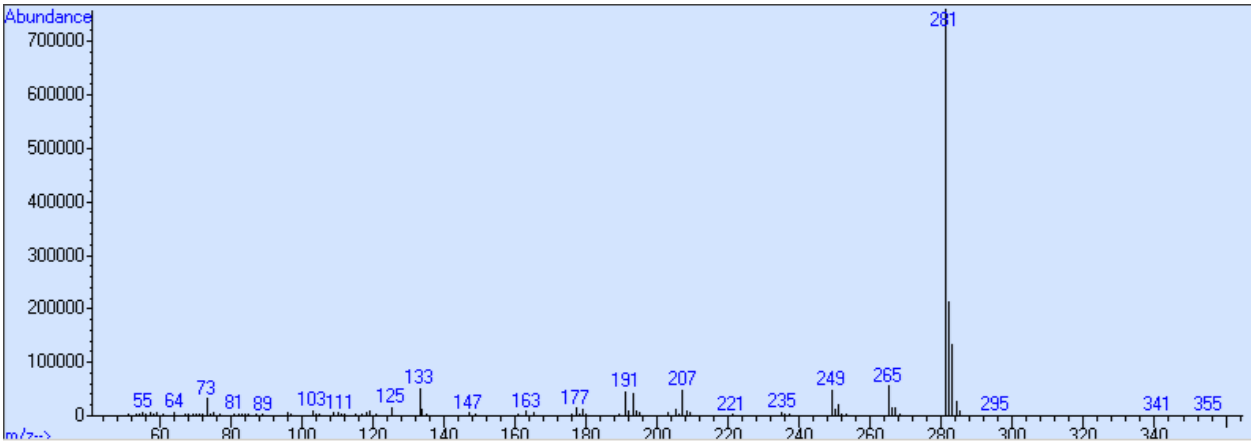
GC Spectrum 12: ECW Extracted in Colorist Secrets™ Hair Color Remover



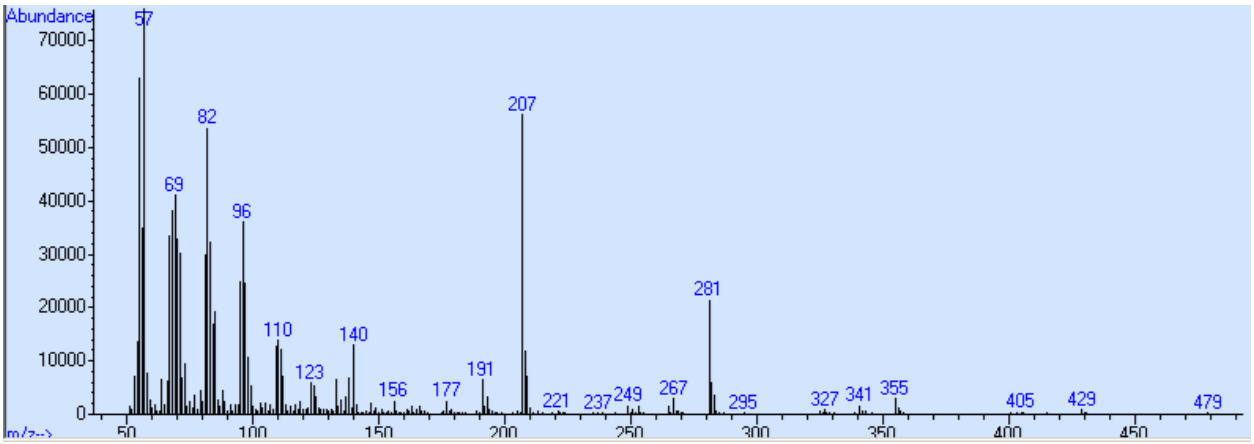
MS Spectrum 62: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 3.487



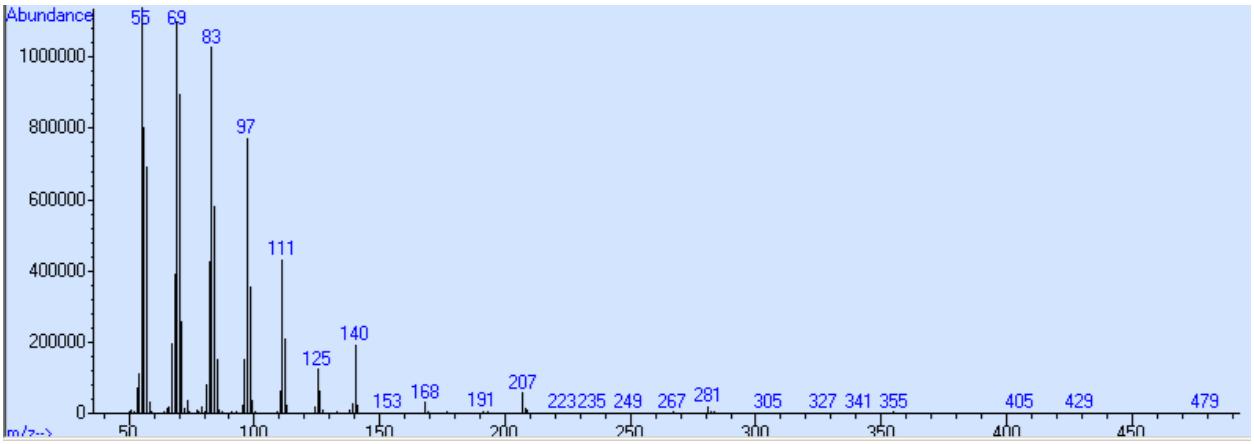
MS Spectrum 63: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 5.358



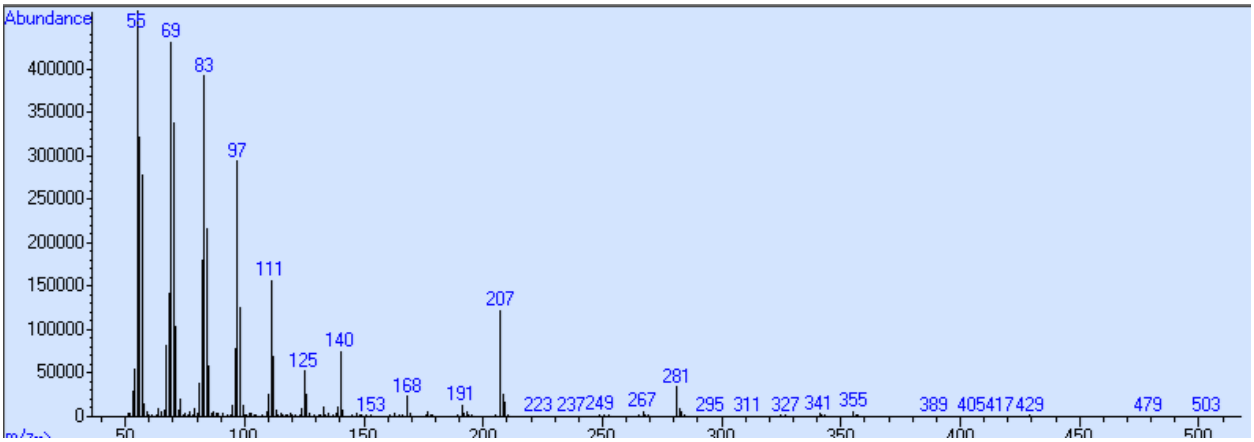
MS Spectrum 64: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 11.172



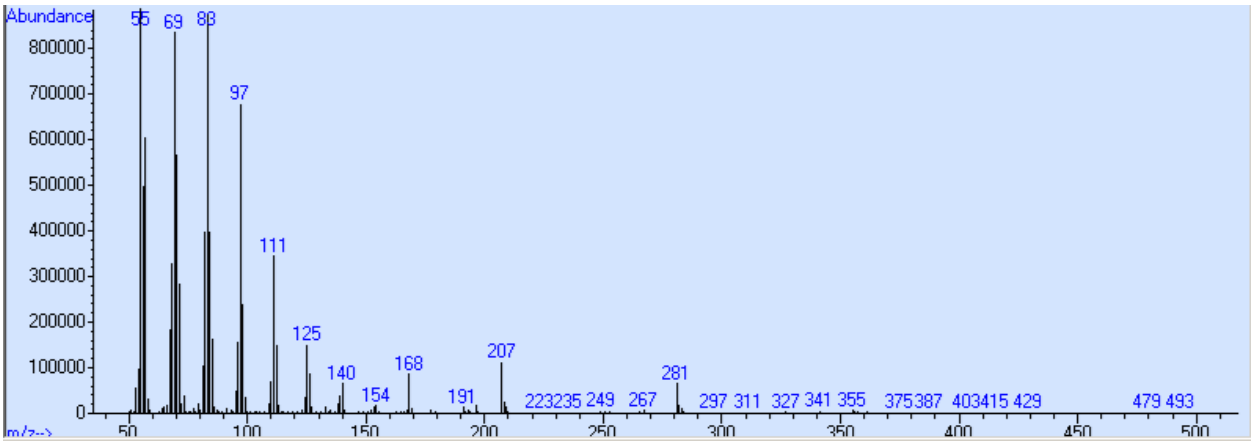
MS Spectrum 65: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 12.024



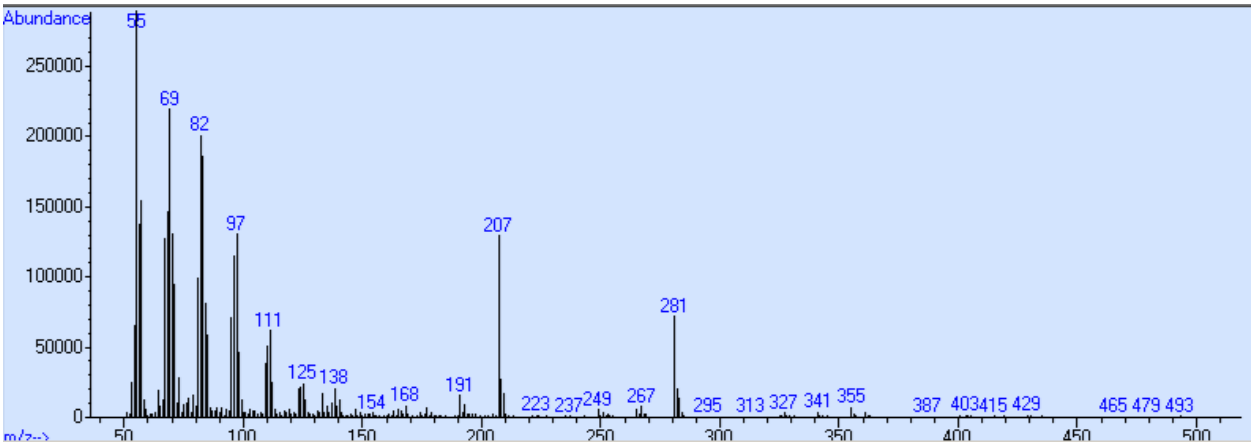
MS Spectrum 66: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 12.700



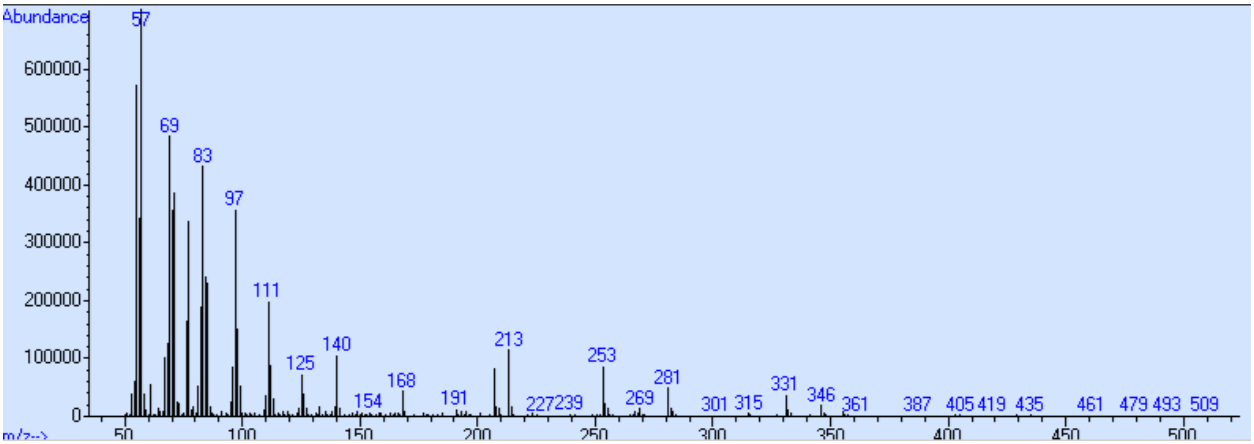
MS Spectrum 67: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 14.439



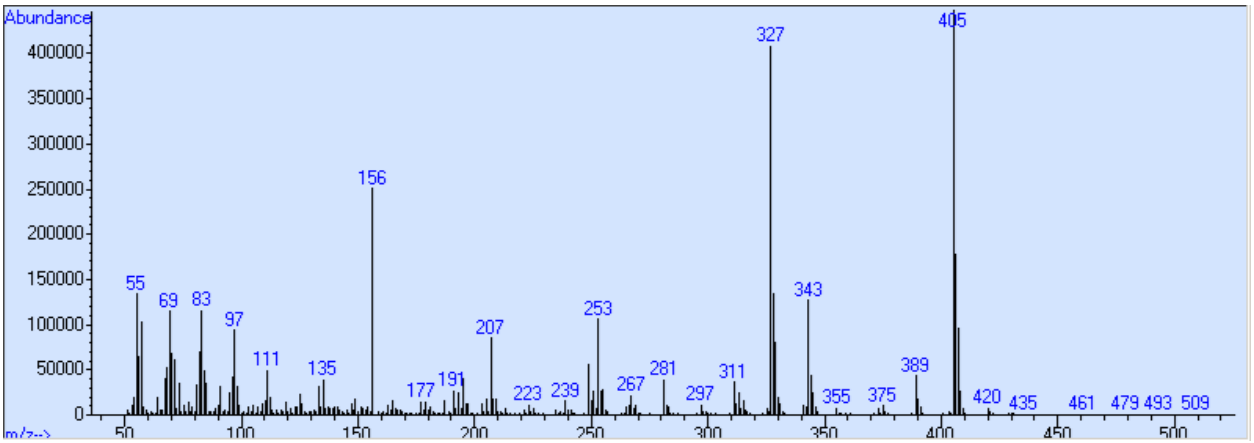
MS Spectrum 68: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 14.594



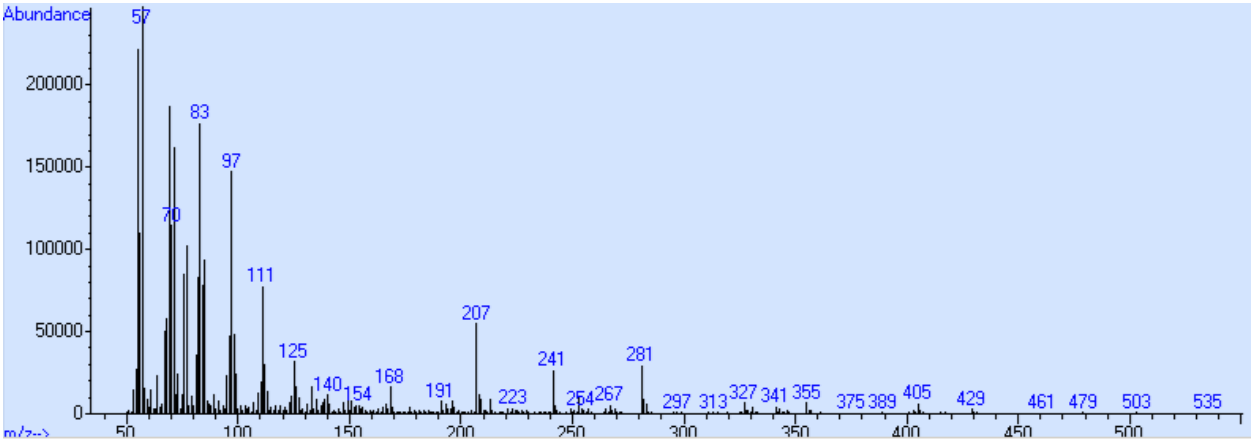
MS Spectrum 69: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 15.704



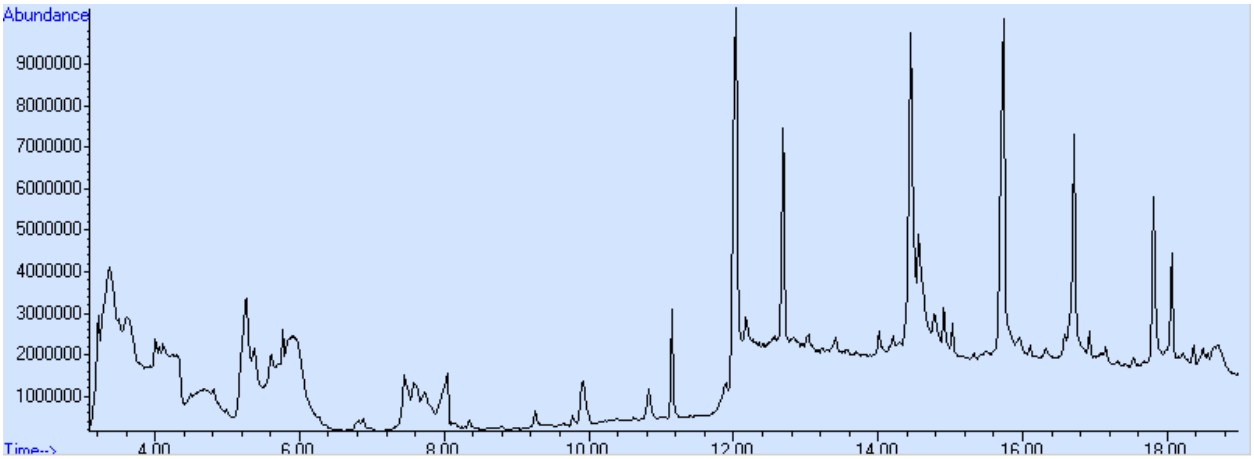
MS Spectrum 70: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 16.705



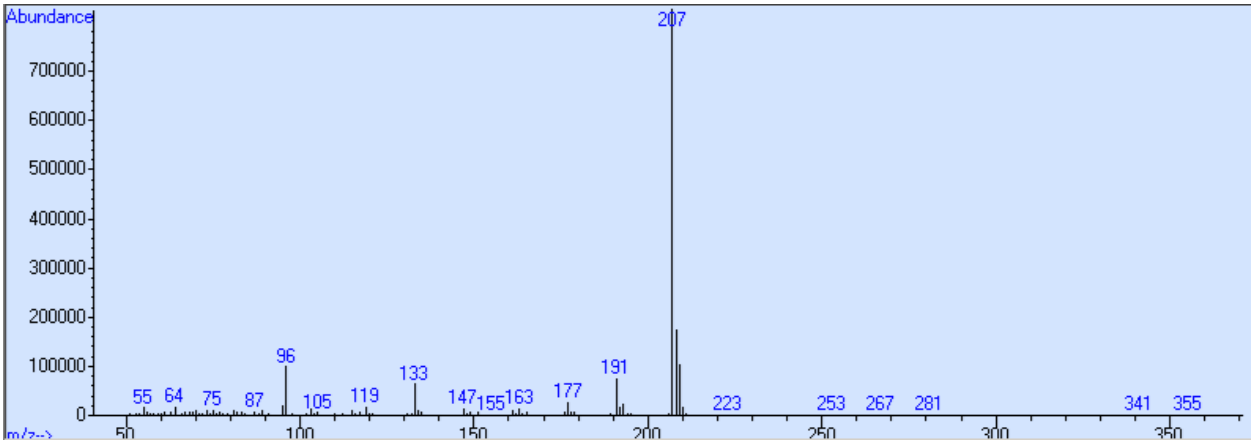
MS Spectrum 71: ECW Extracted in Colorist Secrets™ Hair Color Remover – RT 17.821



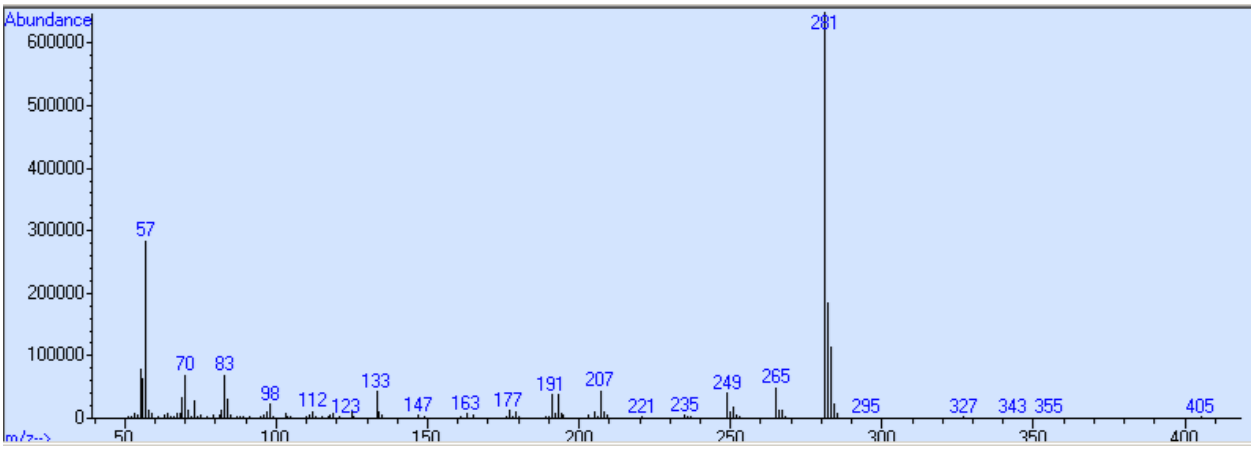
GC Spectrum 13: Que Extracted in Colorist Secrets™ Hair Color Remover



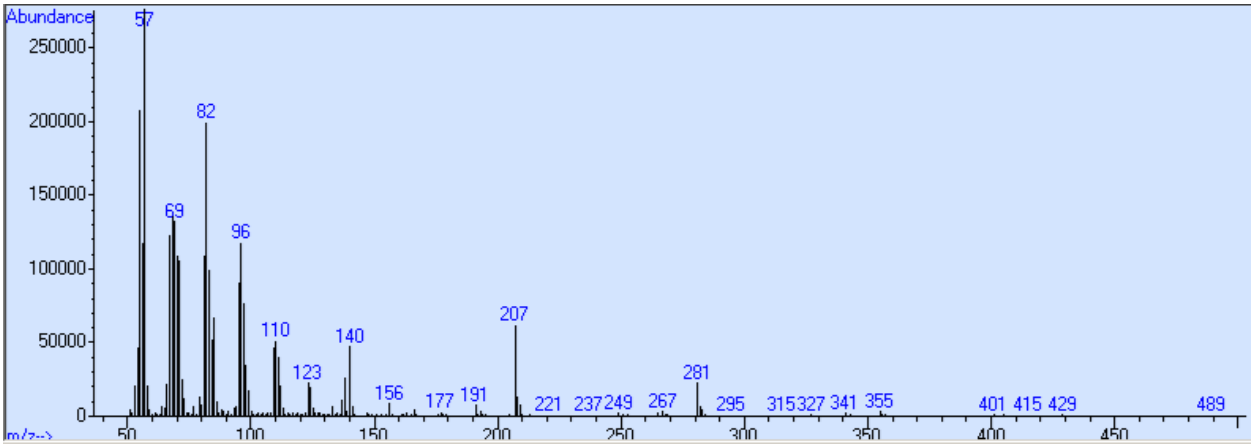
MS Spectrum 72: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 3.745



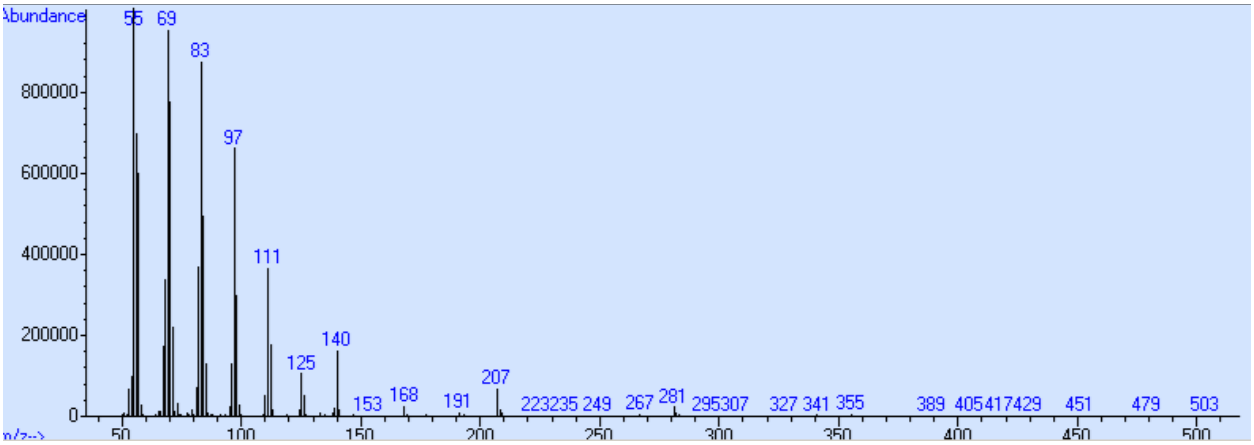
MS Spectrum 73: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 5.839



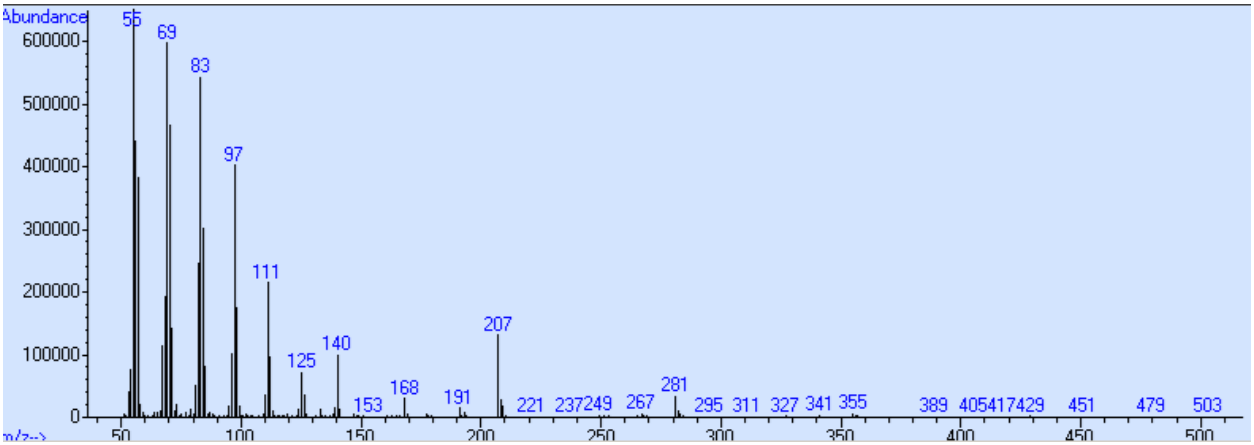
MS Spectrum 74: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 11.155



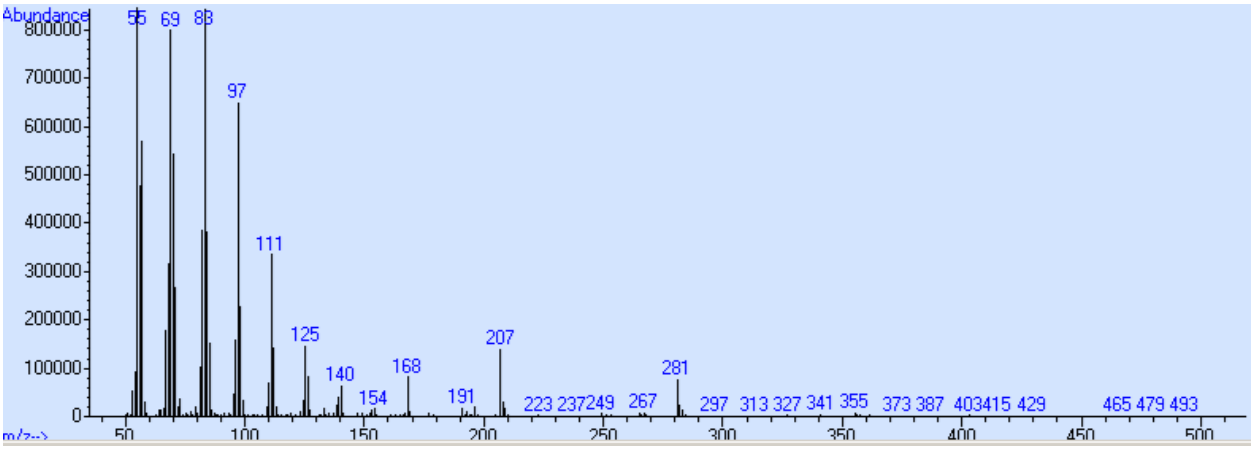
MS Spectrum 75: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 12.047



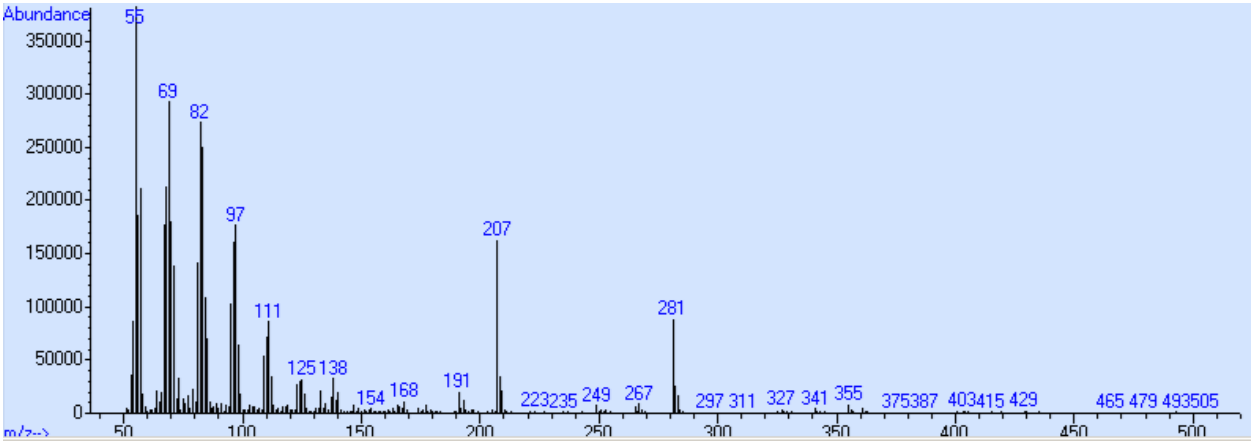
MS Spectrum 76: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 12.677



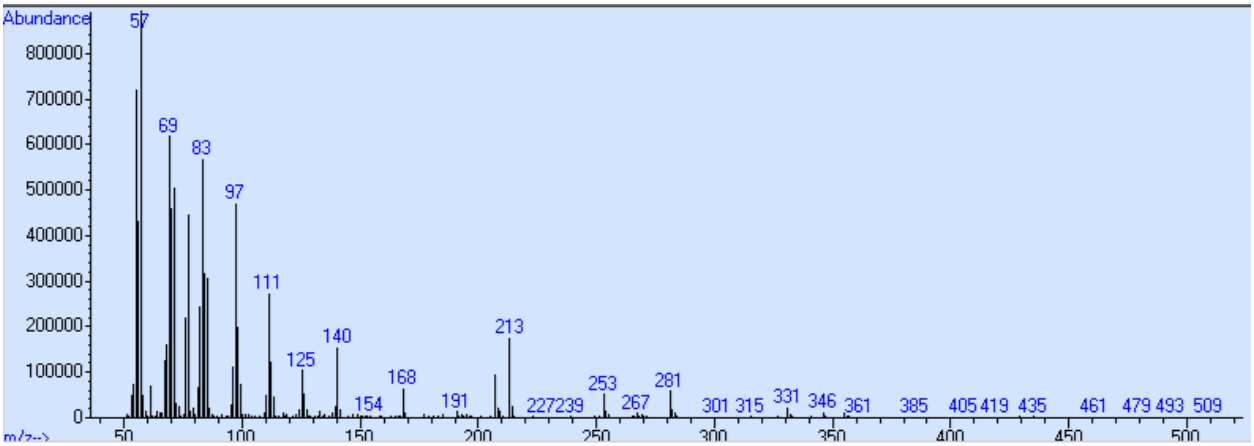
MS Spectrum 77: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 14.462



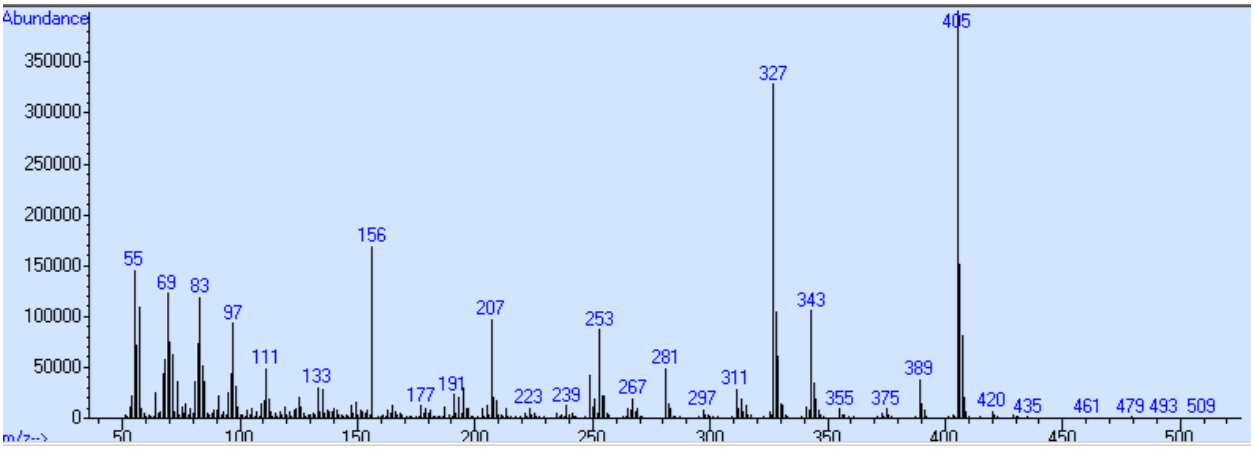
MS Spectrum 78: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 14.571



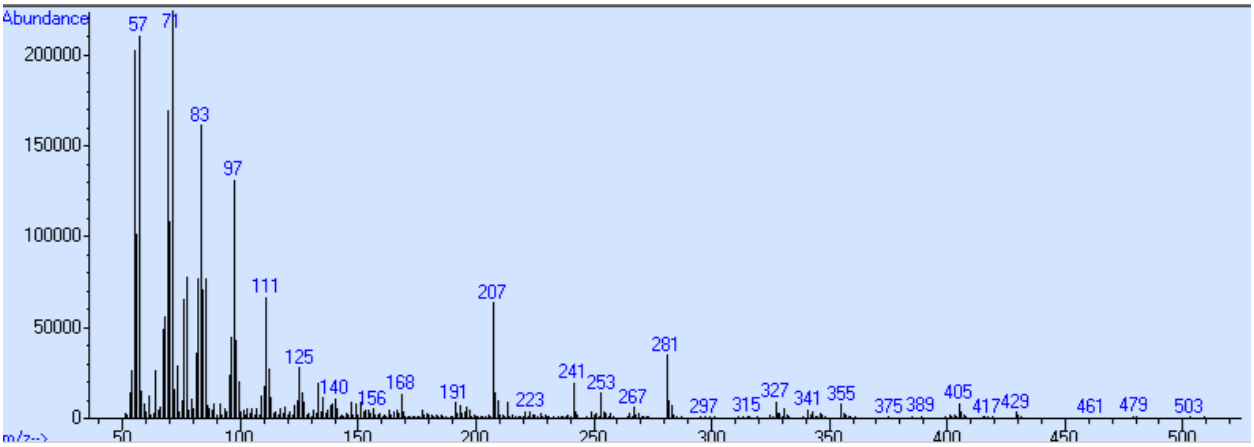
MS Spectrum 79: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 15.727



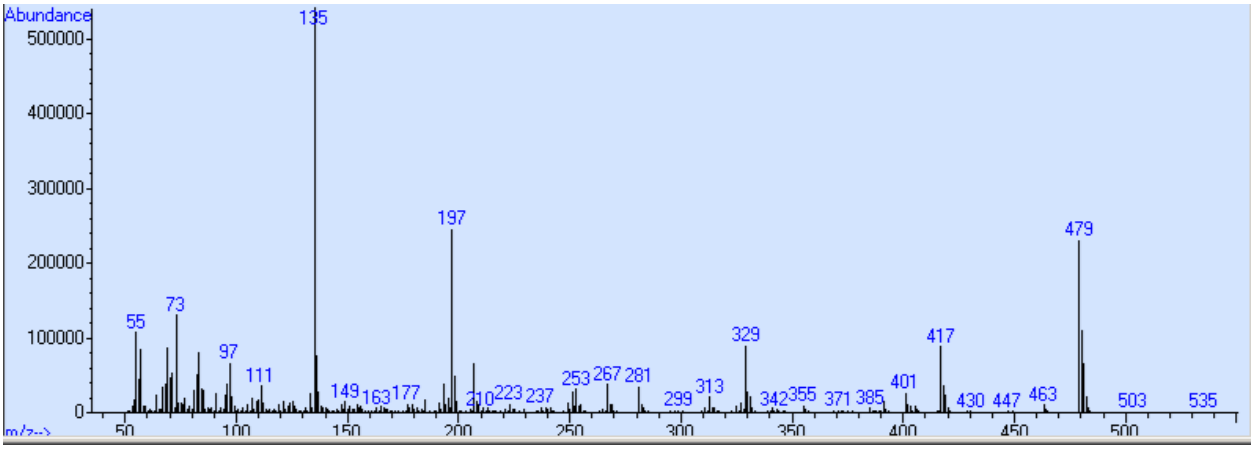
MS Spectrum 80: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 16.728



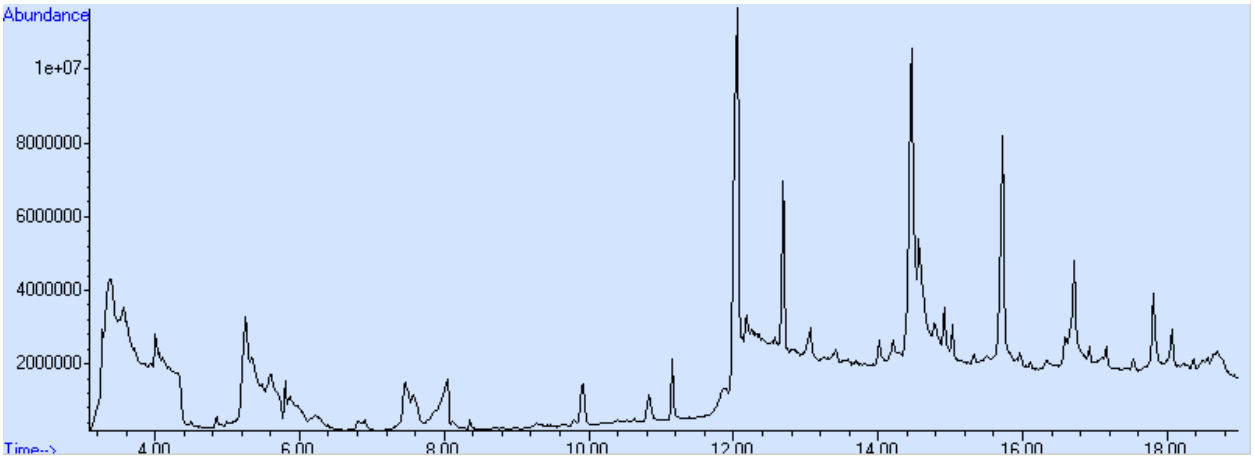
MS Spectrum 81: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 17.838



MS Spectrum 82: Que Extracted in Colorist Secrets™ Hair Color Remover – RT 18.056

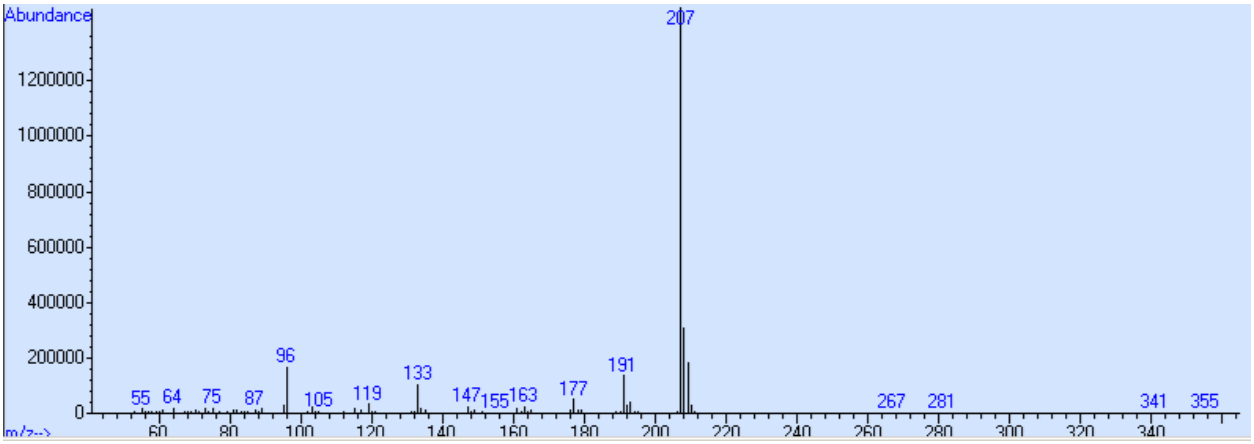


GC Spectrum 14: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover



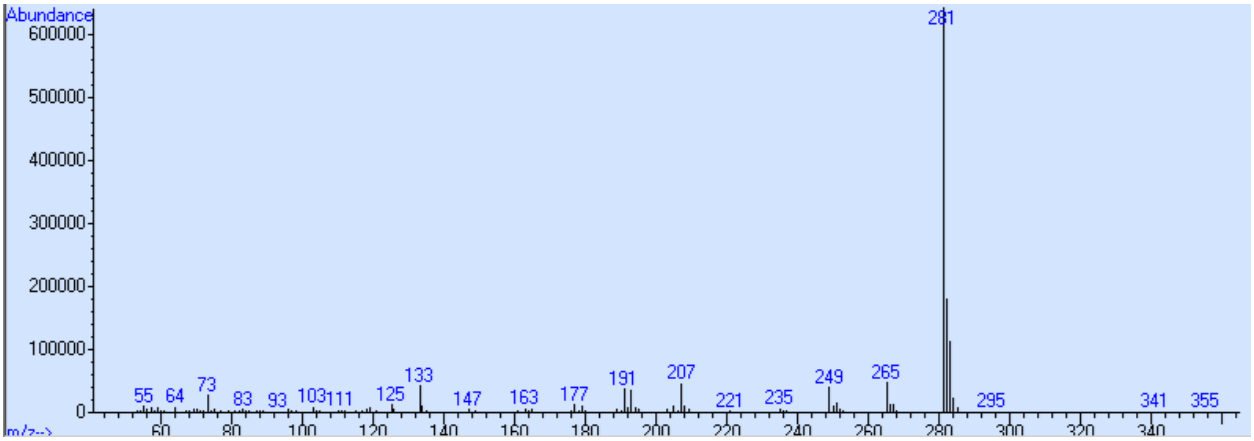
MS Spectrum 83: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 3.613



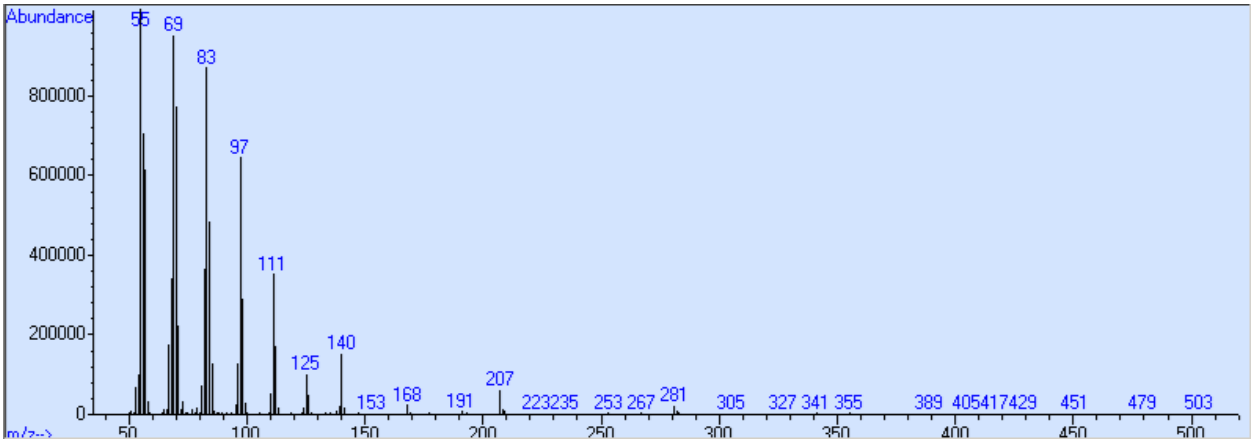
MS Spectrum 84: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 5.404



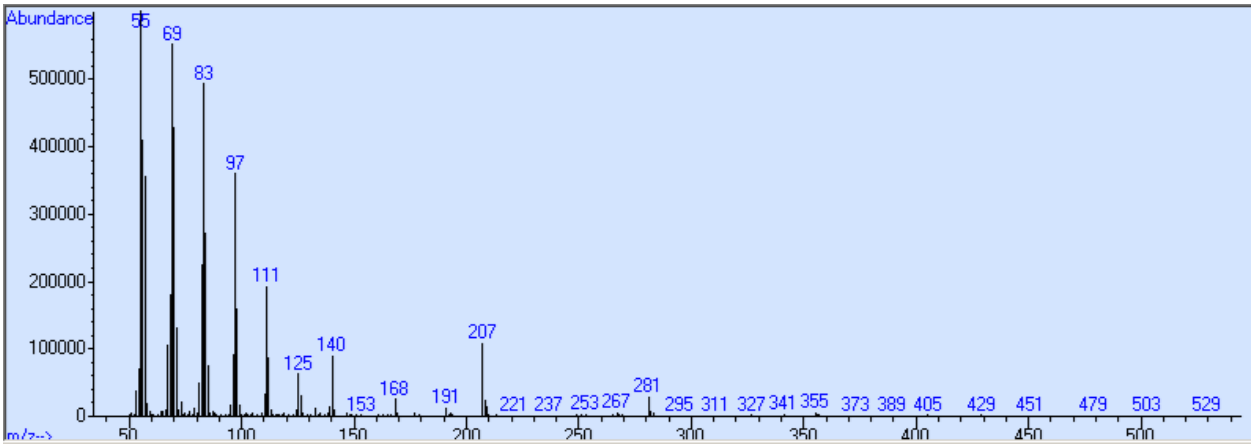
MS Spectrum 85: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 12.024



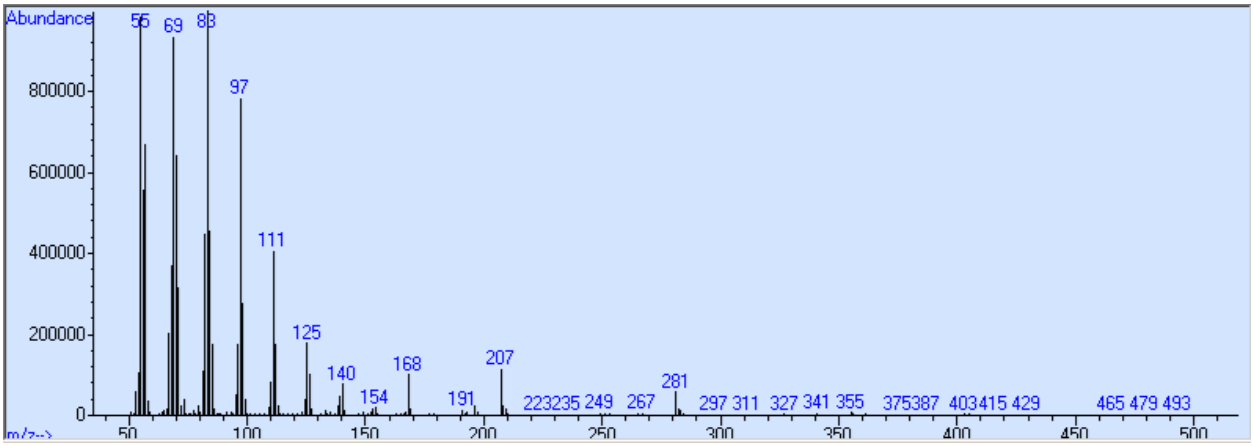
MS Spectrum 86: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 12.677



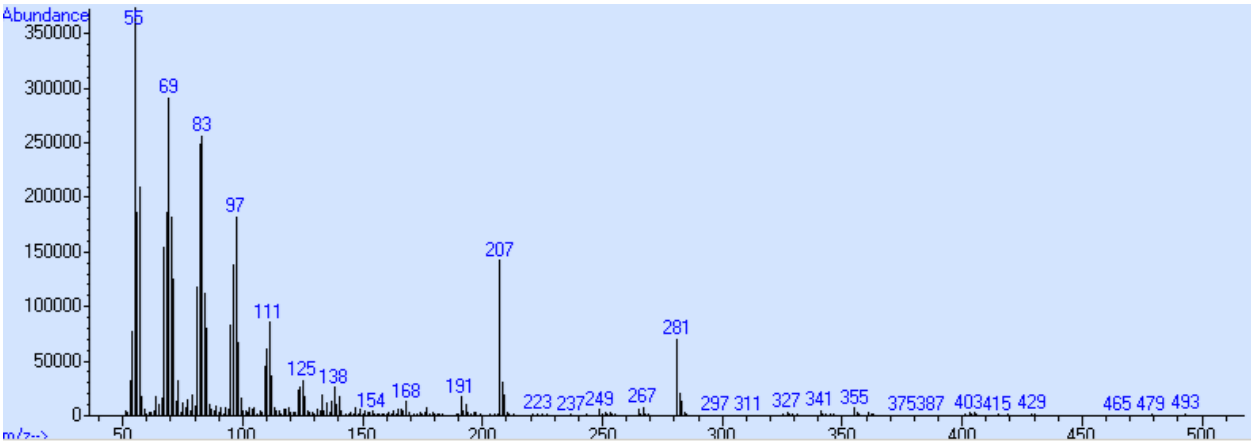
MS Spectrum 87: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 14.462



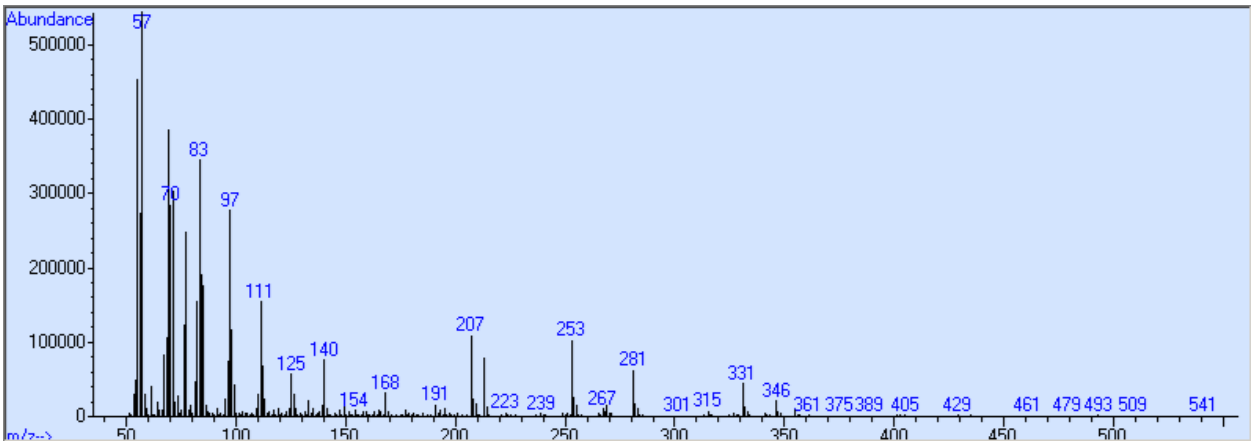
MS Spectrum 88: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 14.594



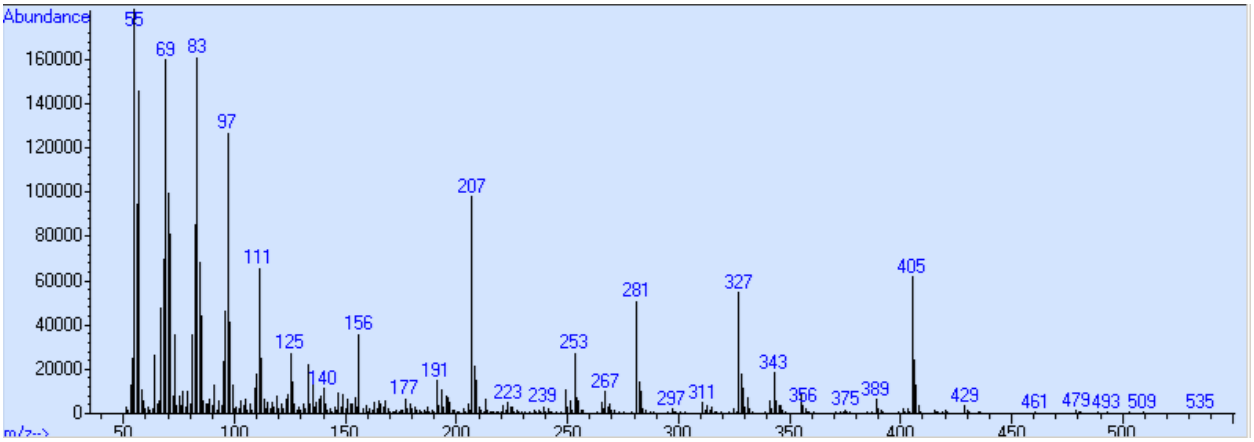
MS Spectrum 89: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 15.704



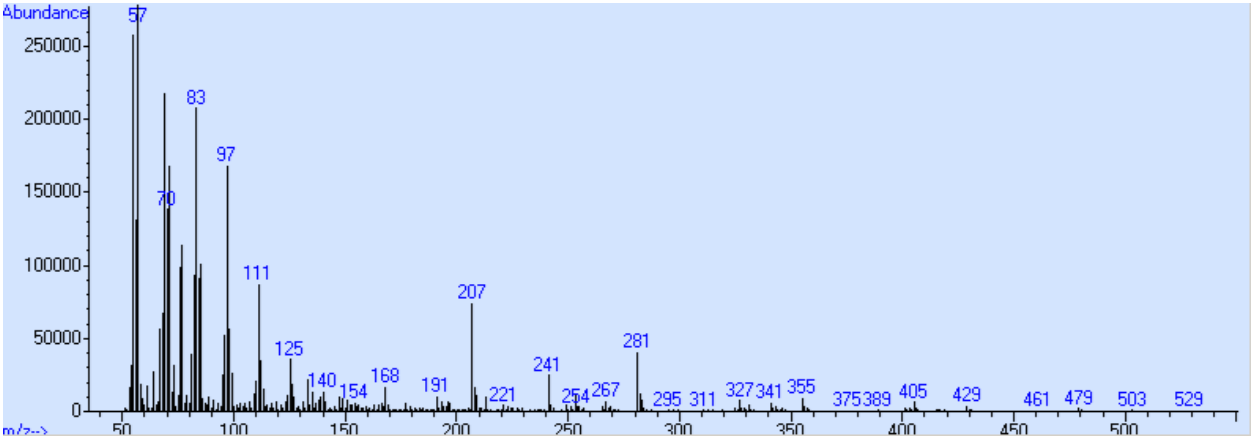
MS Spectrum 90: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 16.665



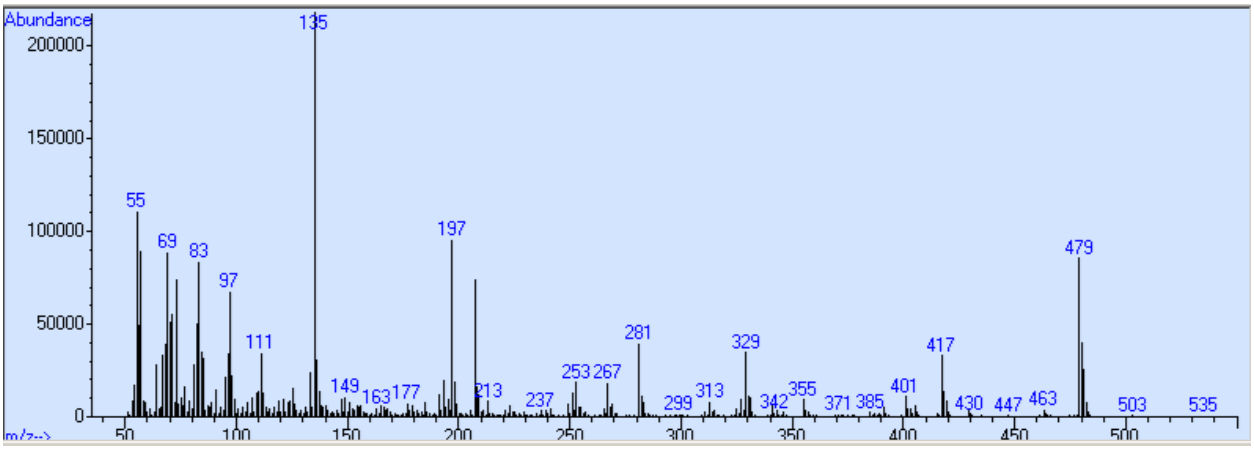
MS Spectrum 91: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 17.798

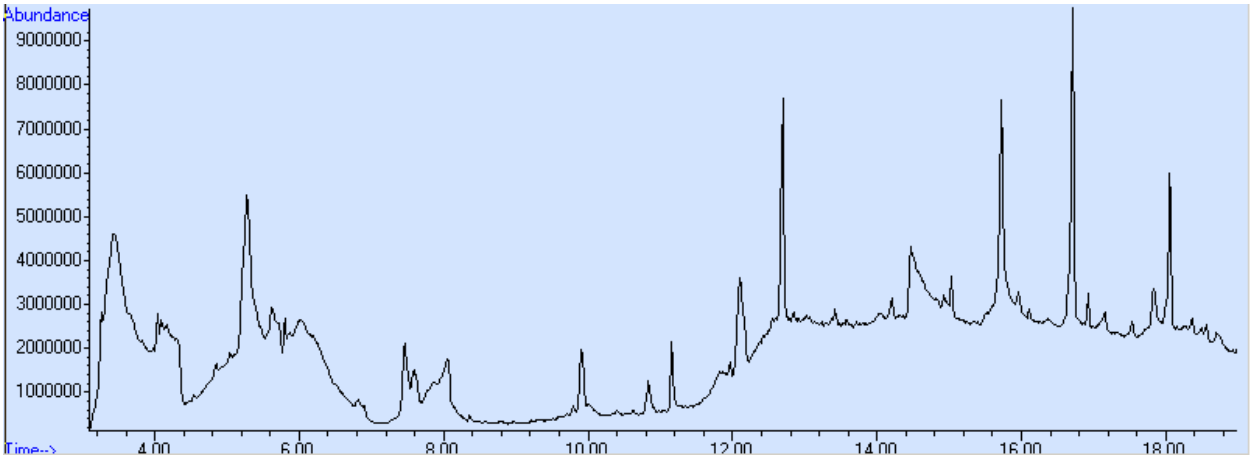


MS Spectrum 92: Undyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 18.055

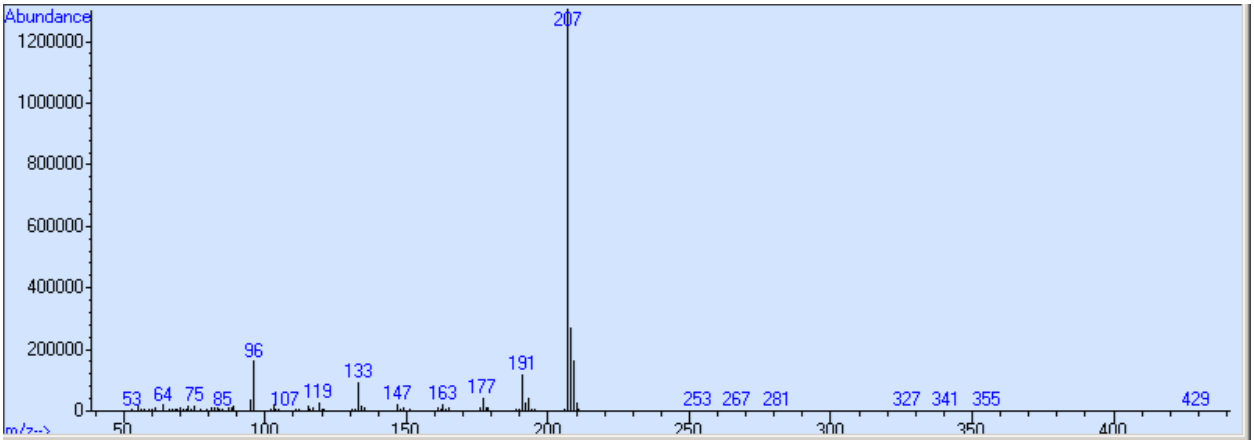


GC Spectrum 15: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover



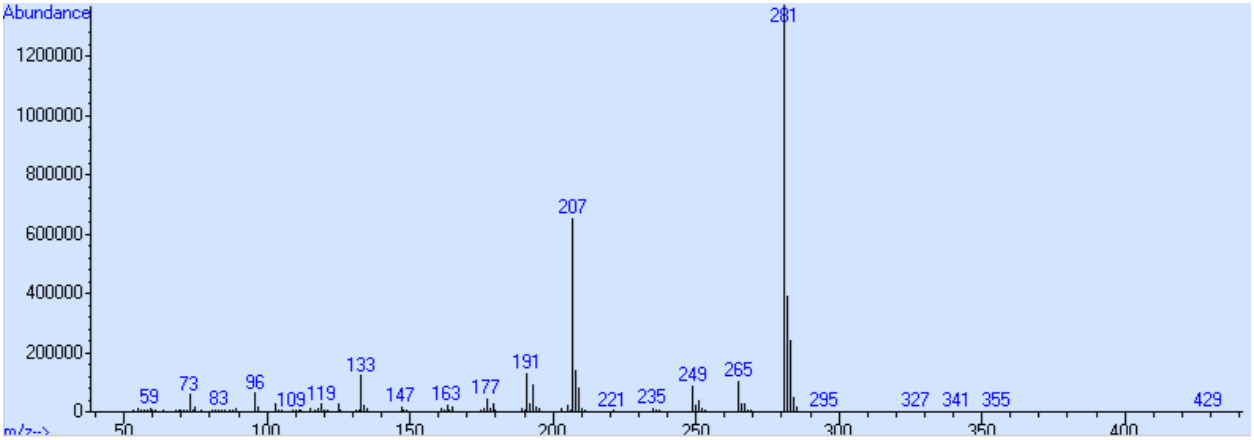
MS Spectrum 93: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 3.613



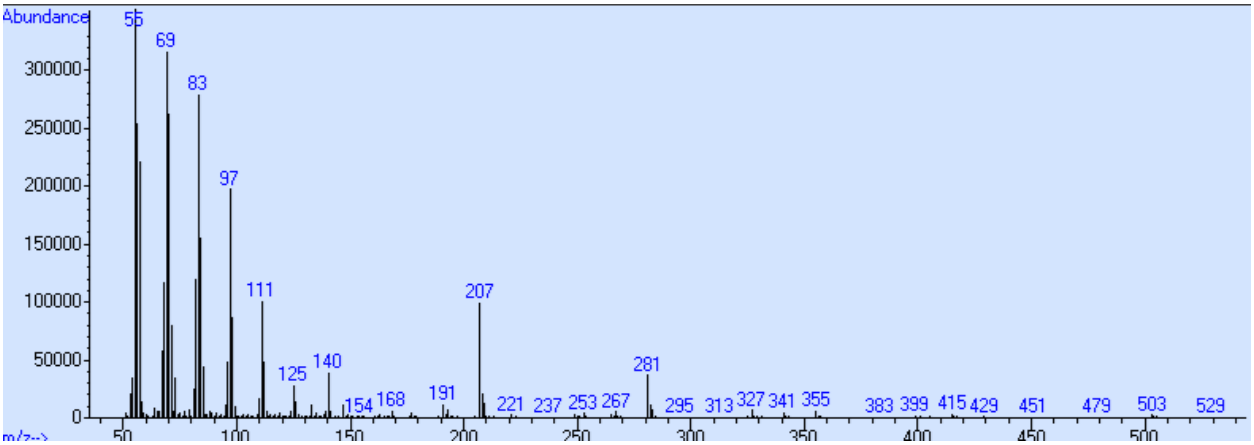
MS Spectrum 94: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 5.313



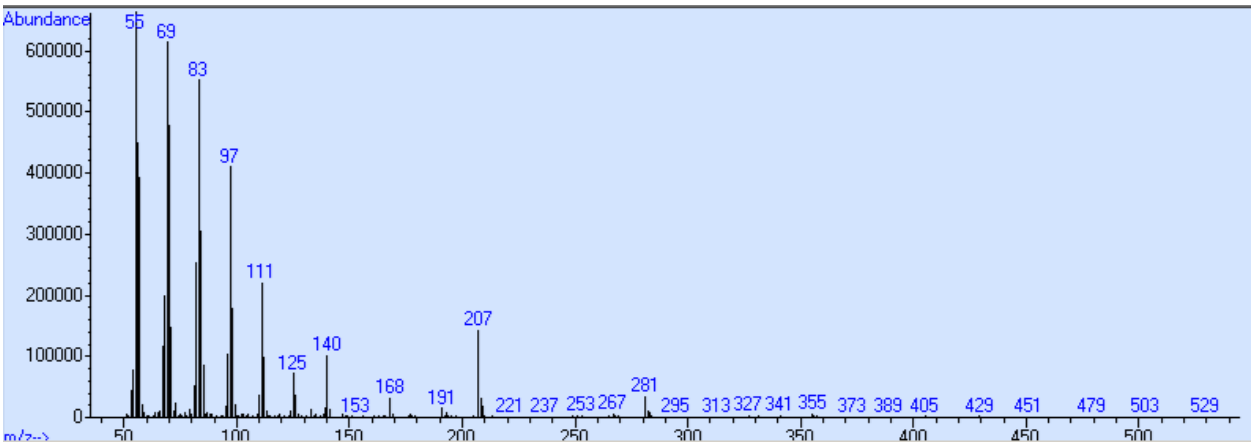
MS Spectrum 95: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 12.110



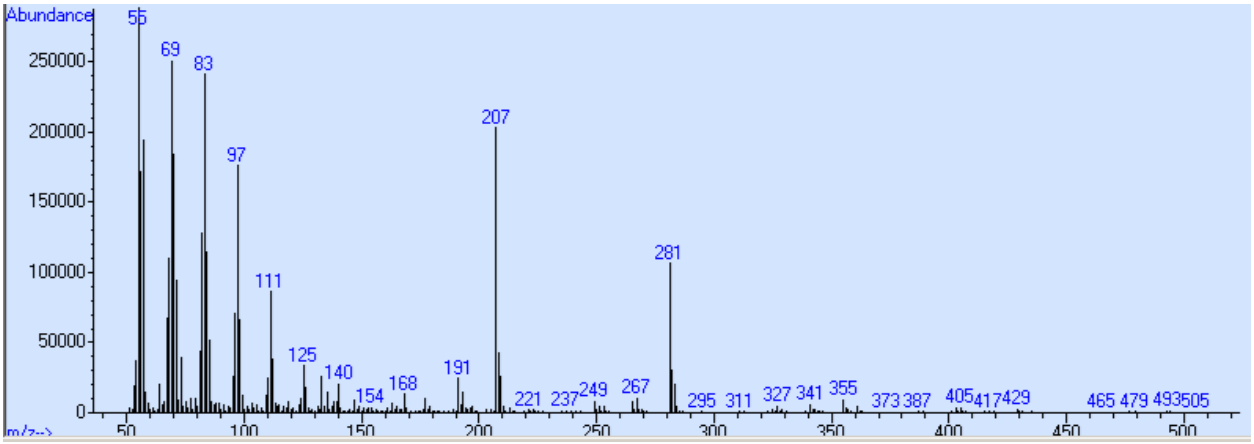
MS Spectrum 96: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 12.677



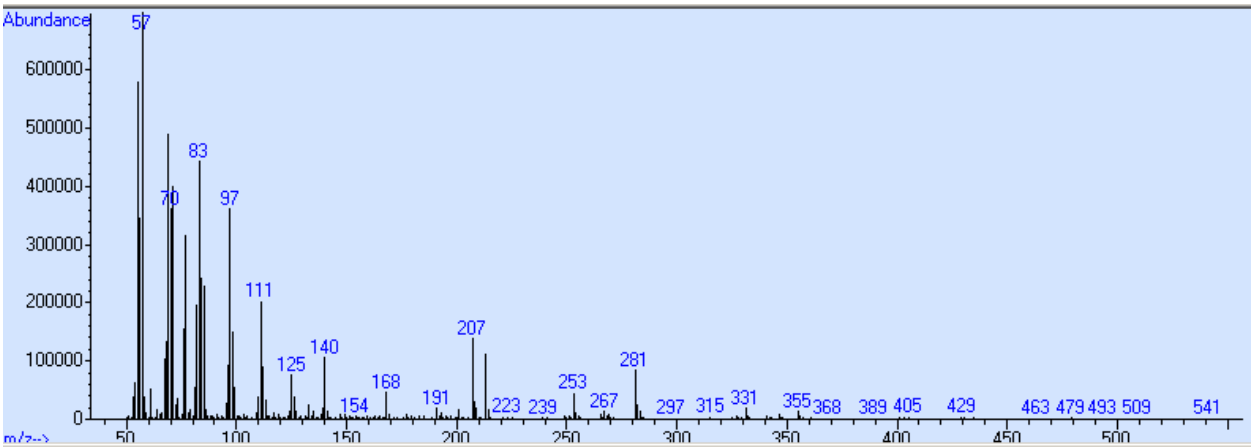
MS Spectrum 97: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 14.571



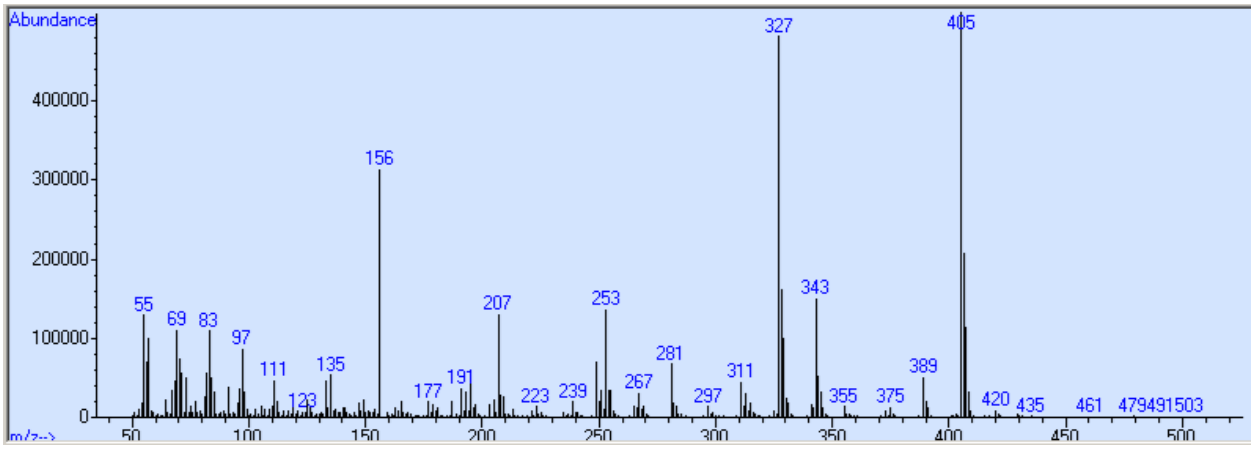
MS Spectrum 98: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 15.727



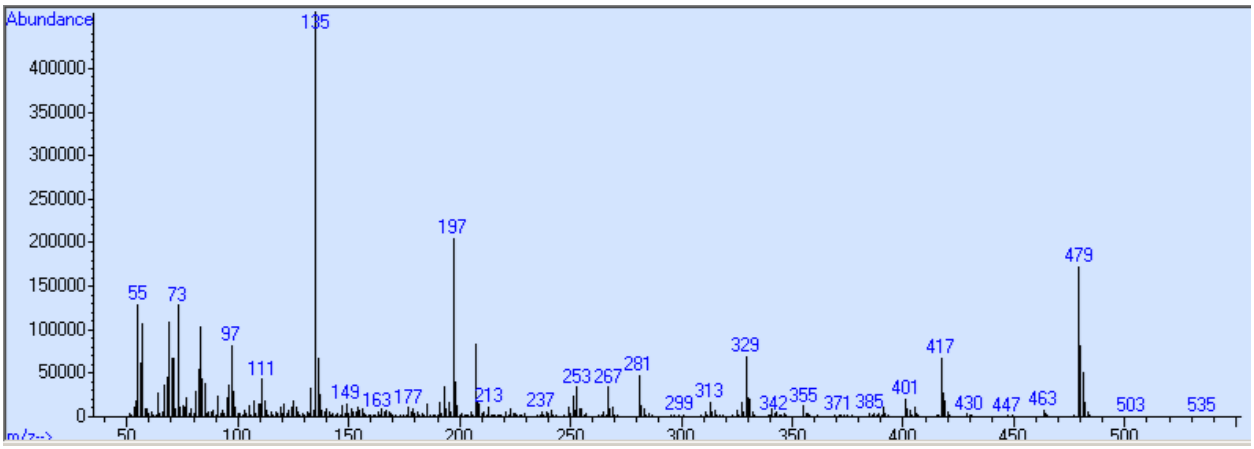
MS Spectrum 99: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

RT 16.688

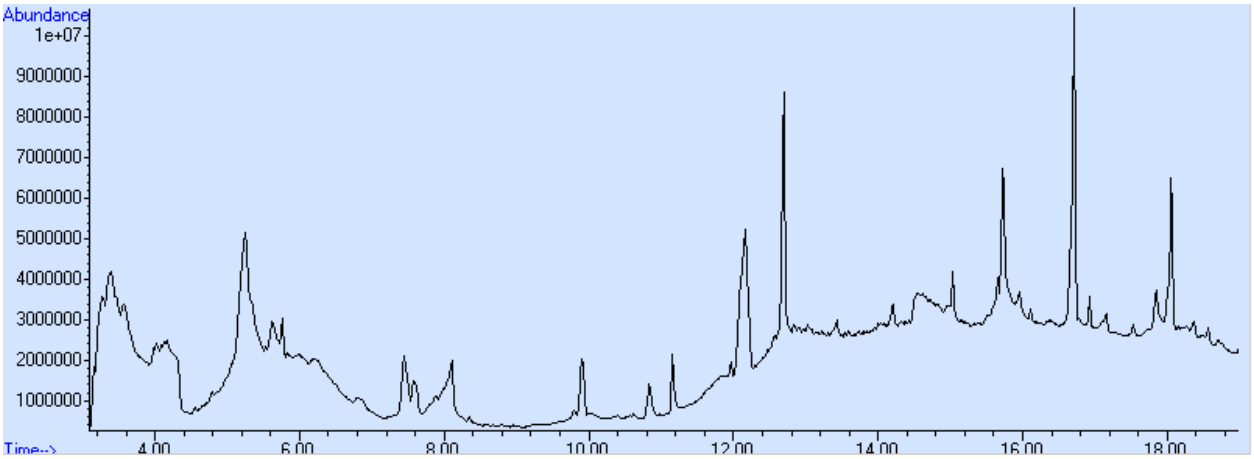


MS Spectrum 100: Dyed Caucasoid Extracted in Colorist Secrets™ Hair Color Remover

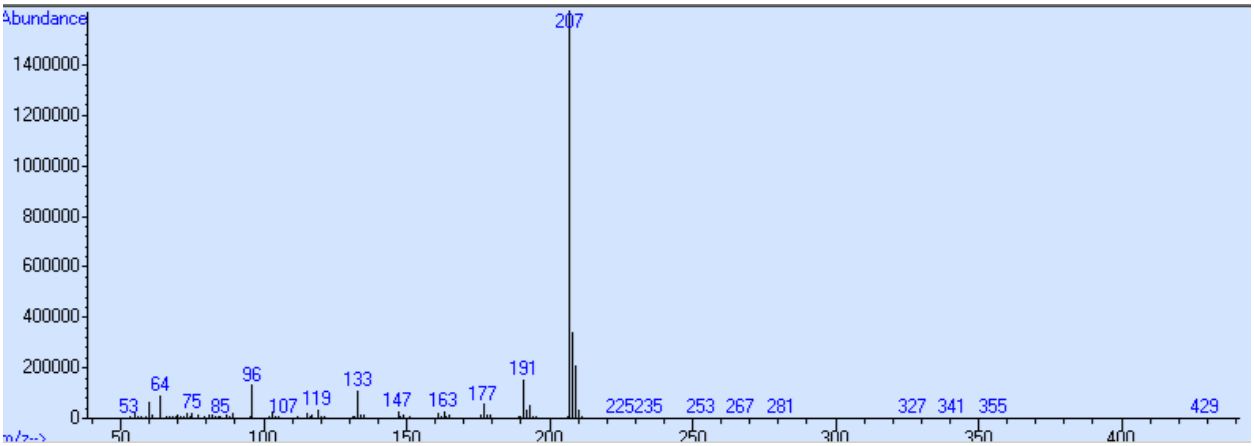
RT 18.038



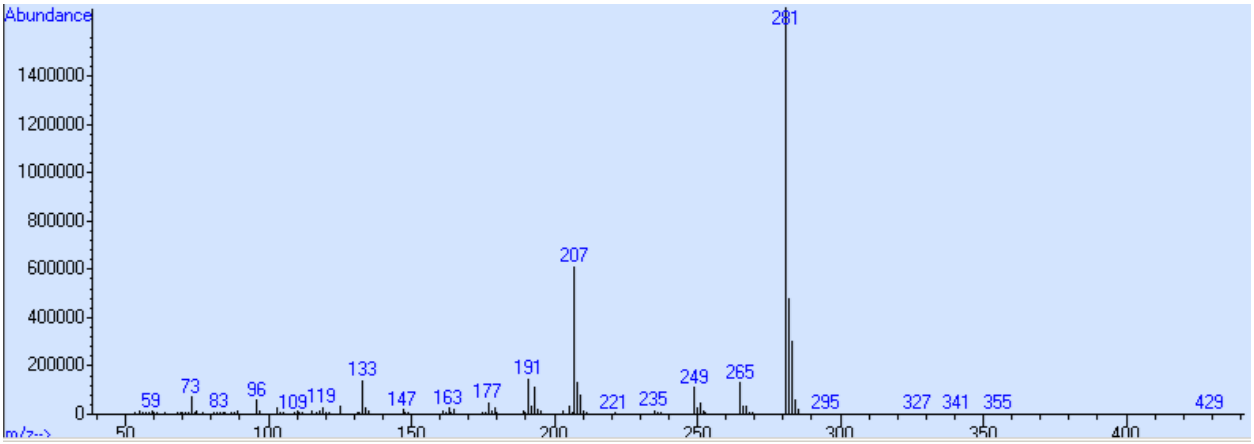
GC Spectrum 16: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover



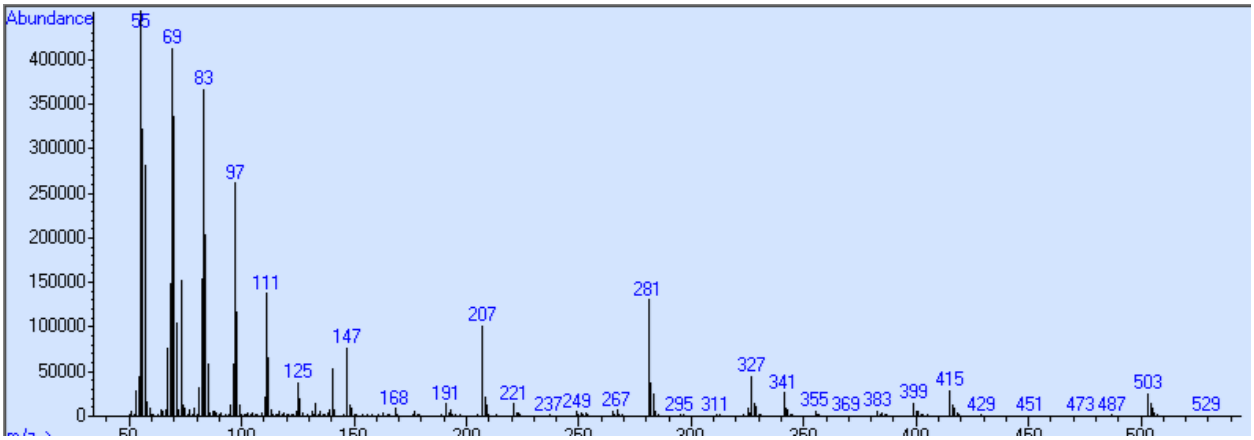
MS Spectrum 101: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover – RT 3.287



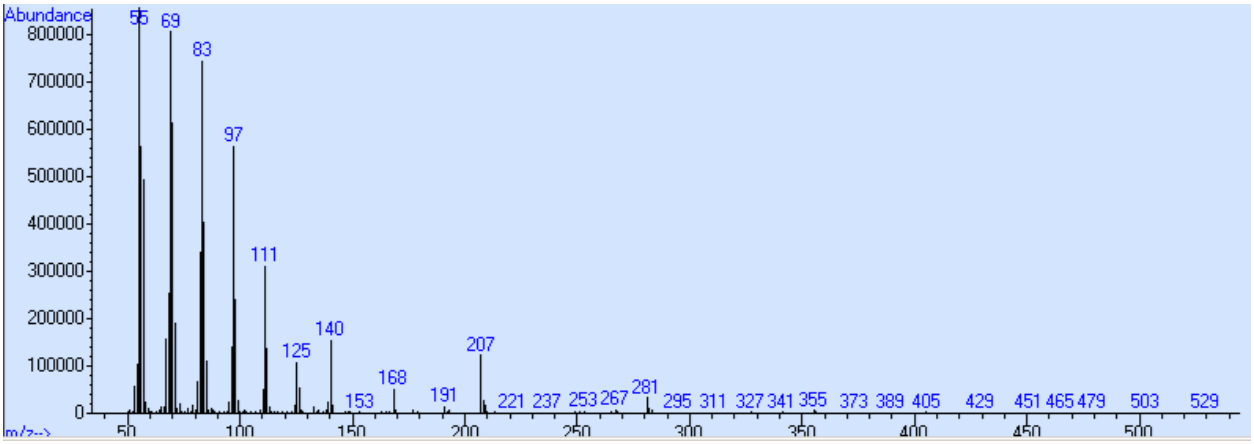
MS Spectrum 102: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover – RT 5.250



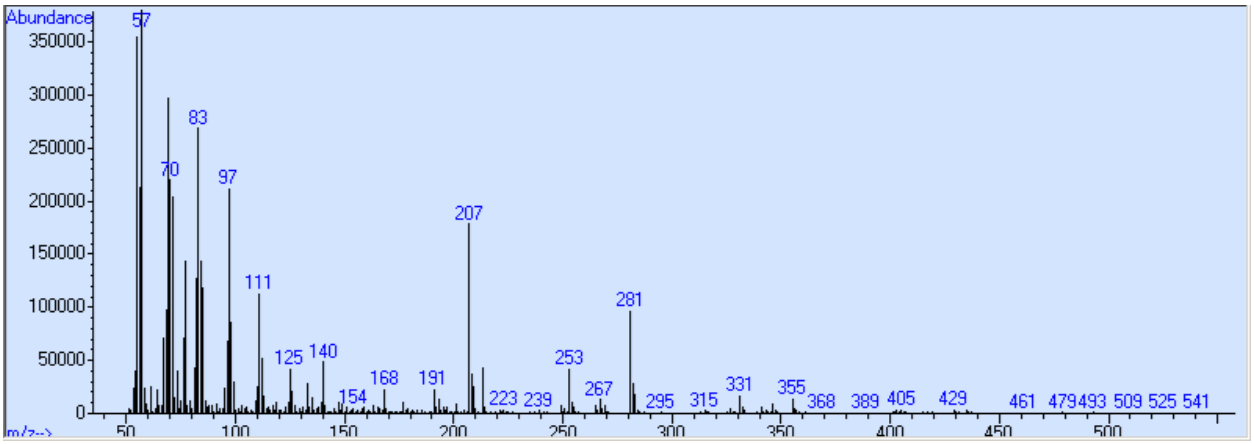
MS Spectrum 103: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover – RT 12.156



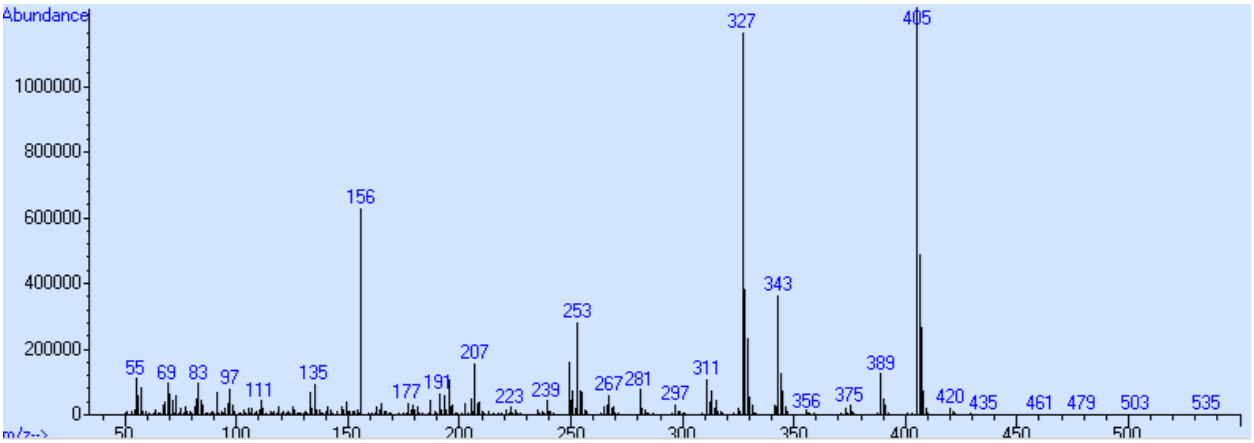
MS Spectrum 104: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover – RT 12.700



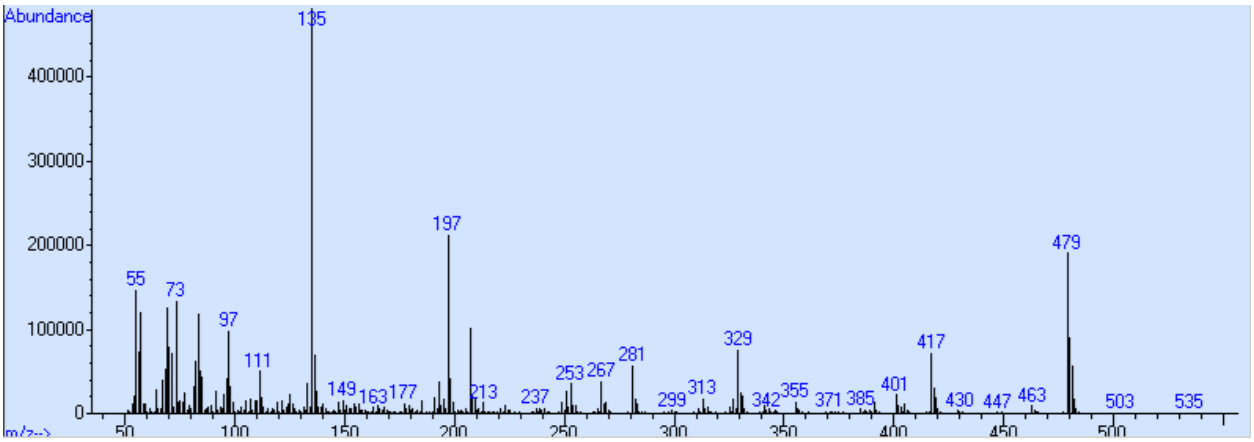
MS Spectrum 105: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover – RT 15.704



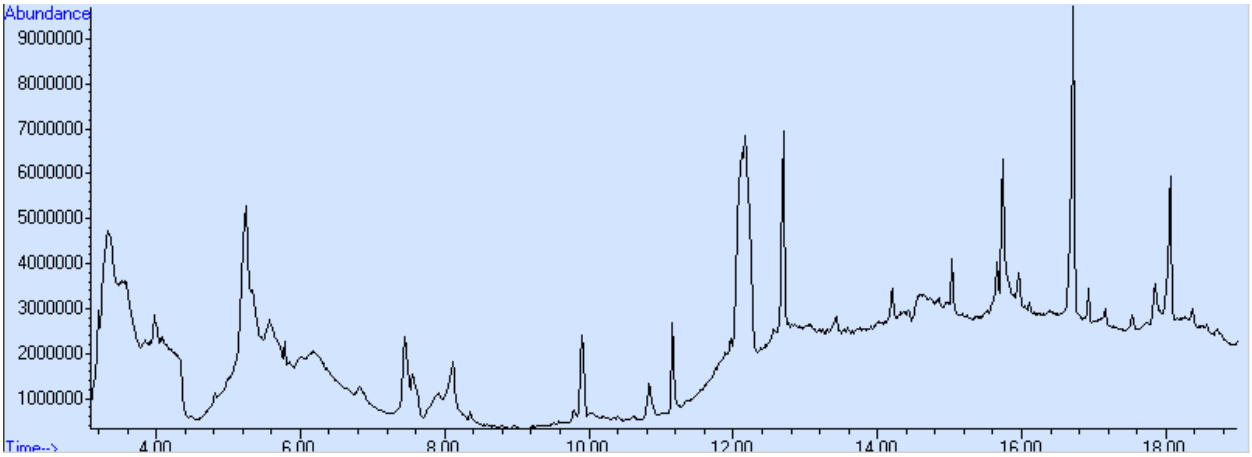
MS Spectrum 106: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover – RT 16.705



MS Spectrum 107: Undyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover – RT 18.038

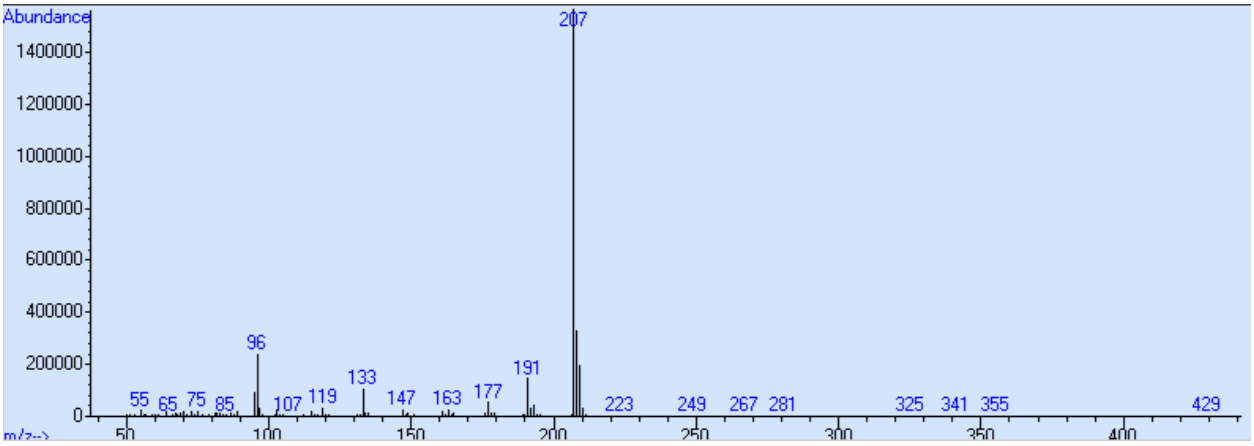


GC Spectrum 17: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover



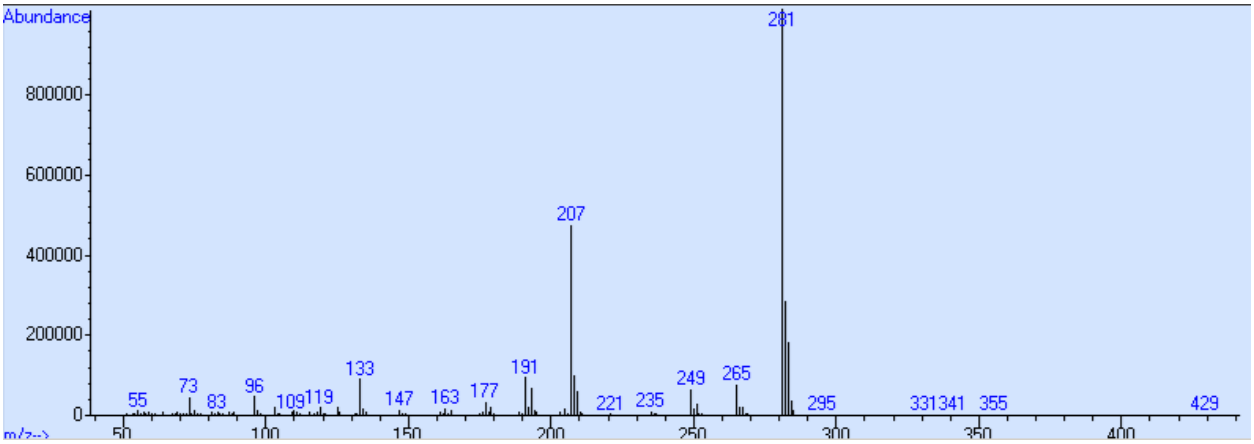
MS Spectrum 108: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 3.487



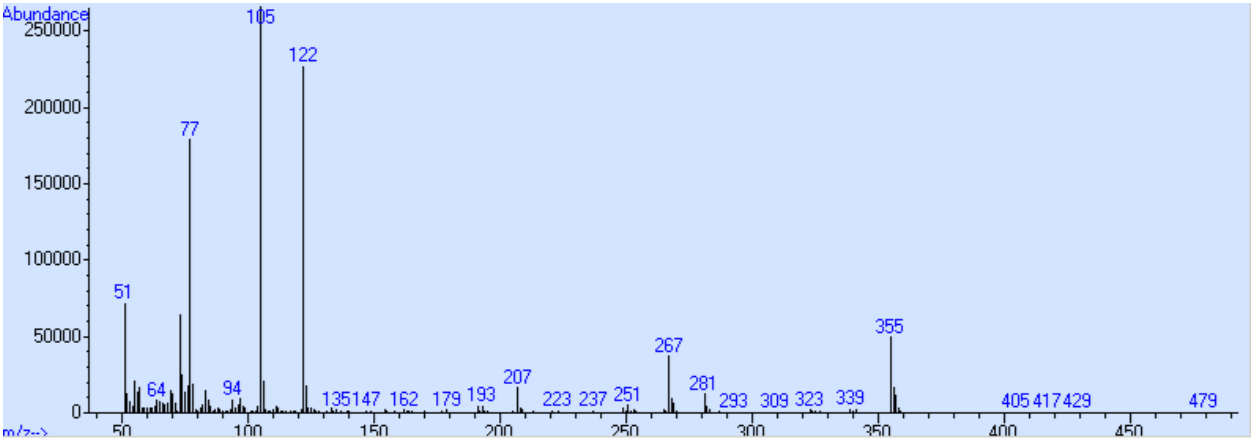
MS Spectrum 109: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 5.313



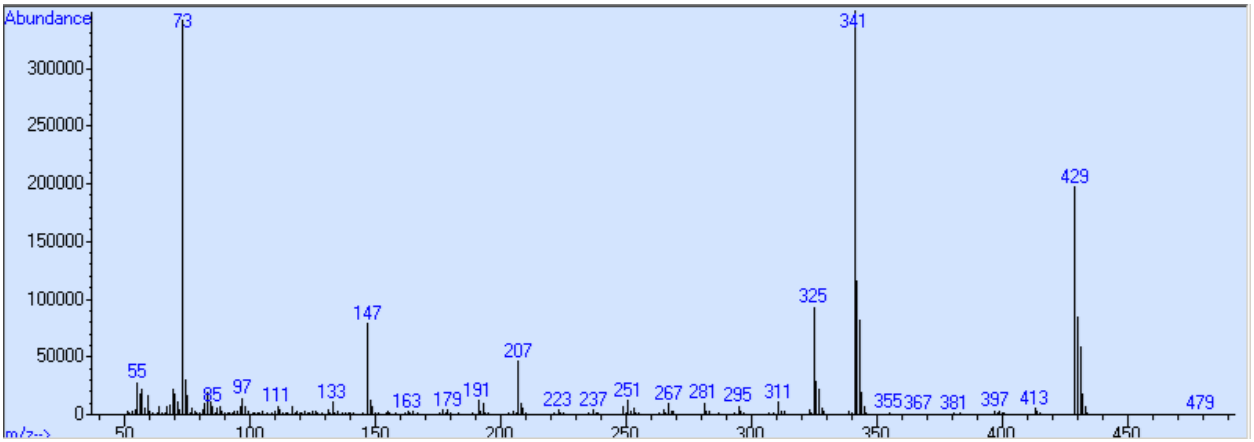
MS Spectrum 110: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 8.082



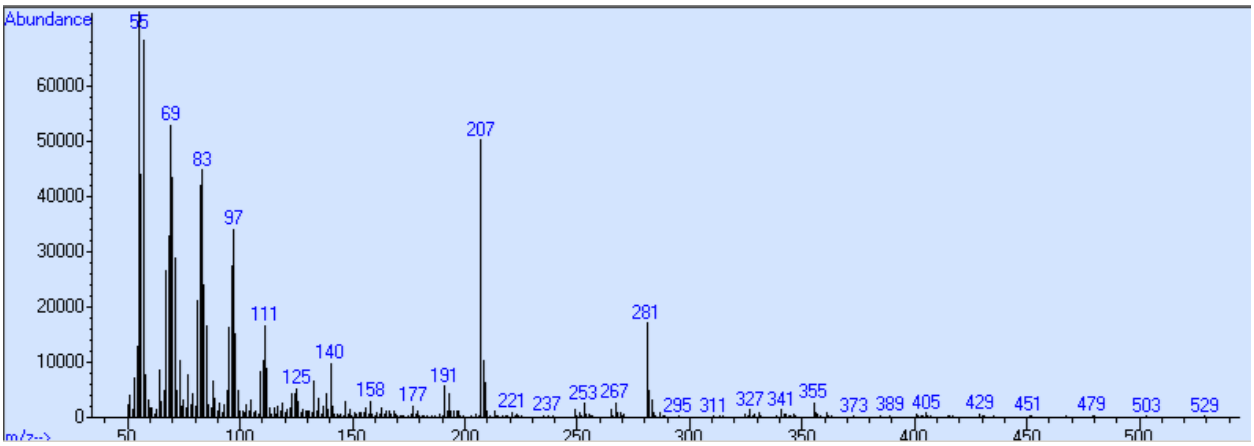
MS Spectrum 111: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 9.913



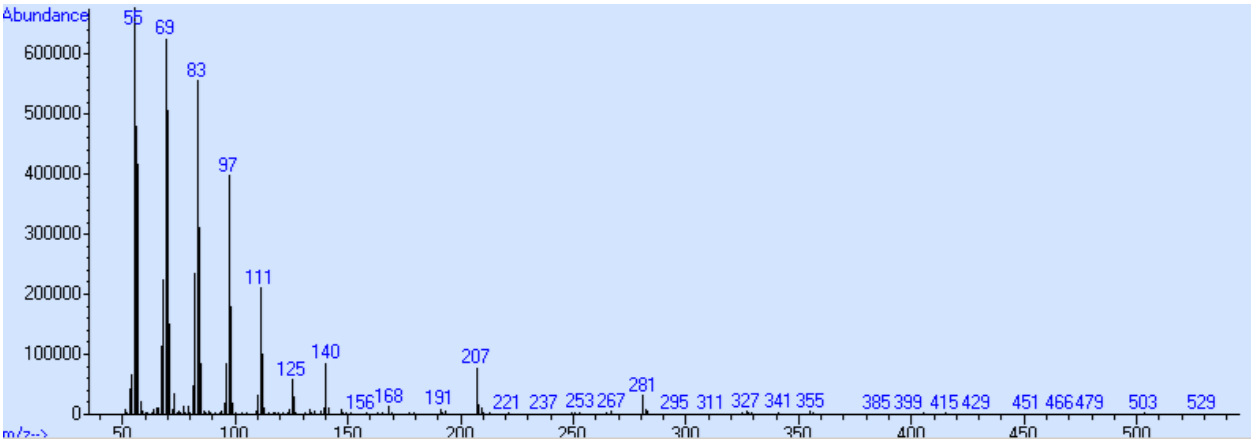
MS Spectrum 112: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 11.195



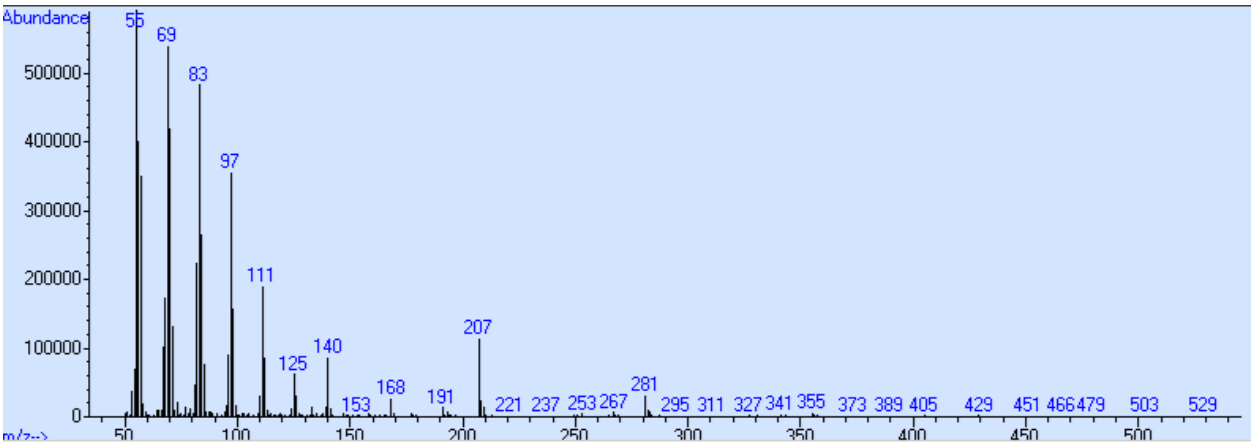
MS Spectrum 113: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 12.110



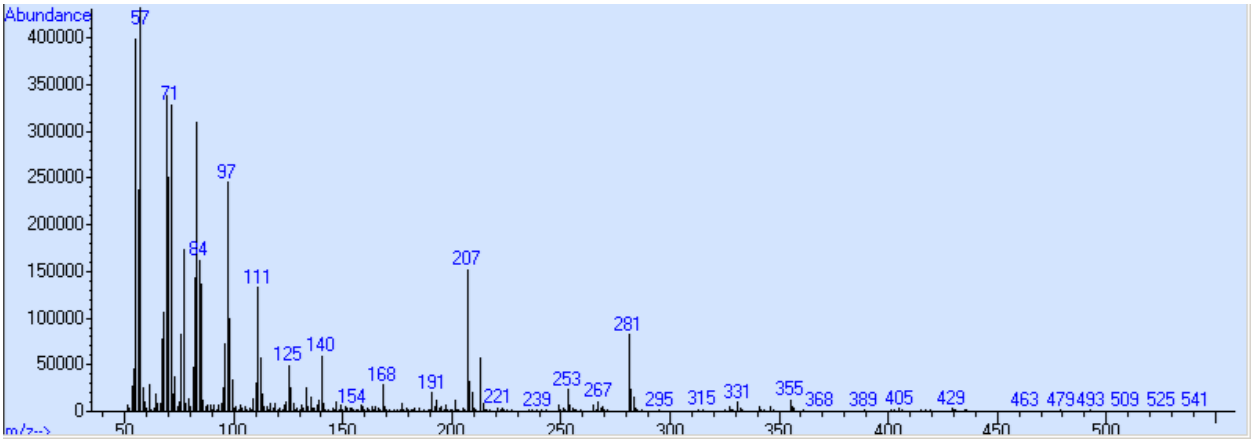
MS Spectrum 114: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 12.677



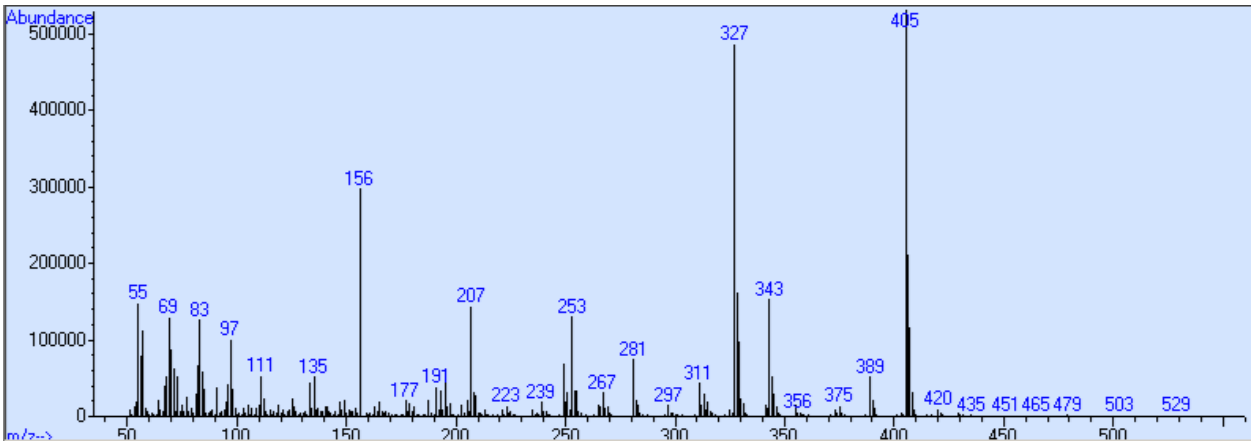
MS Spectrum 115: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 15.749



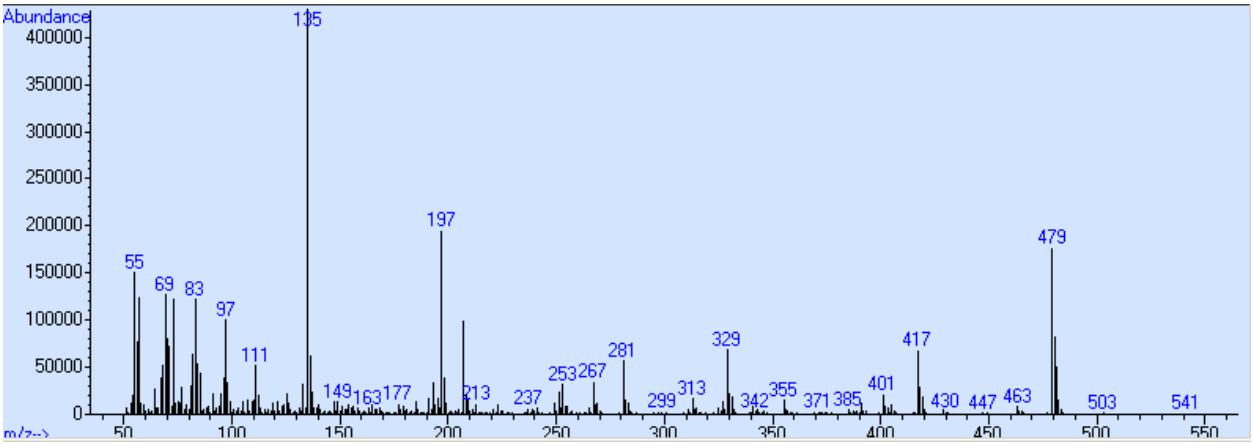
MS Spectrum 116: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 16.688

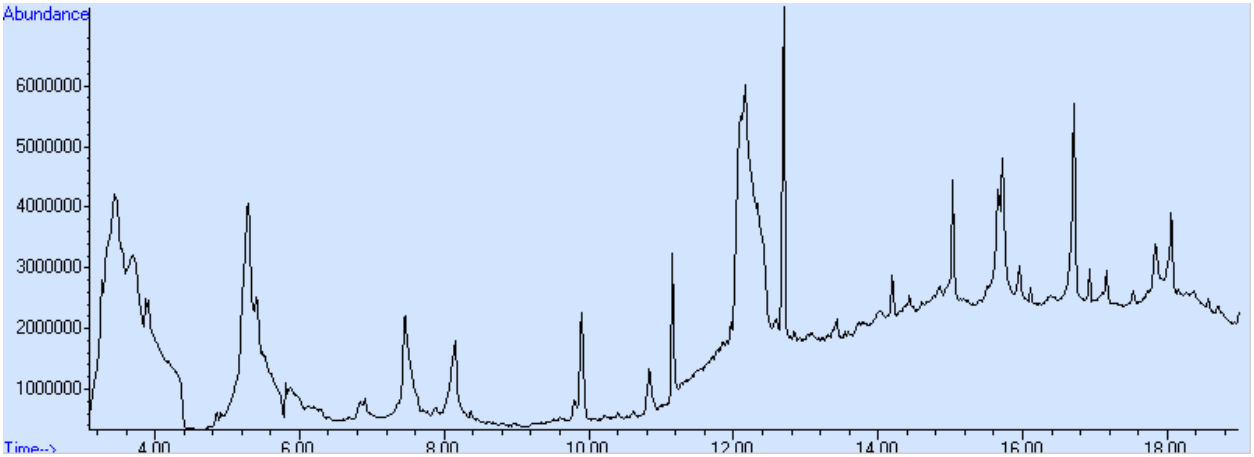


MS Spectrum 117: Dyed Mongoloid Extracted in Colorist Secrets™ Hair Color Remover

RT 18.038

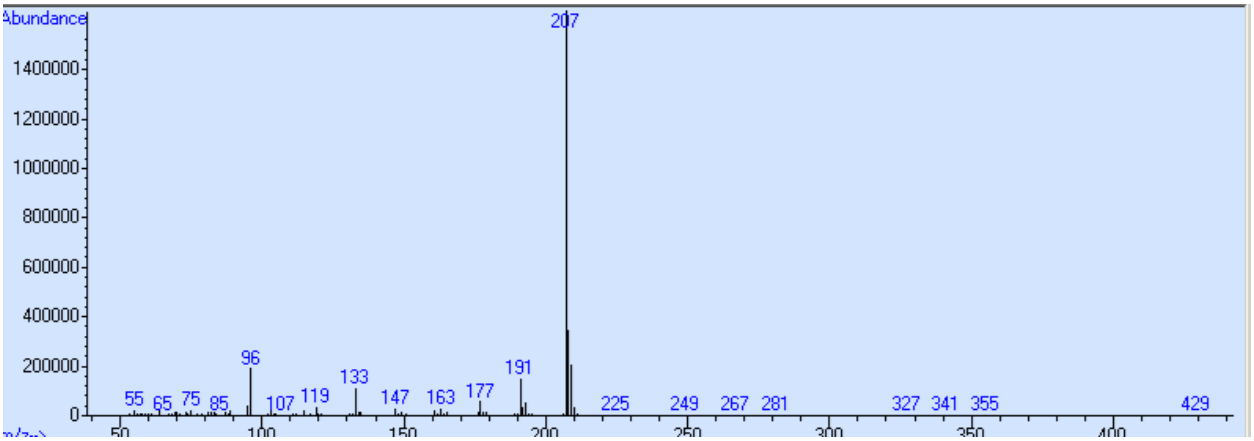


GC Spectrum 18: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover



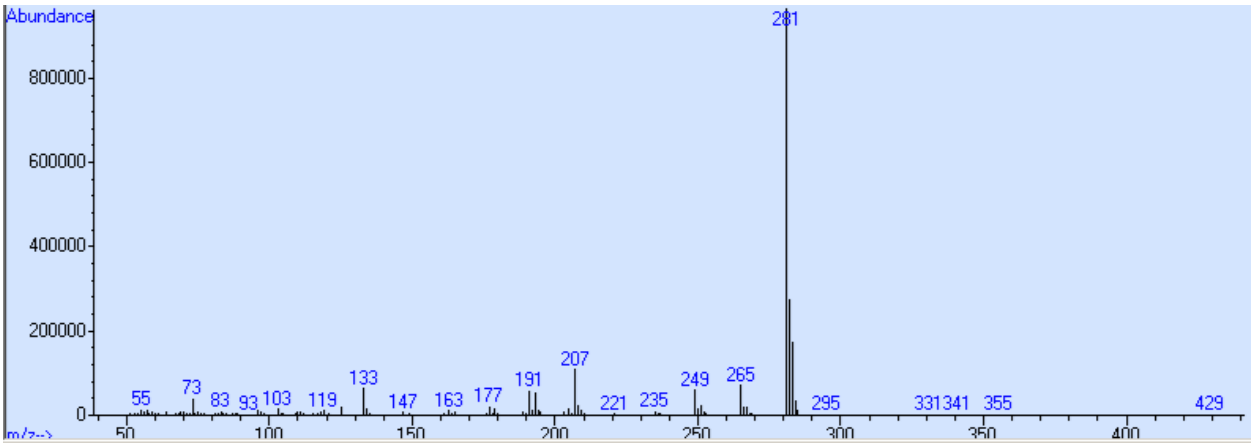
MS Spectrum 118: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 3.505



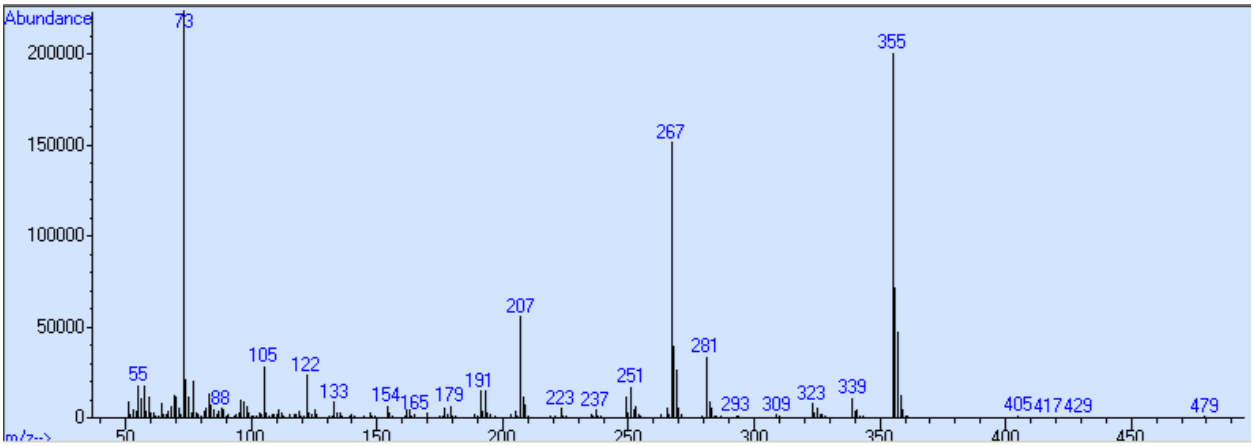
MS Spectrum 119: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 5.336



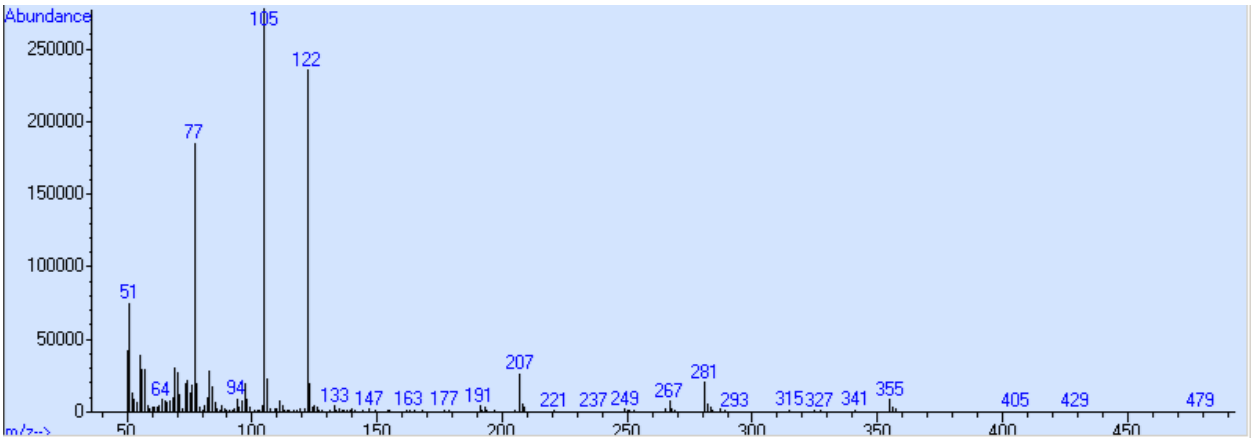
MS Spectrum 120: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 7.516



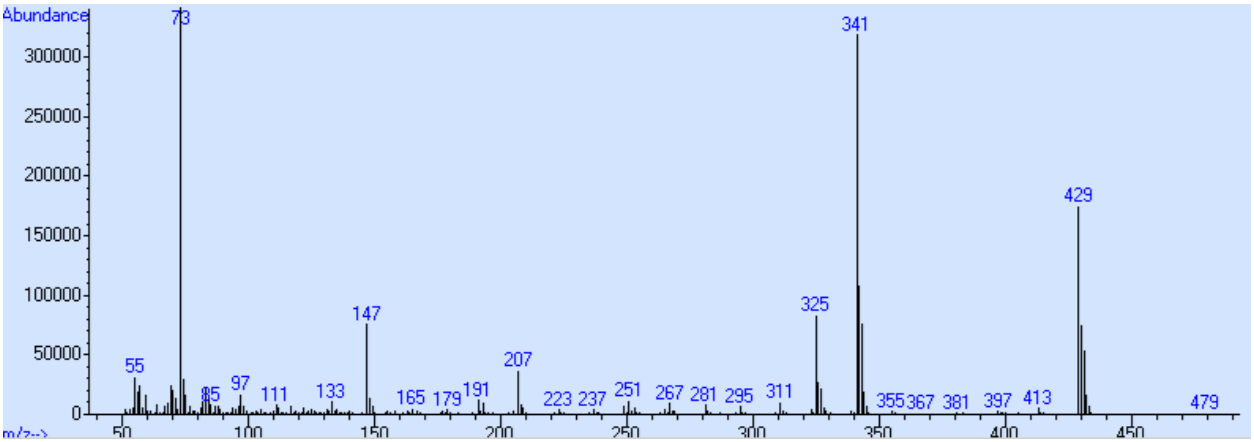
MS Spectrum 121: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 8.122



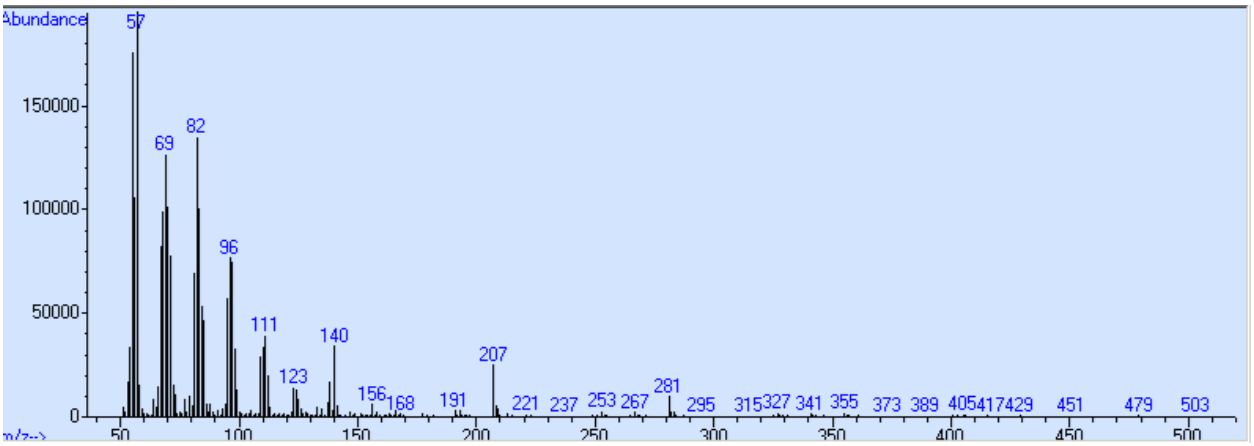
MS Spectrum 122: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 9.890



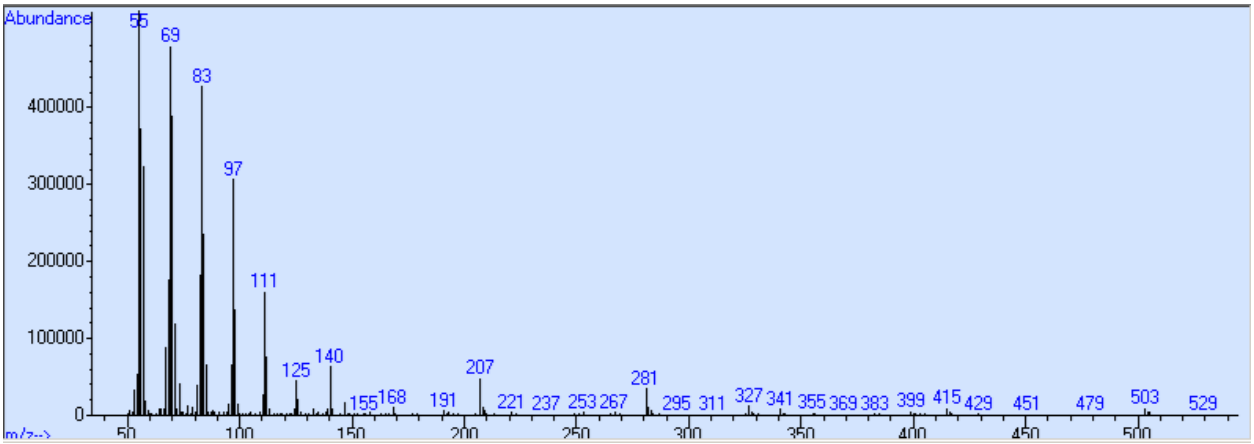
MS Spectrum 123: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 11.172



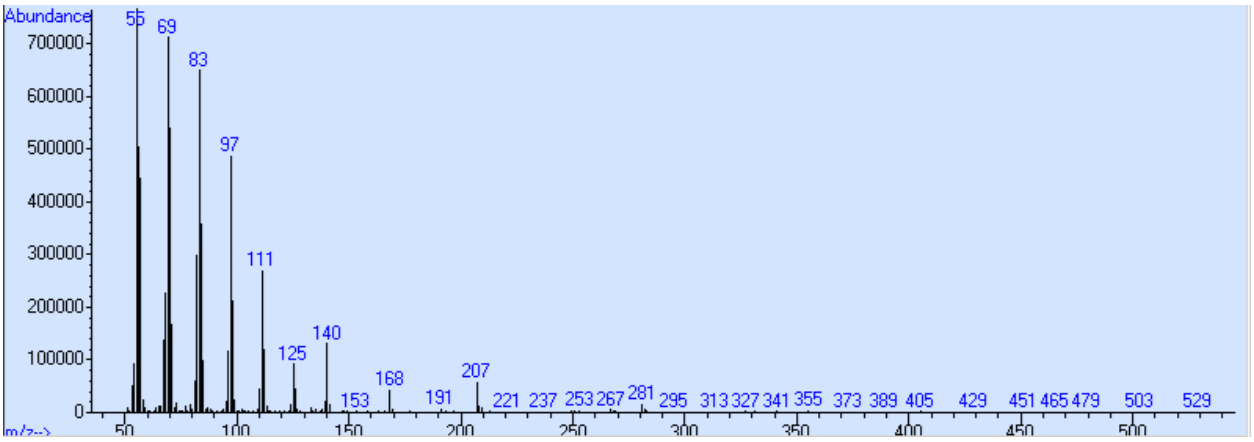
MS Spectrum 124: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 12.196



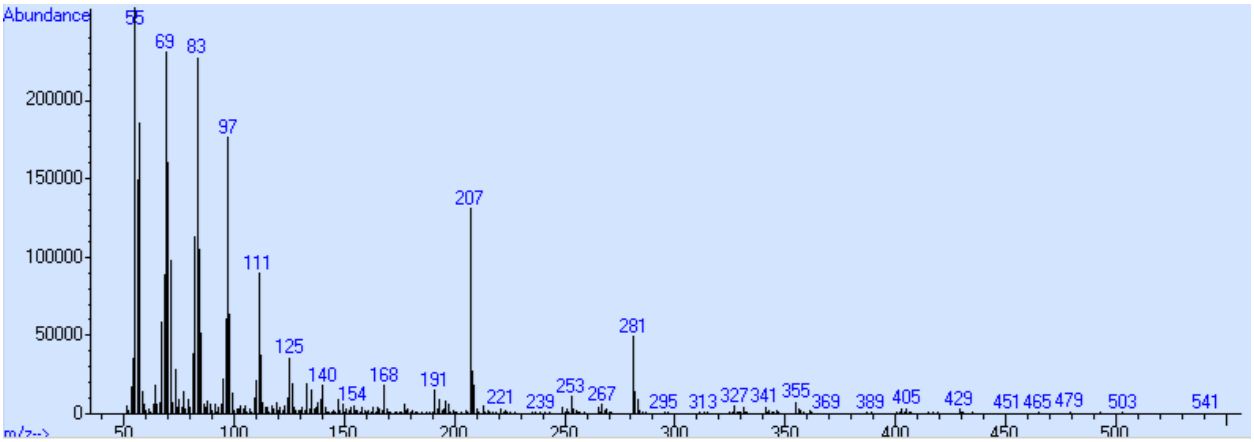
MS Spectrum 125: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 12.700



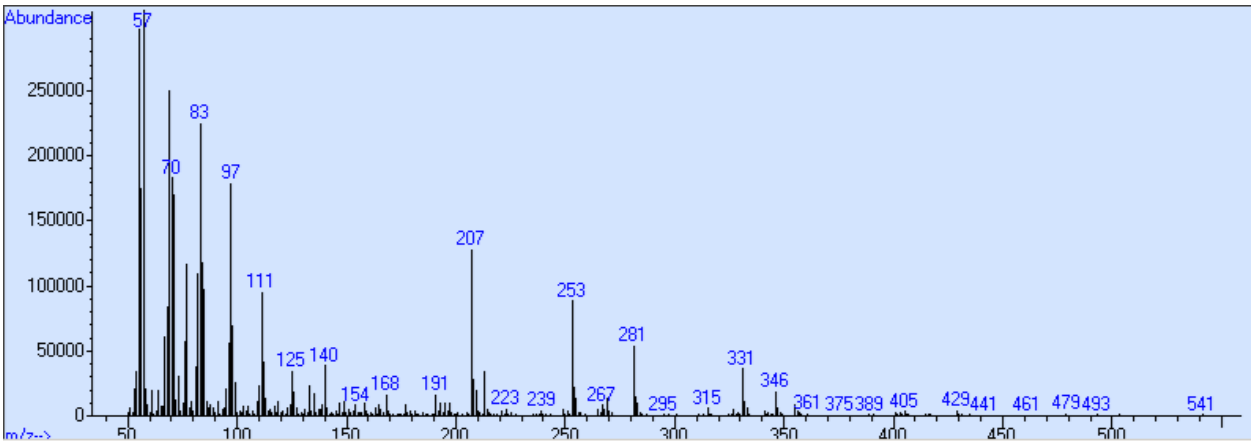
MS Spectrum 126: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 15.052



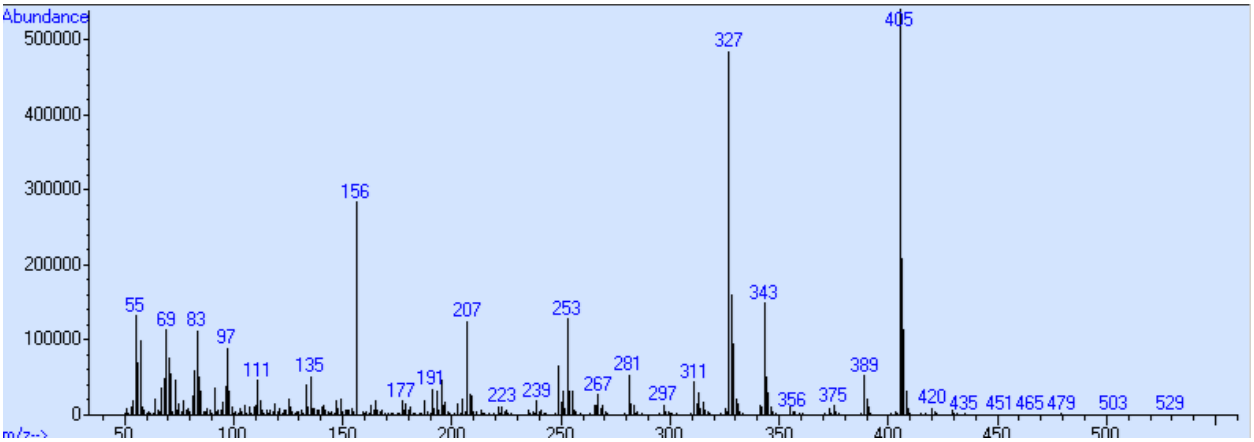
MS Spectrum 127: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 15.704



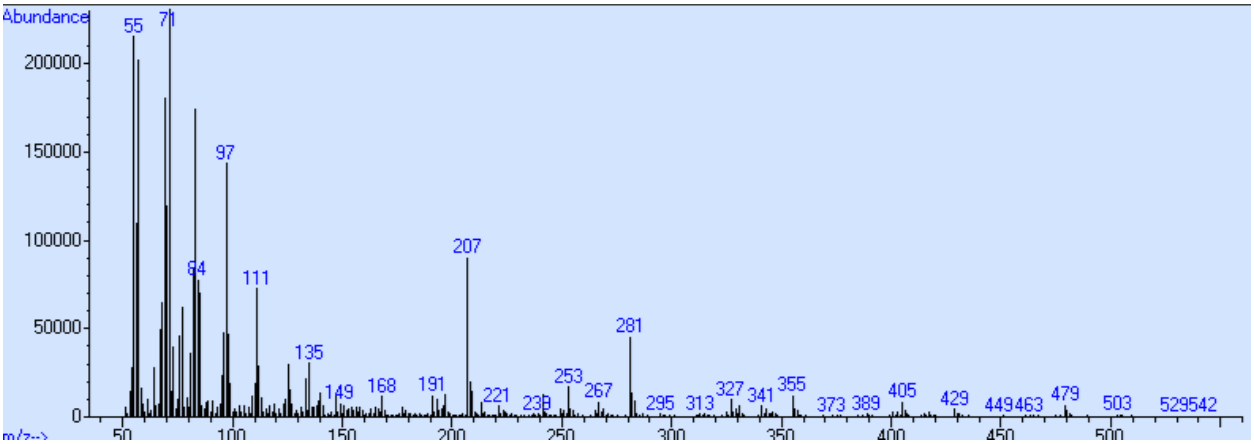
MS Spectrum 128: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 16.705



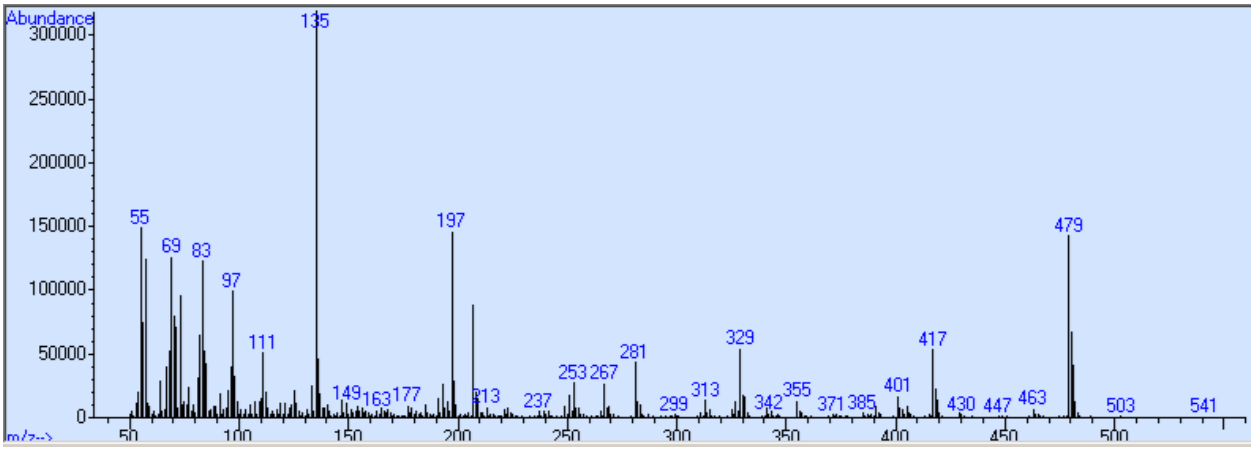
MS Spectrum 129: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

RT 17.838

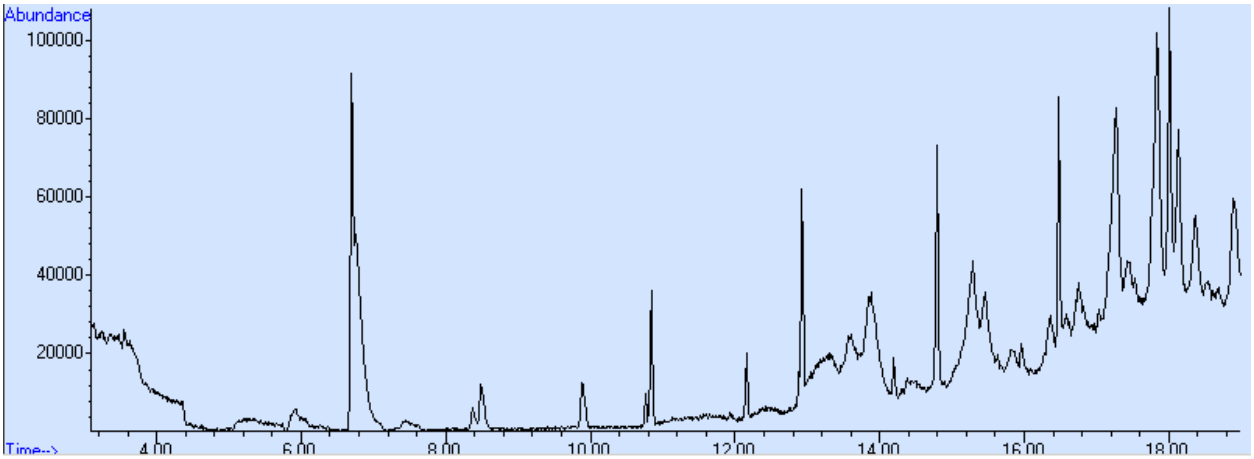


MS Spectrum 130: Undyed Negroid Extracted in Colorist Secrets™ Hair Color Remover

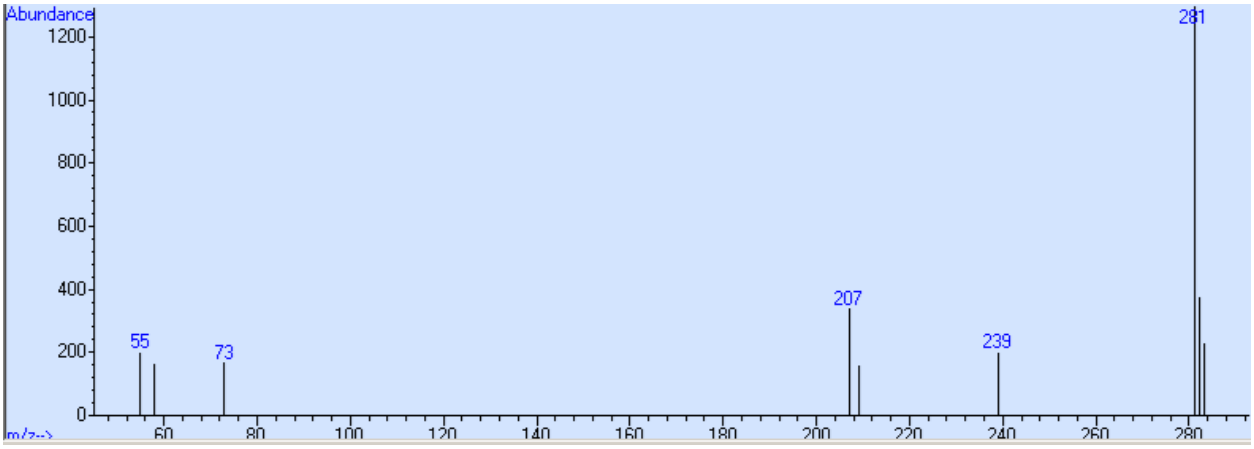
RT 18.056



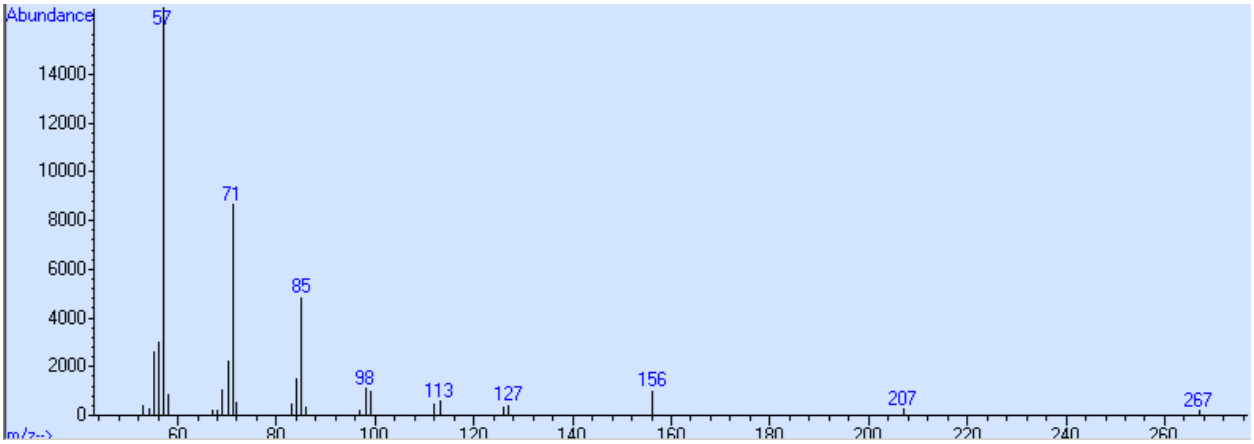
GC Spectrum 19: Solvent Methanol



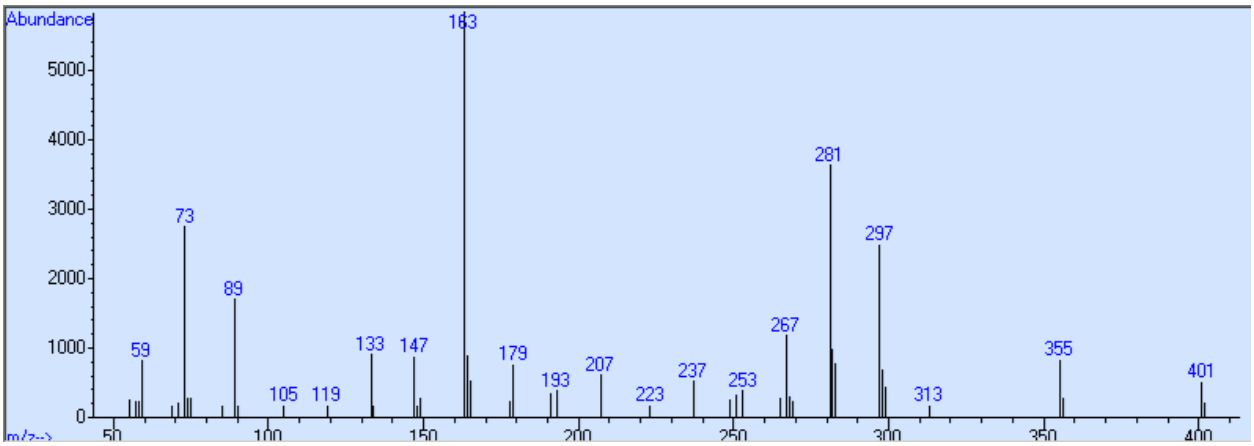
MS Spectrum 131: Solvent Methanol – RT 5.227



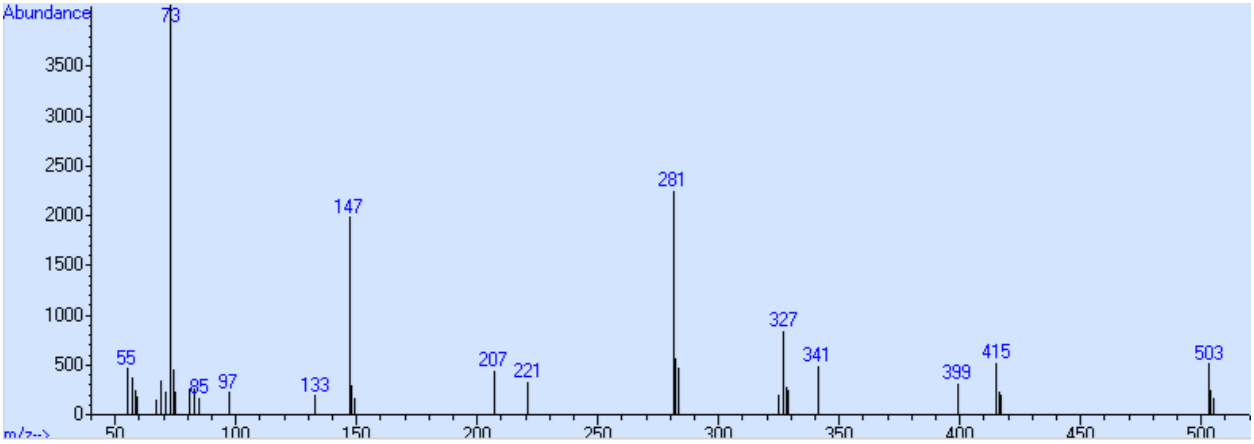
MS Spectrum 132: Solvent Methanol – RT 6.755



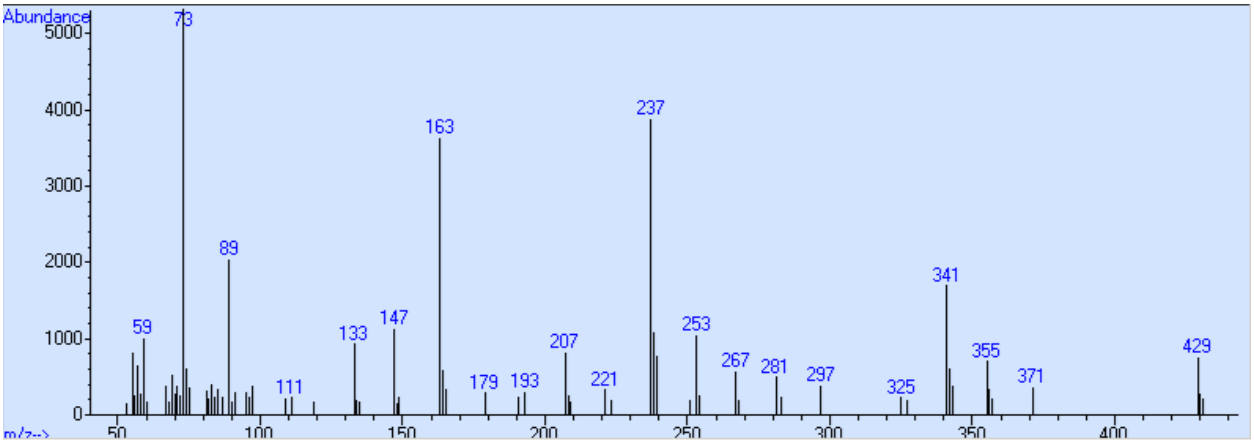
MS Spectrum 133: Solvent Methanol – RT 10.846



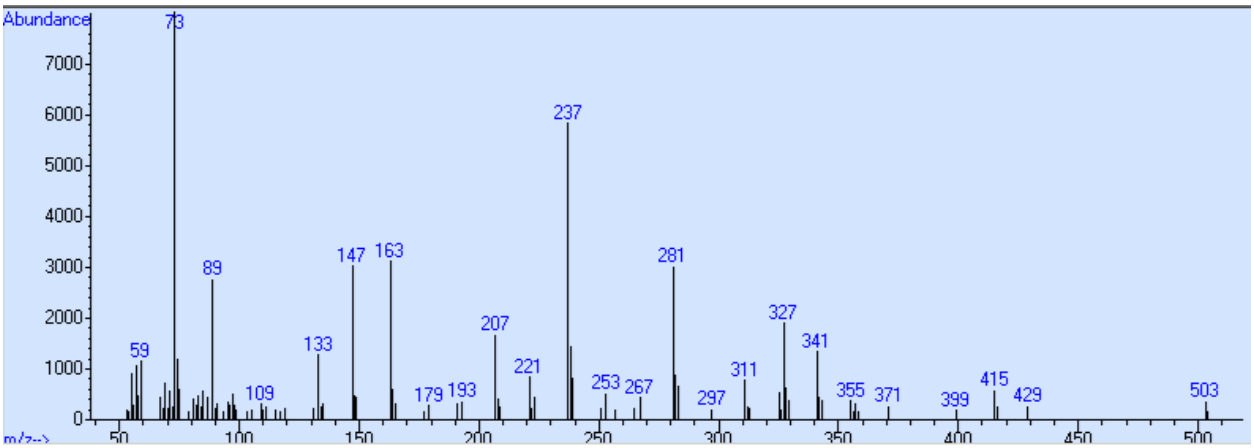
MS Spectrum 134: Solvent Methanol – RT 12.156



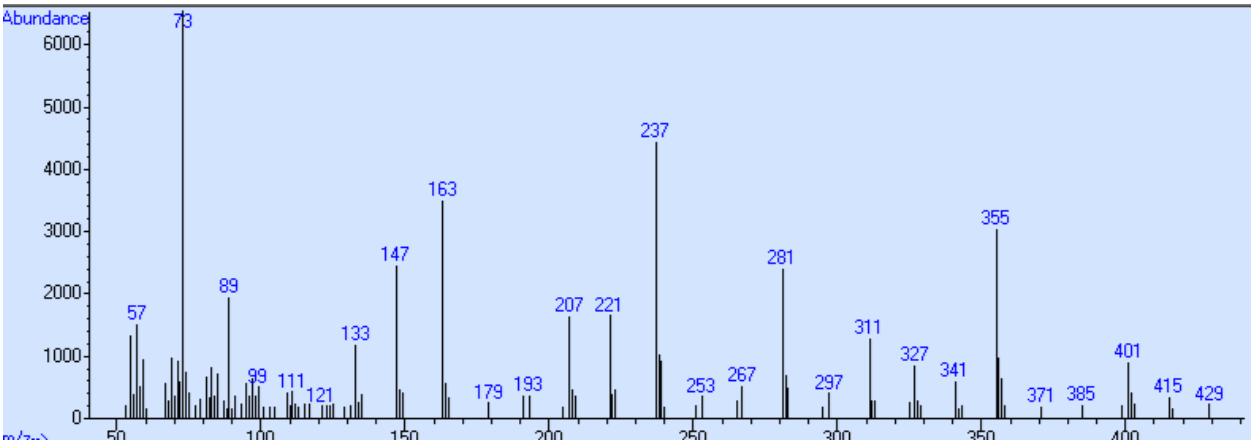
MS Spectrum 135: Solvent Methanol – RT 12.917



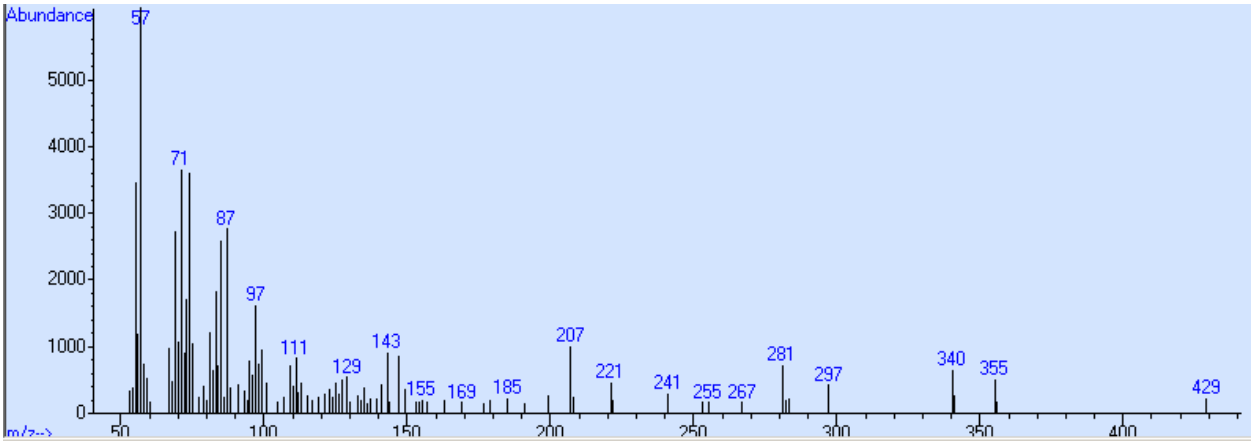
MS Spectrum 136: Solvent Methanol – RT 14.788



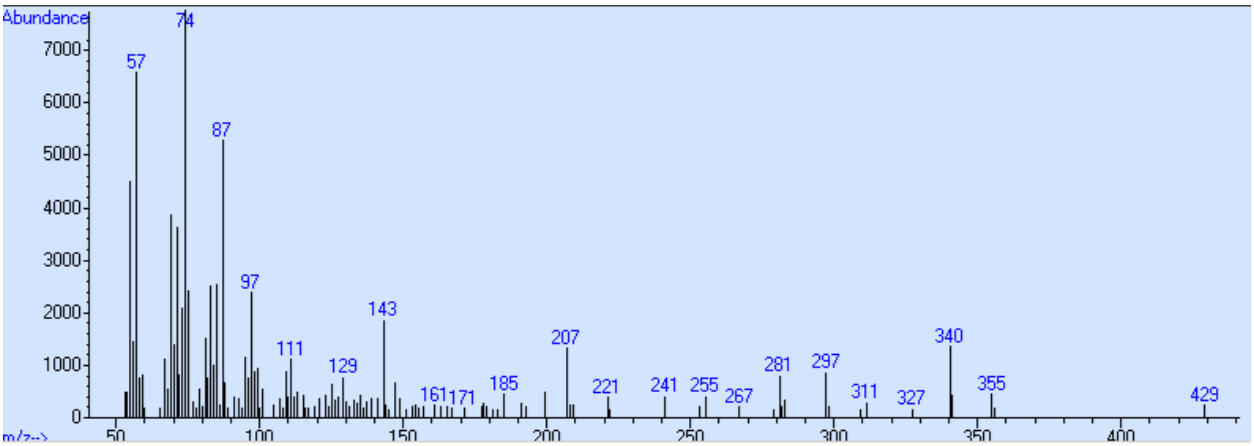
MS Spectrum 137: Solvent Methanol – RT 16.488



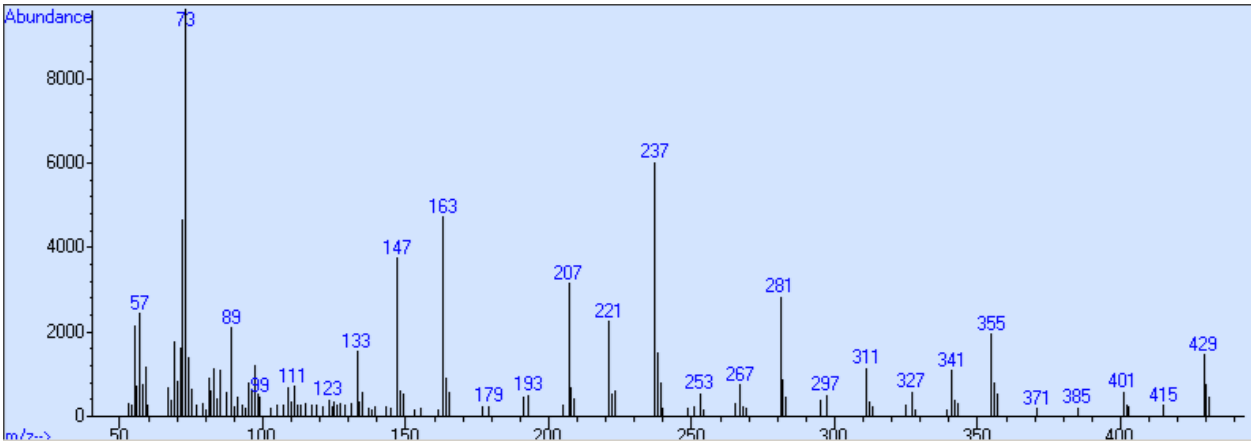
MS Spectrum 138: Solvent Methanol – RT 17.232



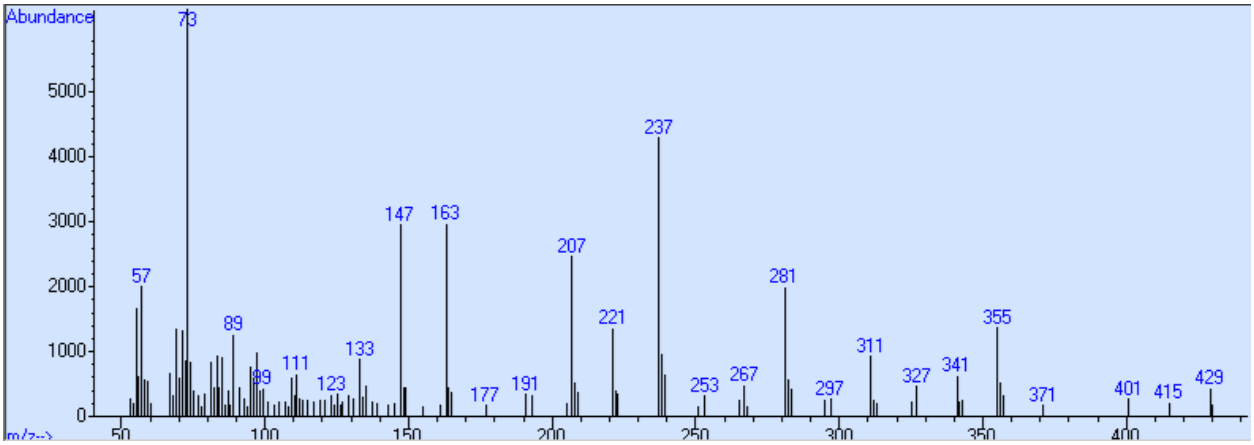
MS Spectrum 139: Solvent Methanol – RT 17.821



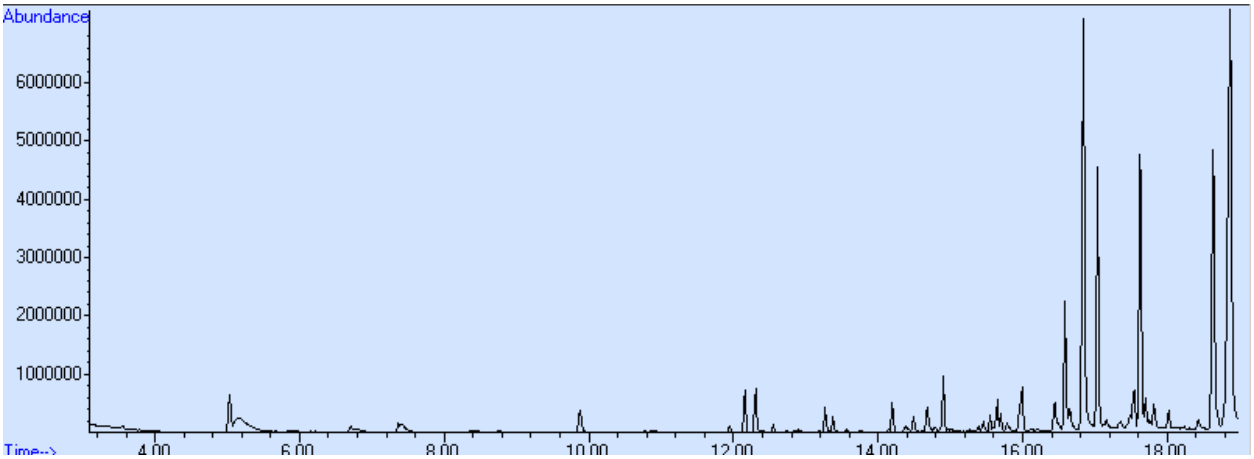
MS Spectrum 140: Solvent Methanol – RT 18.016



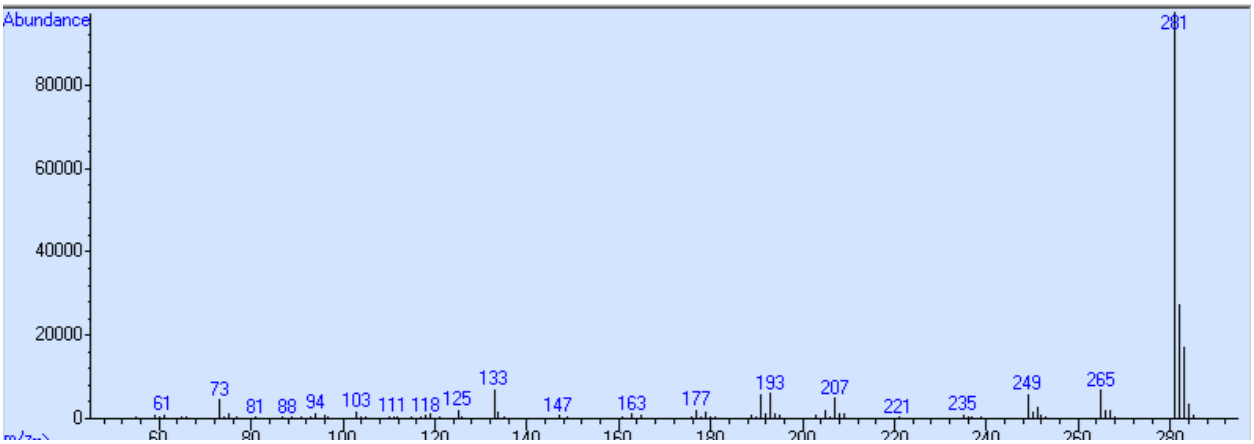
MS Spectrum 141: Solvent Methanol – RT 18.147



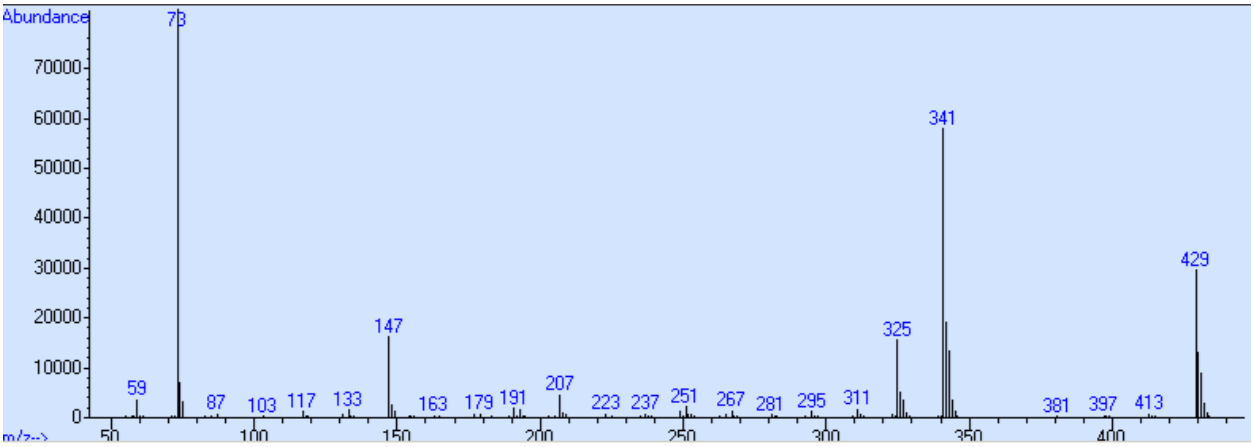
GC Spectrum 20: Remy XQ Extracted in Methanol



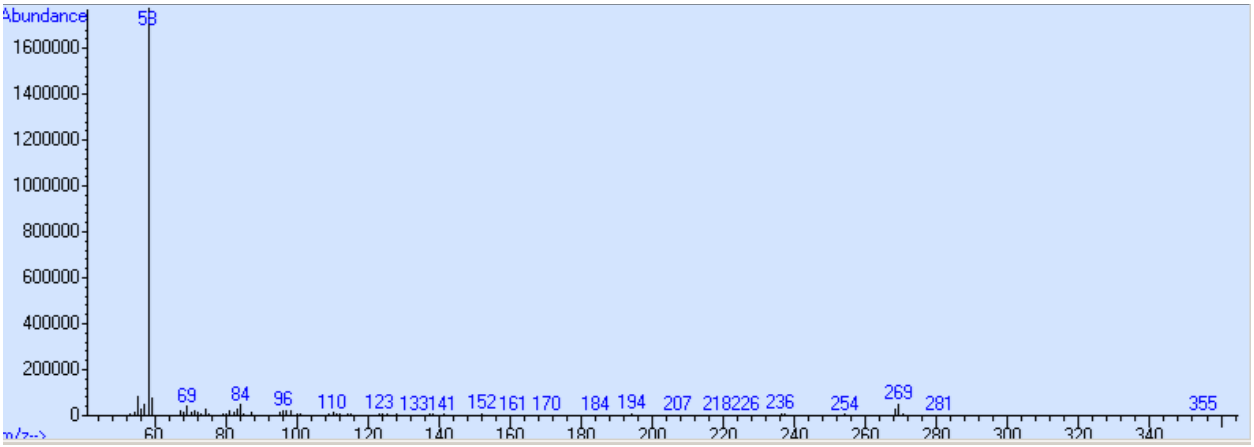
MS Spectrum 142: Remy XQ Extracted in Methanol – RT 5.181



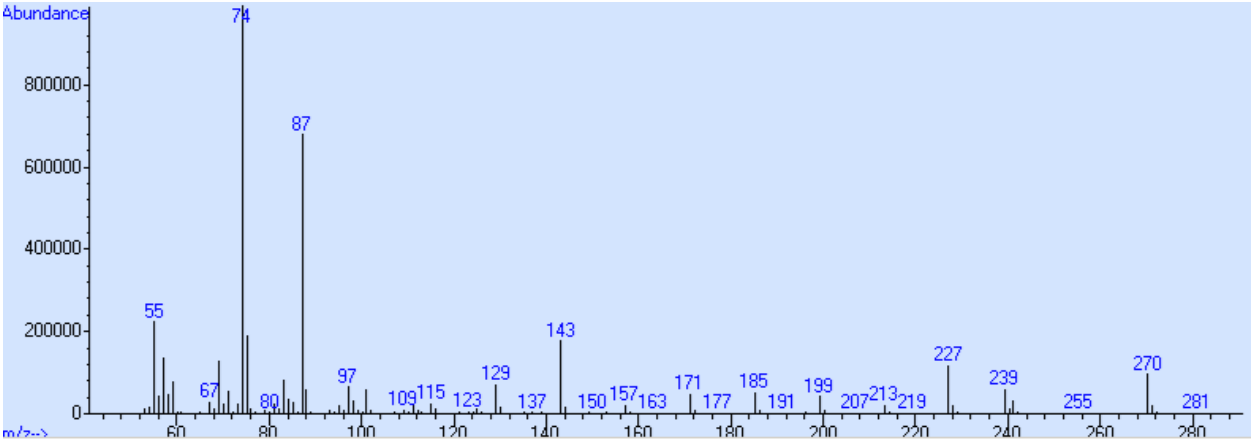
MS Spectrum 143: Remy XQ Extracted in Methanol – RT 9.867



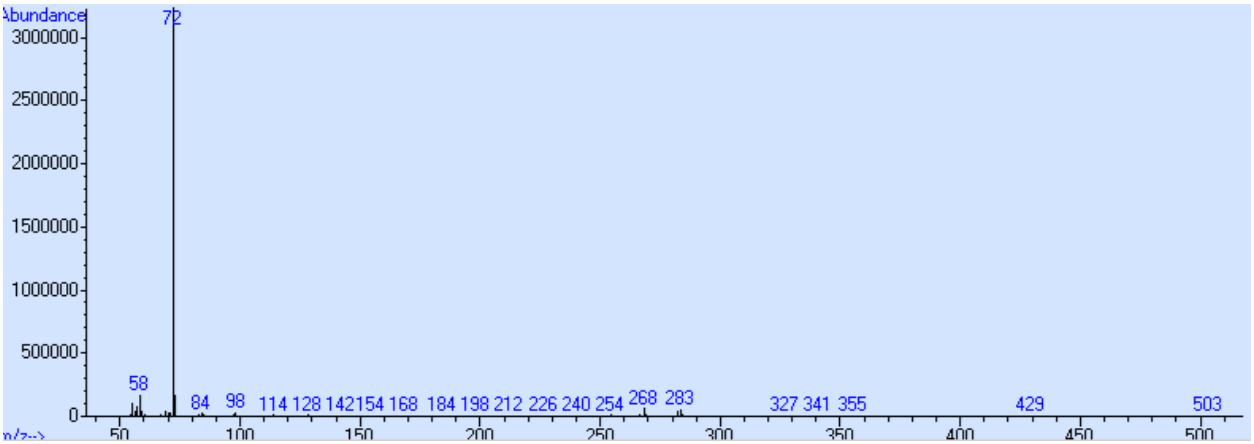
MS Spectrum 144: Remy XQ Extracted in Methanol – RT 16.860



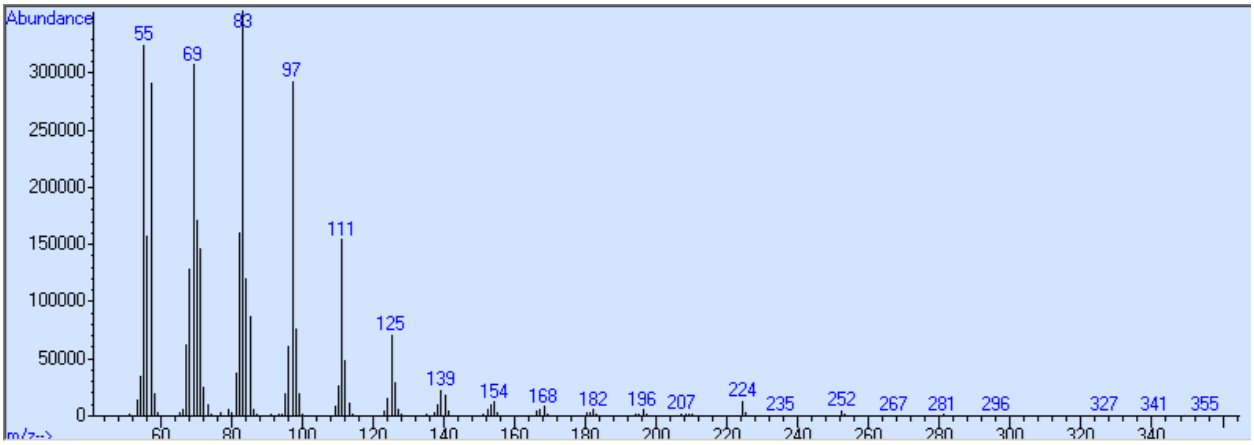
MS Spectrum 145: Remy XQ Extracted in Methanol – RT 17.031



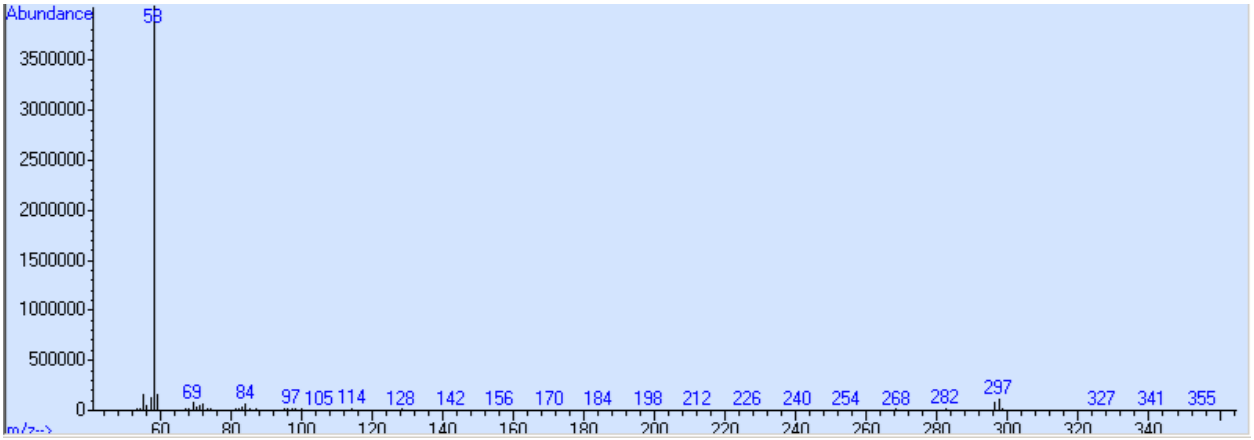
MS Spectrum 146: Remy XQ Extracted in Methanol – RT 17.621



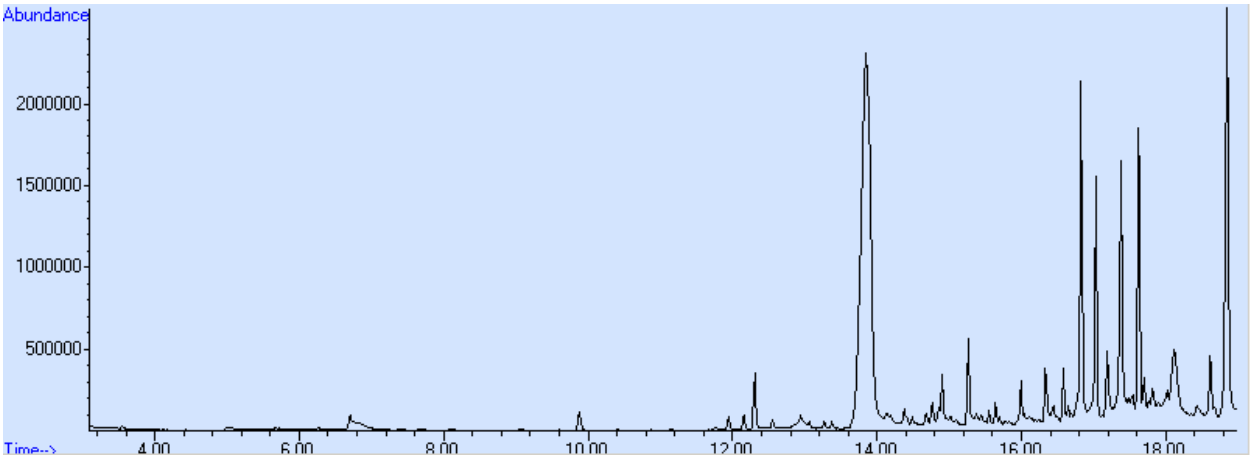
MS Spectrum 147: Remy XQ Extracted in Methanol – RT 18.622



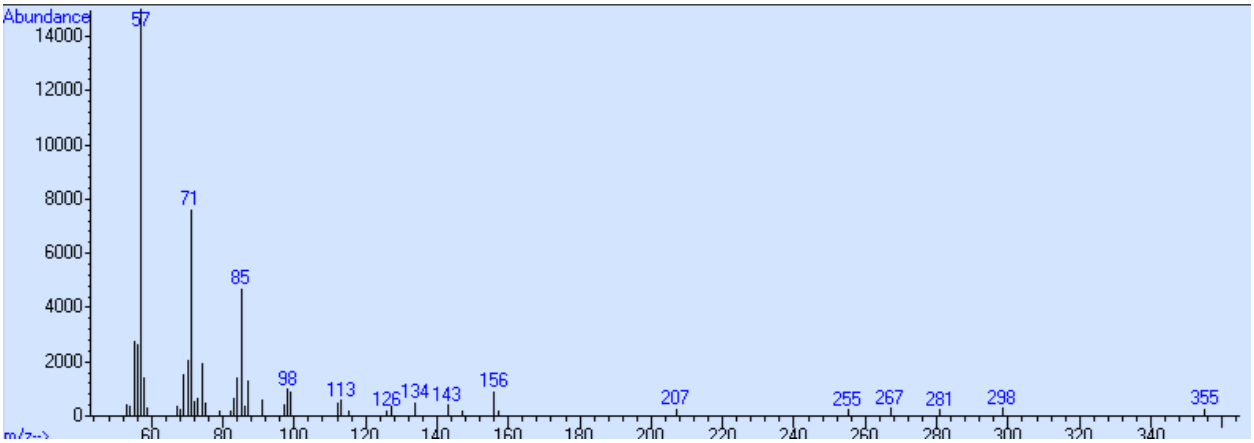
MS Spectrum 148: Remy XQ Extracted in Methanol – RT 18.885



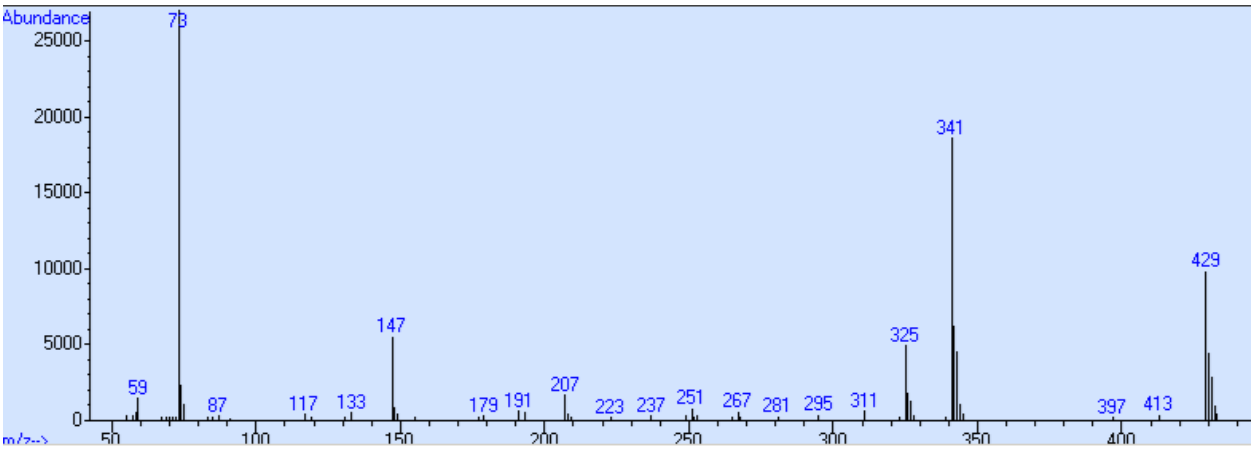
GC Spectrum 21: ECW Extracted in Methanol



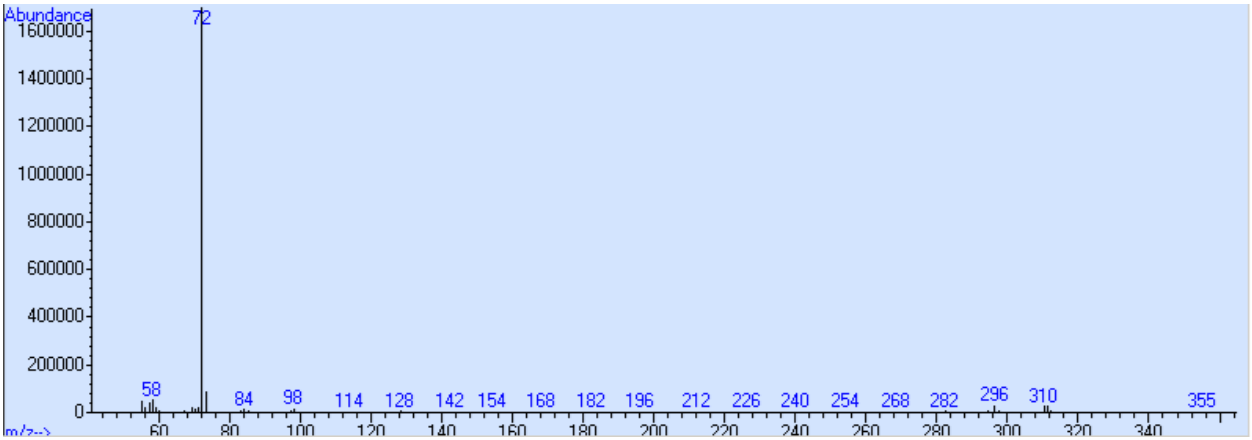
MS Spectrum 149: ECW Extracted in Methanol – RT 6.732



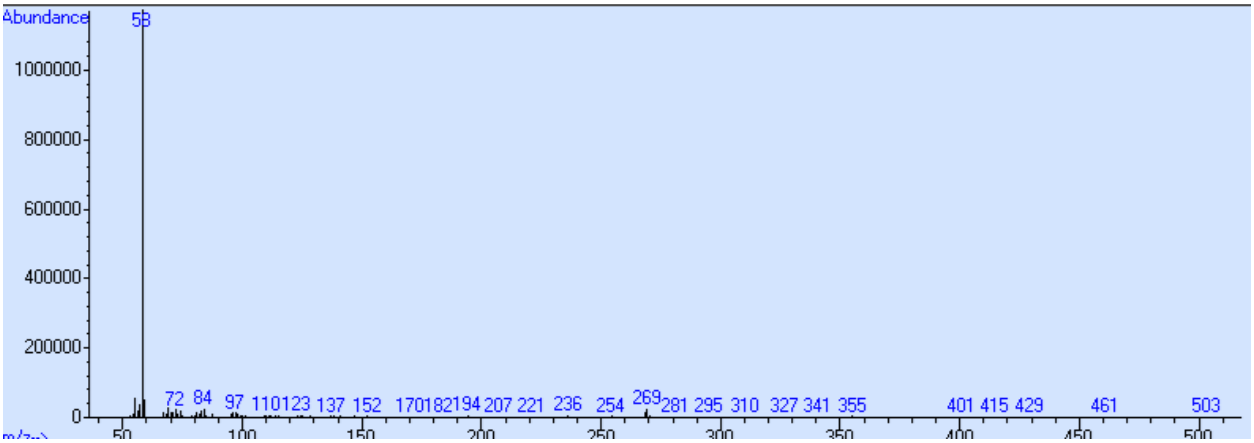
MS Spectrum 150: ECW Extracted in Methanol – RT 9.867



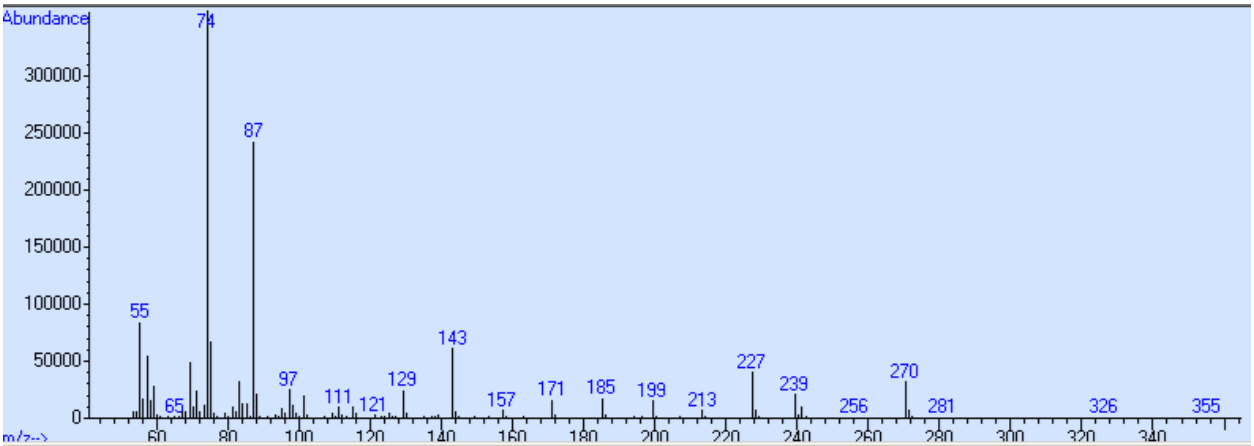
MS Spectrum 151: ECW Extracted in Methanol – RT 13.856



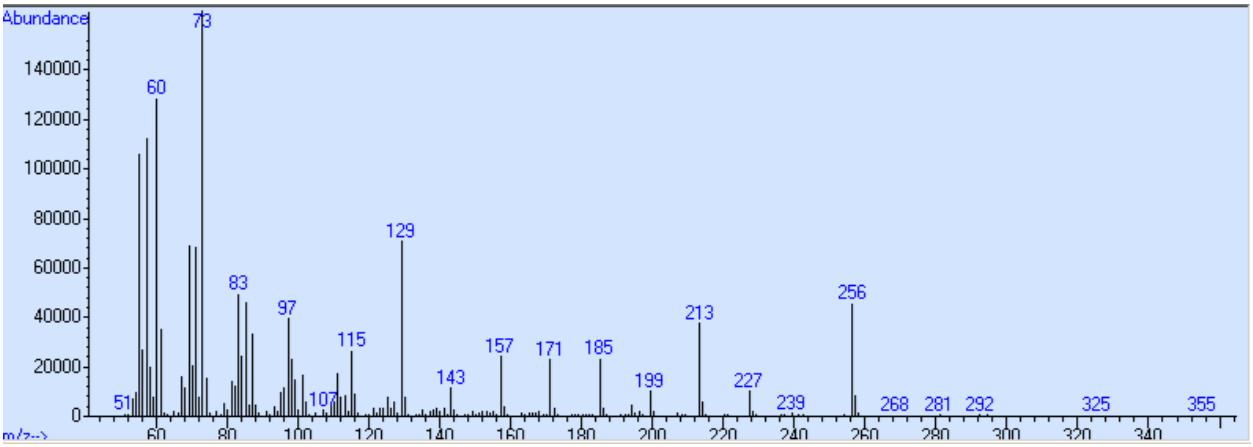
MS Spectrum 152: ECW Extracted in Methanol – RT 16.837



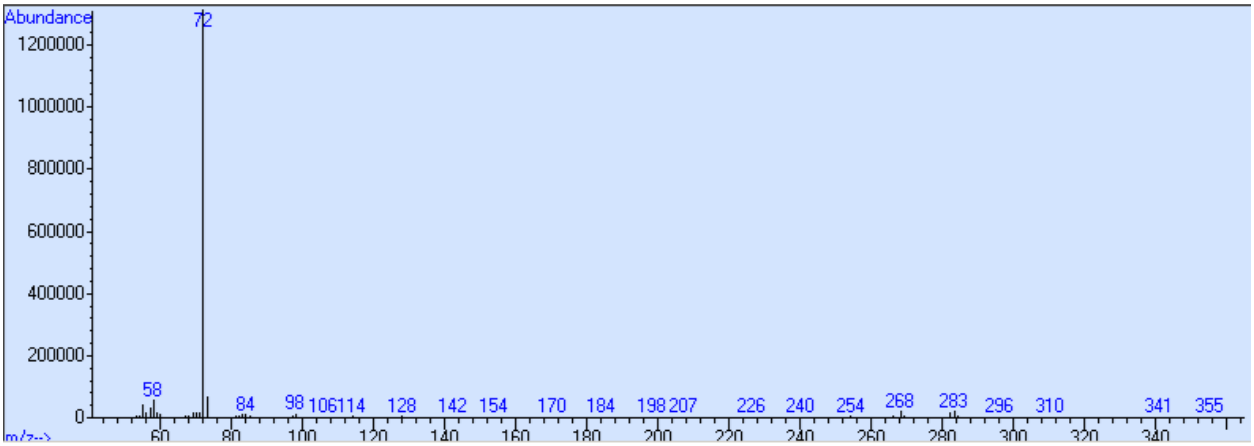
MS Spectrum 153: ECW Extracted in Methanol – RT 17.031



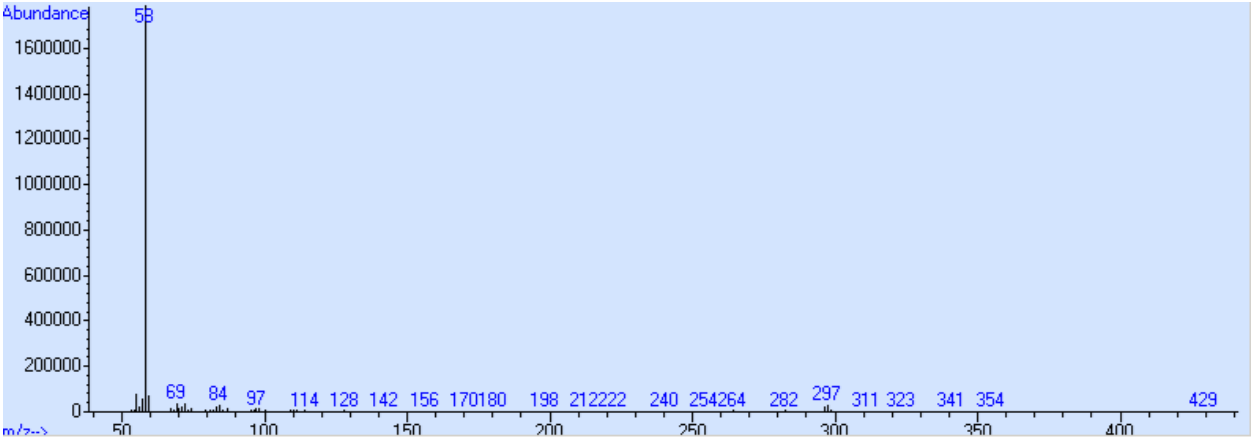
MS Spectrum 154: ECW Extracted in Methanol – RT 17.380



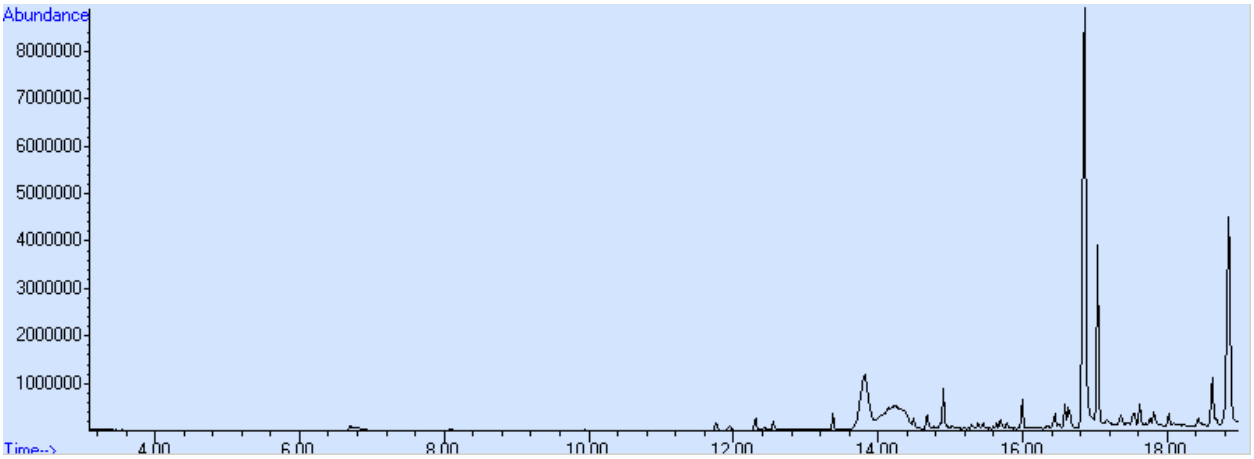
MS Spectrum 155: ECW Extracted in Methanol – RT 17.621



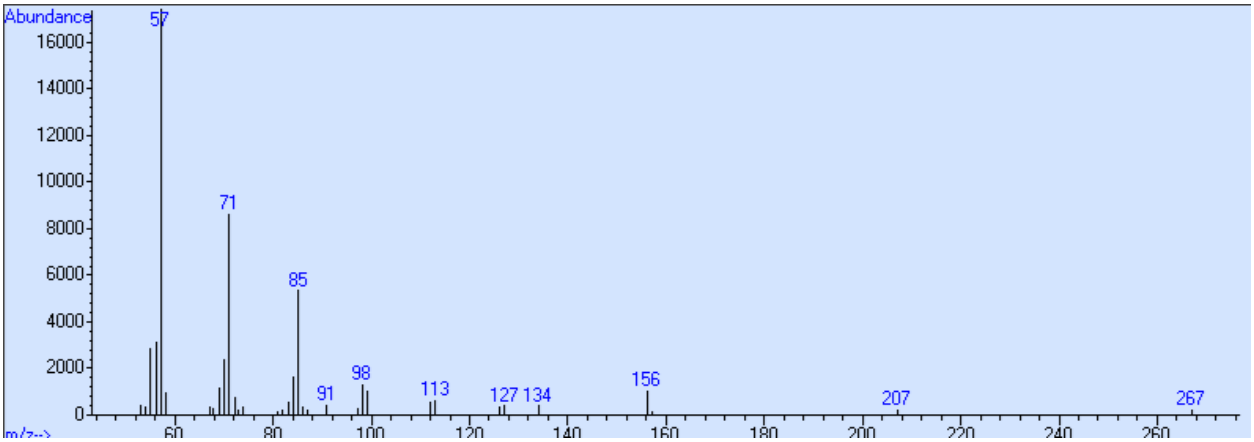
MS Spectrum 156: ECW Extracted in Methanol – RT 18.845



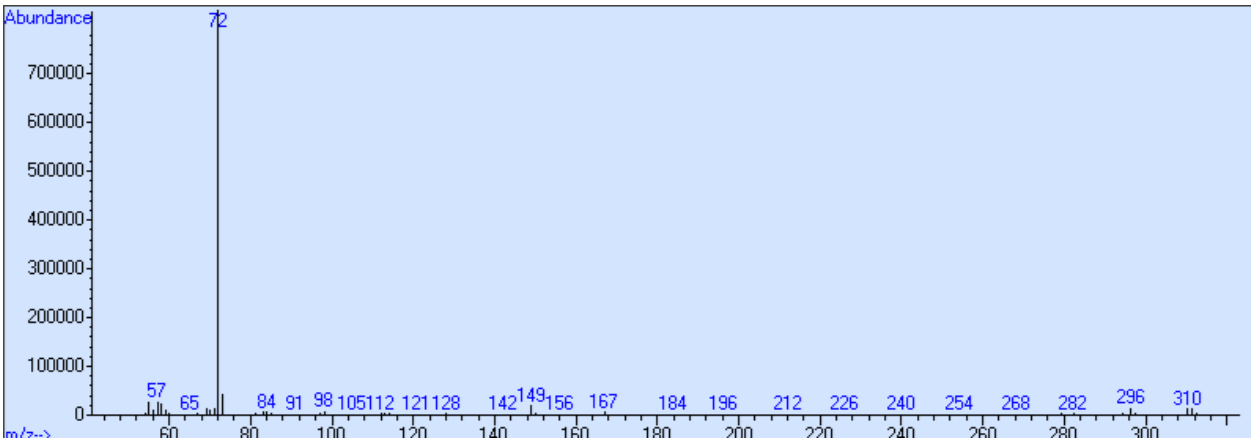
GC Spectrum 22: Que Extracted in Methanol



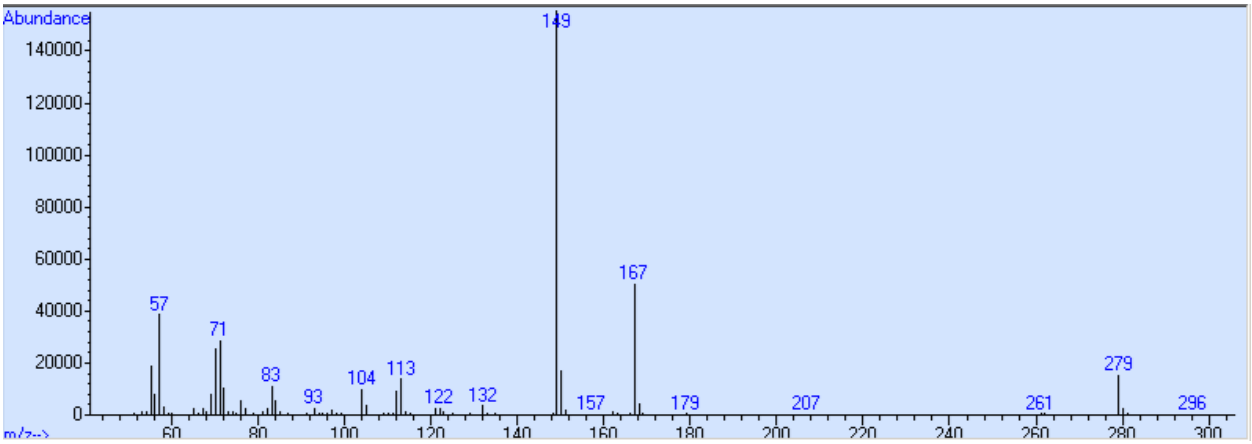
MS Spectrum 157: Que Extracted in Methanol – RT 6.732



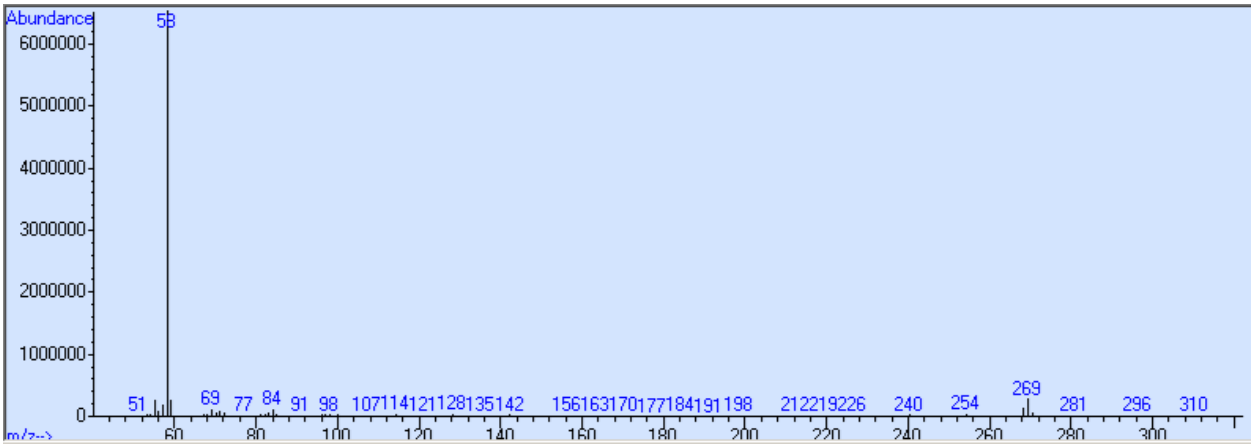
MS Spectrum 158: Que Extracted in Methanol – RT 13.810



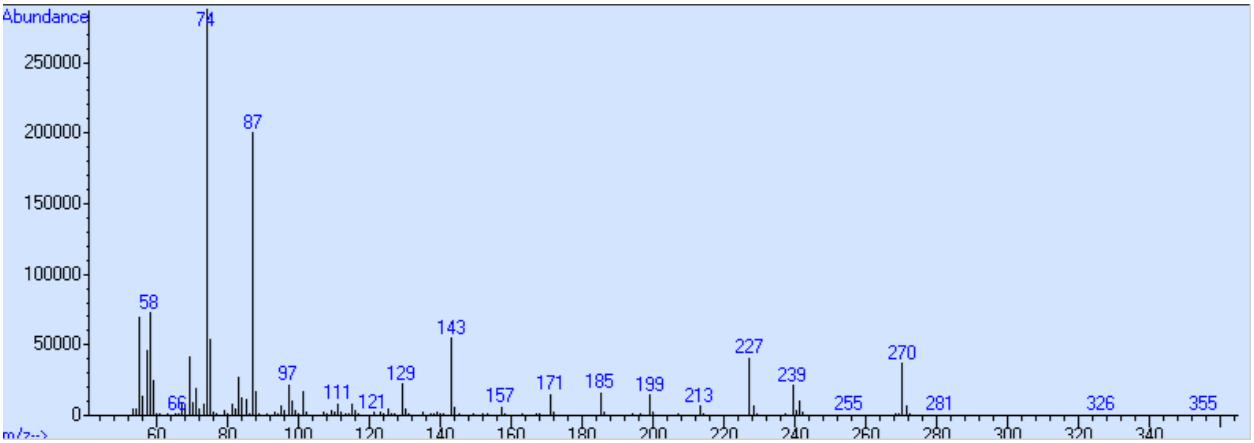
MS Spectrum 159: Que Extracted in Methanol – RT 14.268



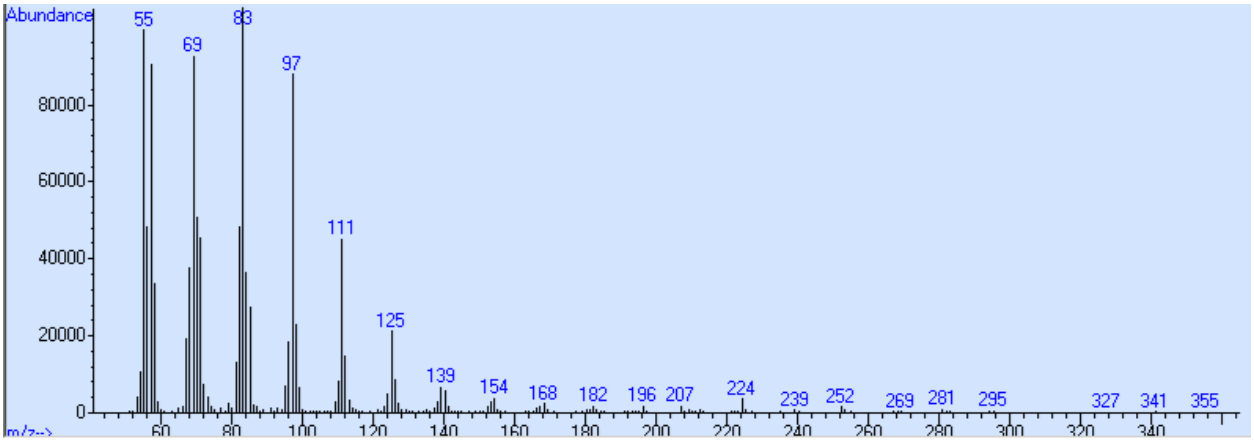
MS Spectrum 160: Que Extracted in Methanol – RT 16.860



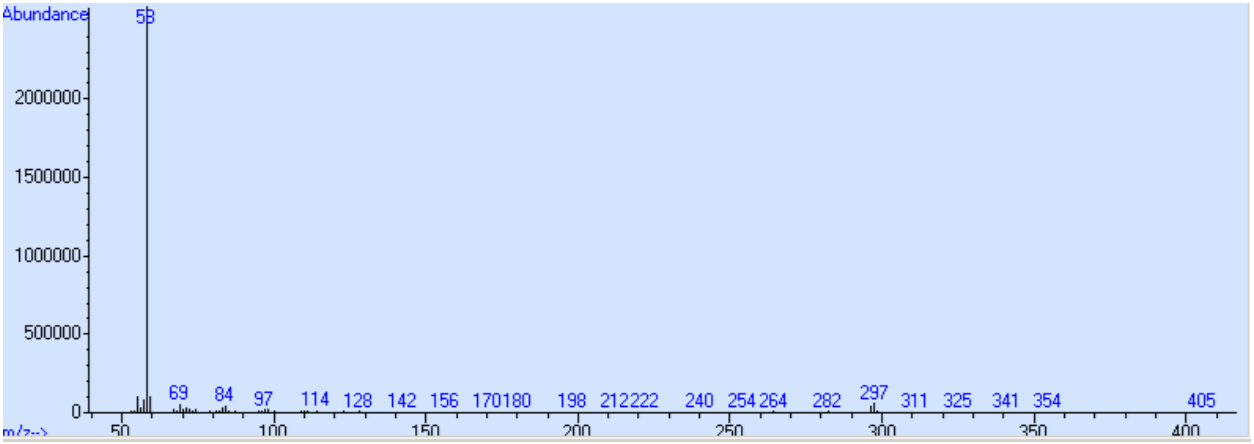
MS Spectrum 161: Que Extracted in Methanol – RT 17.054



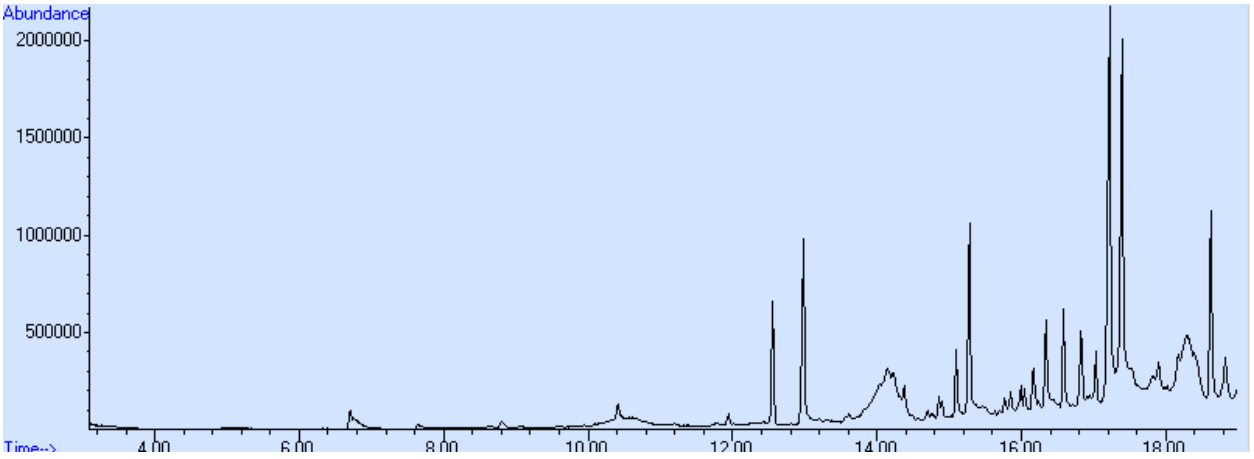
MS Spectrum 162: Que Extracted in Methanol – RT 18.622



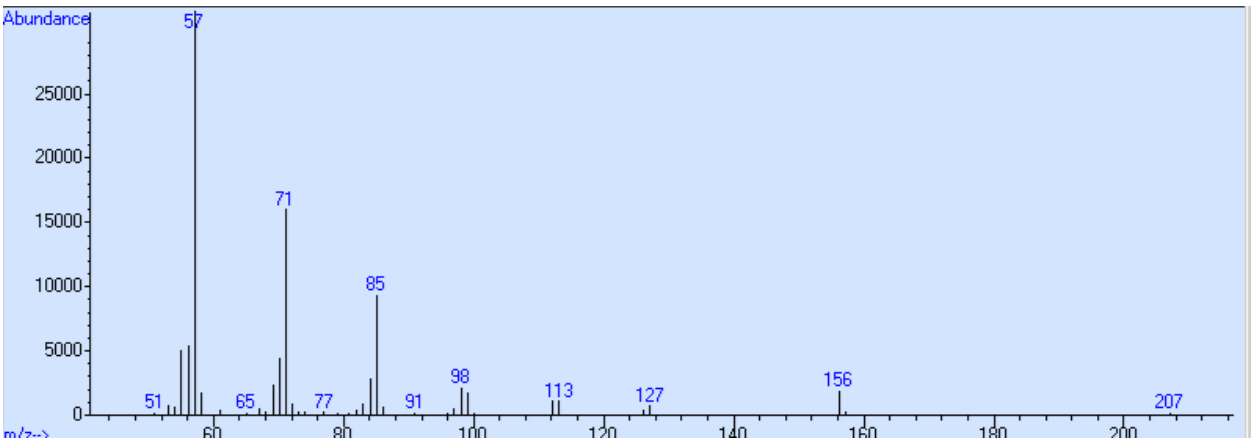
MS Spectrum 163: Que Extracted in Methanol – RT 18.862



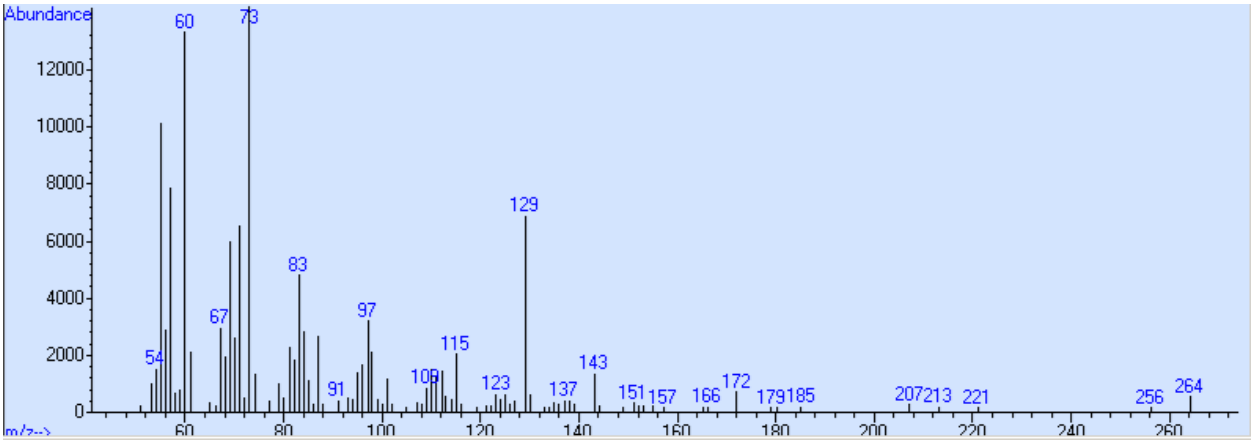
GC Spectrum 23: Undyed Caucasoid Extracted in Methanol



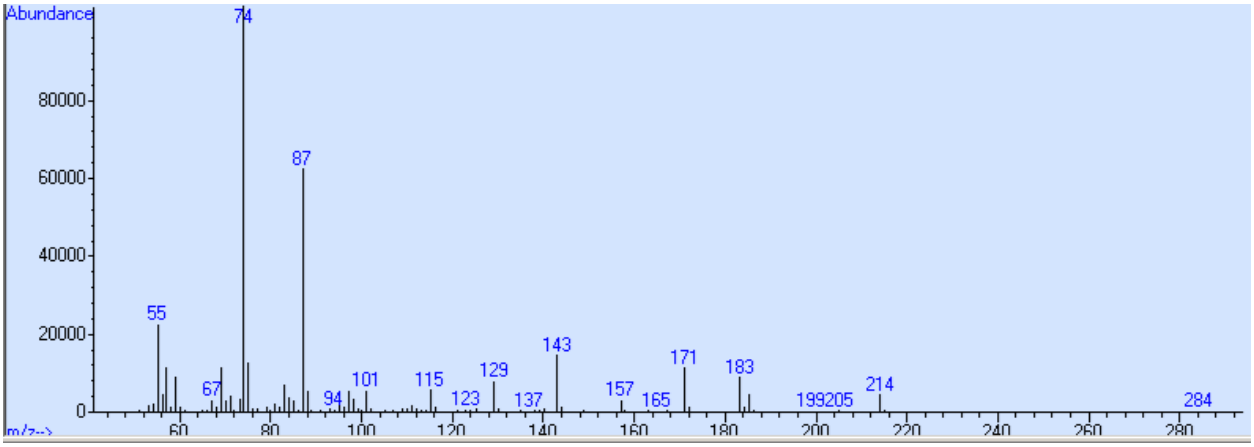
MS Spectrum 164: Undyed Caucasoid Extracted in Methanol – RT 6.709



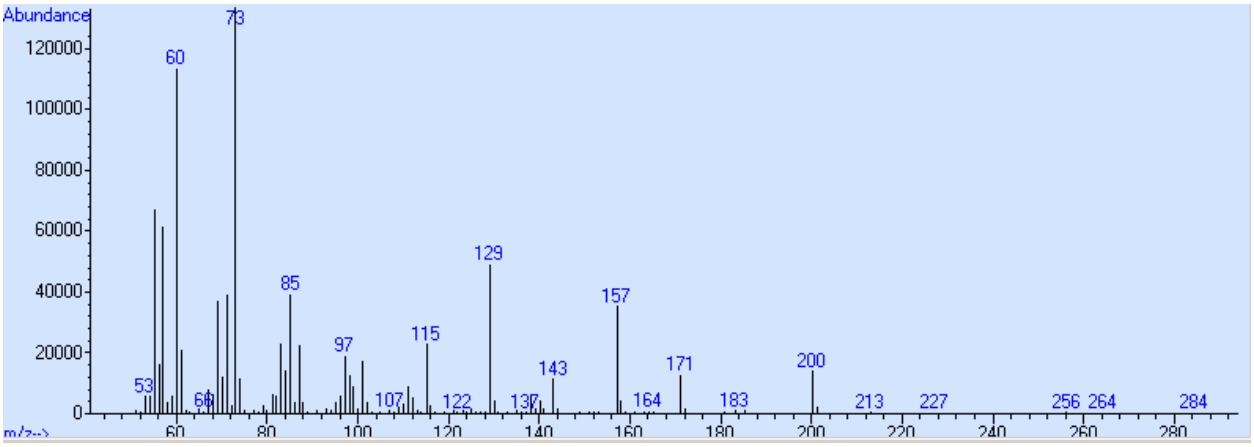
MS Spectrum 165: Undyed Caucasoid Extracted in Methanol – RT 10.411



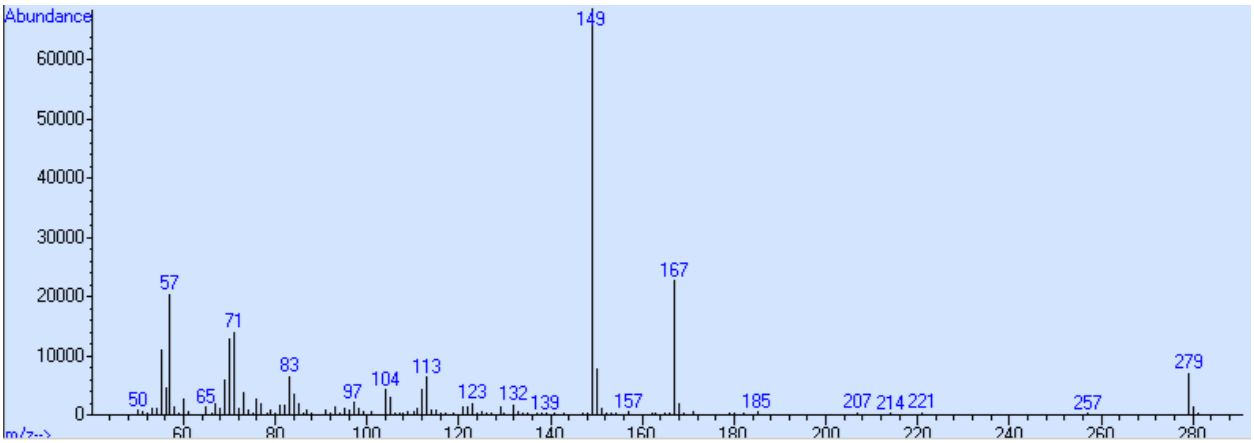
MS Spectrum 166: Undyed Caucasoid Extracted in Methanol – RT 12.568



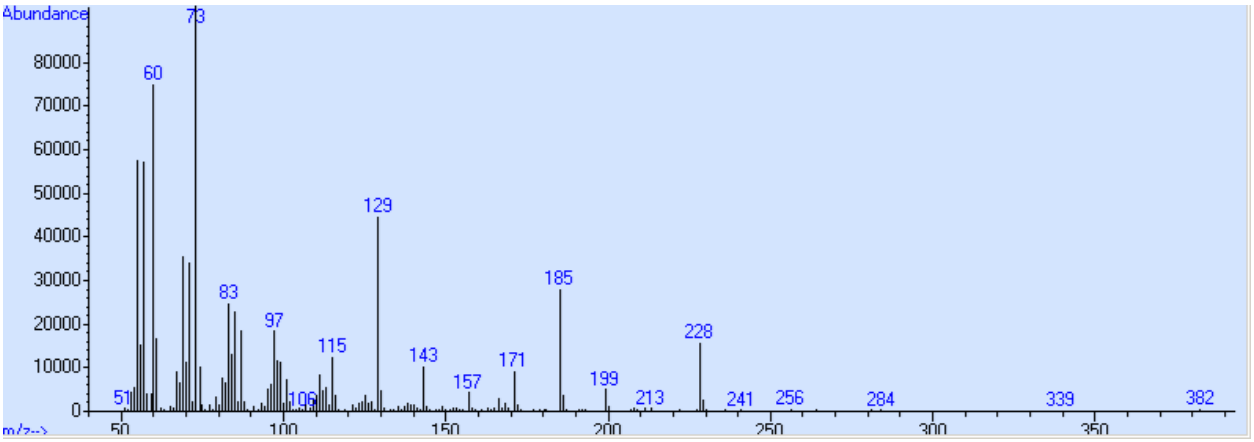
MS Spectrum 167: Undyed Caucasoid Extracted in Methanol – RT 12.980



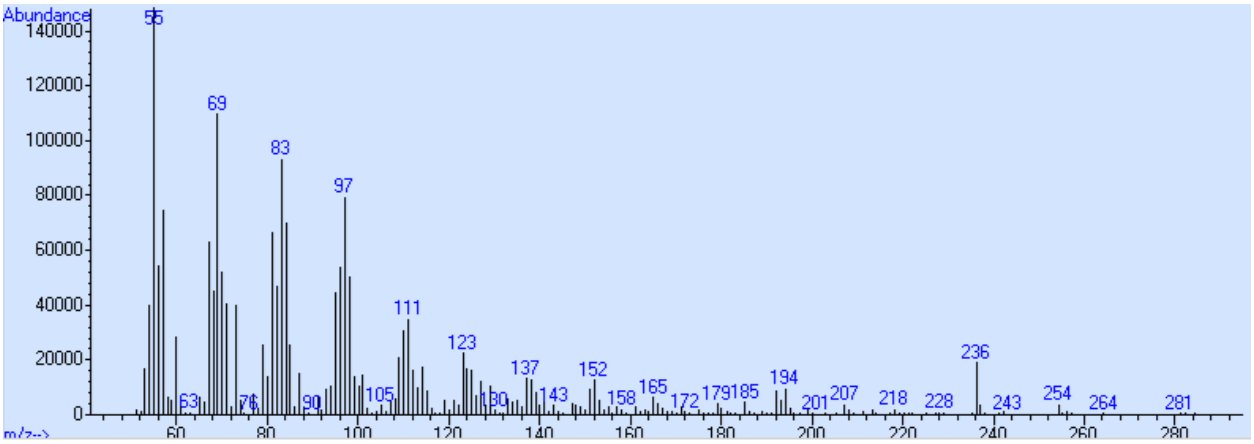
MS Spectrum 168: Undyed Caucasoid Extracted in Methanol – RT 14.113



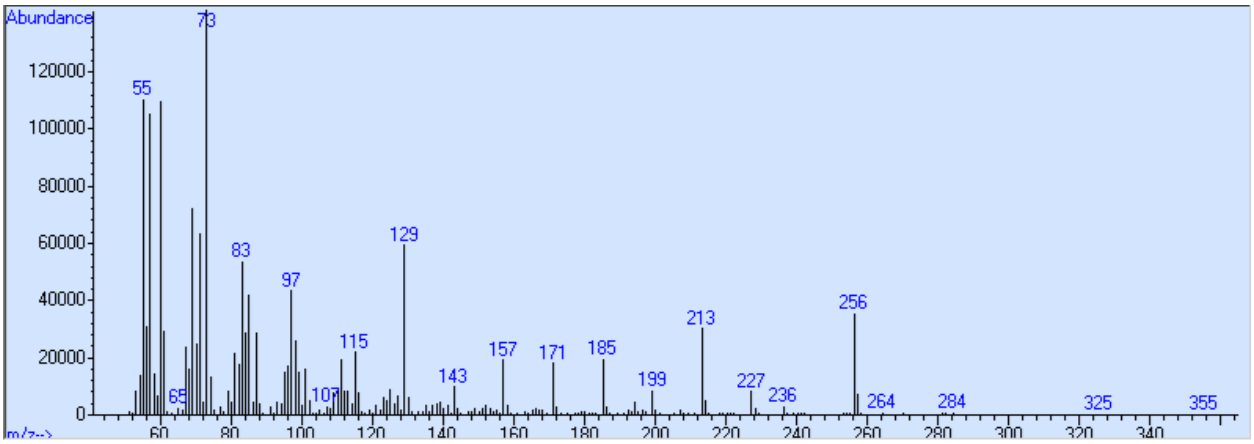
MS Spectrum 169: Undyed Caucasoid Extracted in Methanol – RT 15.269



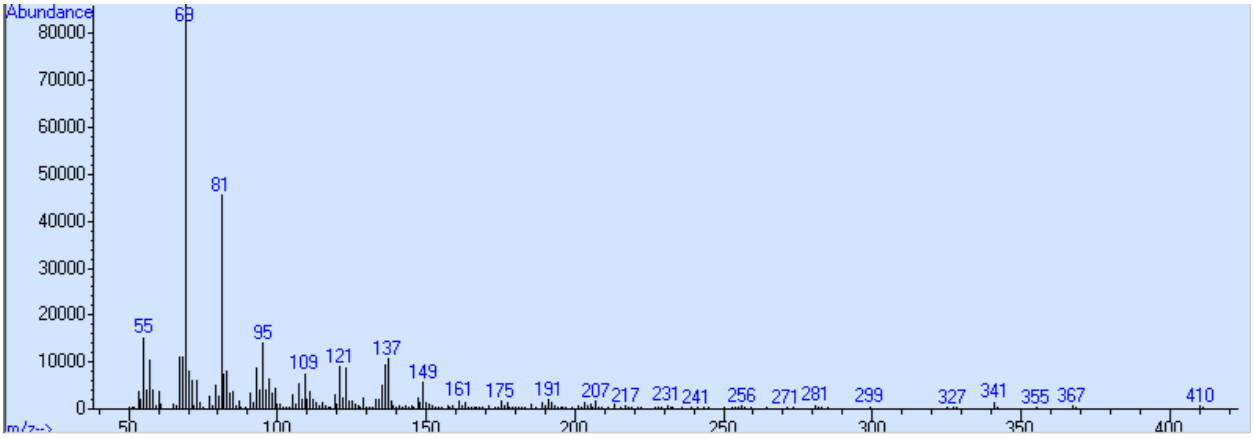
MS Spectrum 170: Undyed Caucasoid Extracted in Methanol – RT 17.209



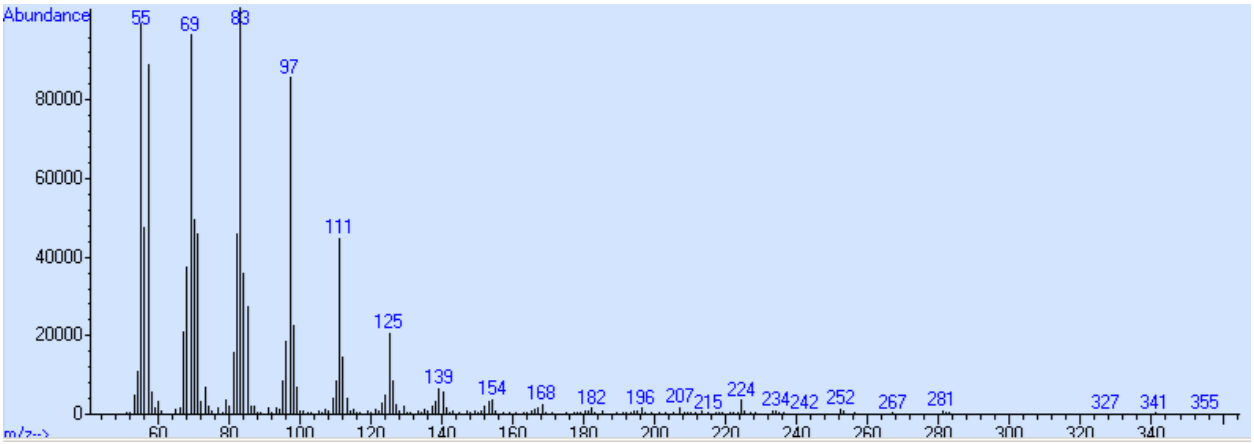
MS Spectrum 171: Undyed Caucasoid Extracted in Methanol – RT 17.380



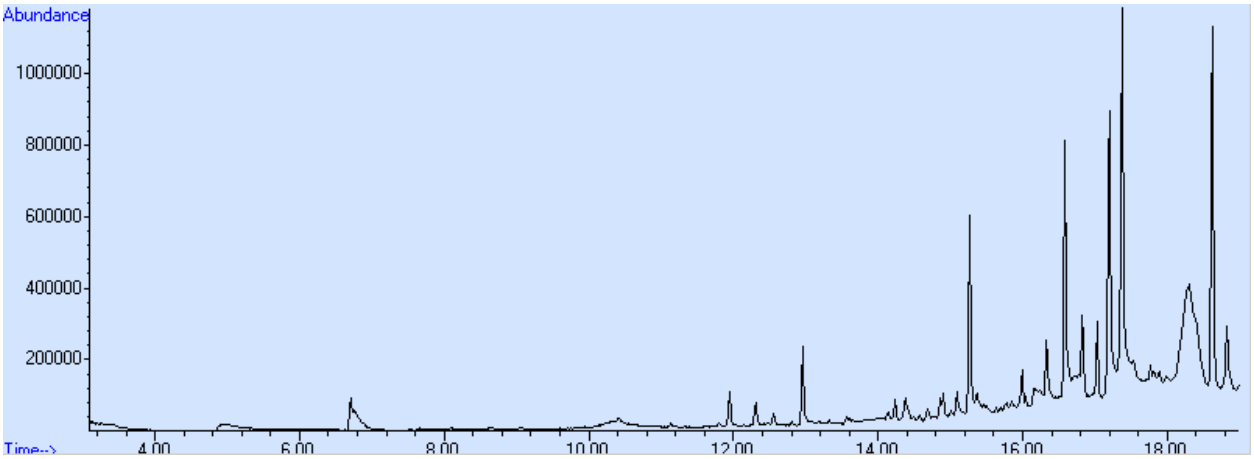
MS Spectrum 172: Undyed Caucasoid Extracted in Methanol – RT 18.296



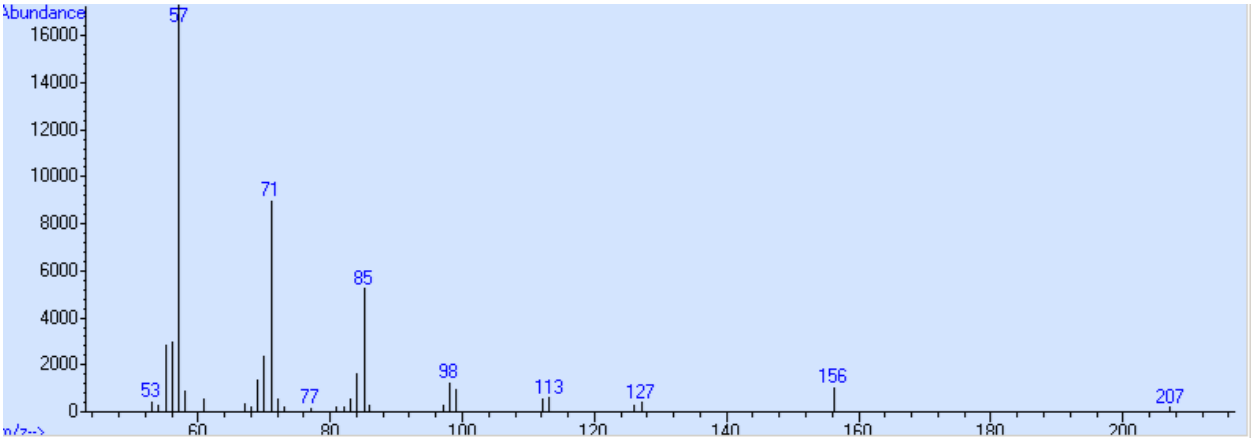
MS Spectrum 173: Undyed Caucasoid Extracted in Methanol – RT 18.622



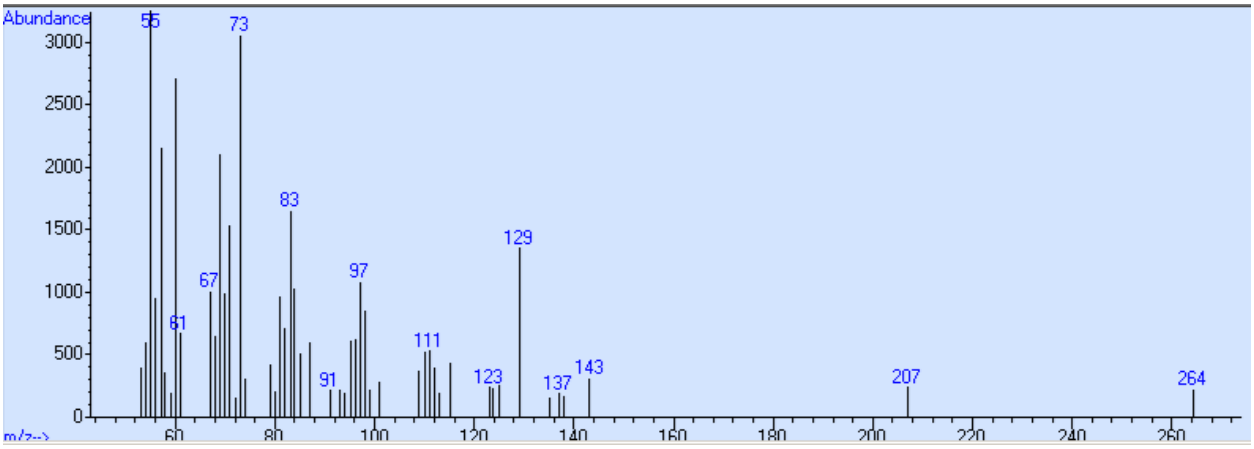
GC Spectrum 24: Dyed Caucasoid Extracted in Methanol



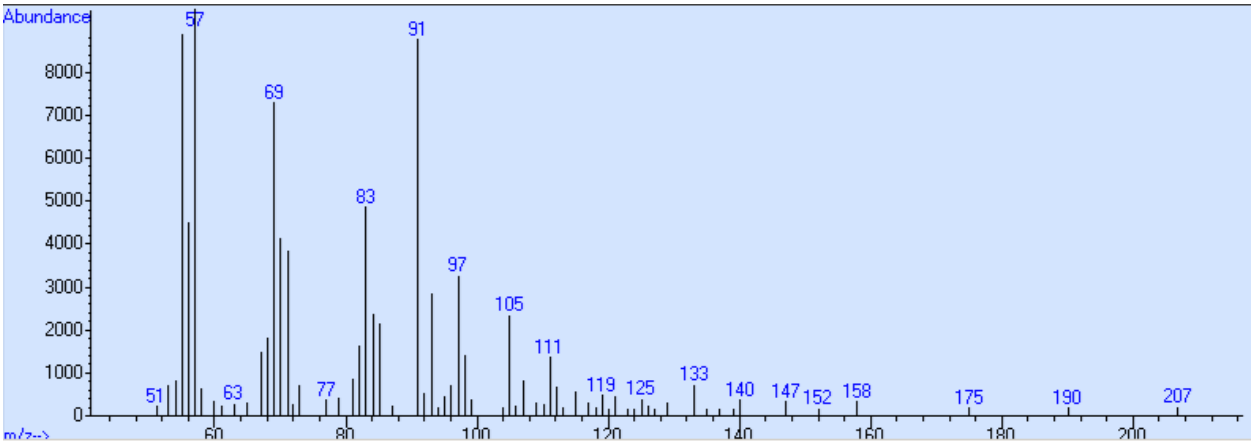
MS Spectrum 174: Dyed Caucasoid Extracted in Methanol – RT 6.772



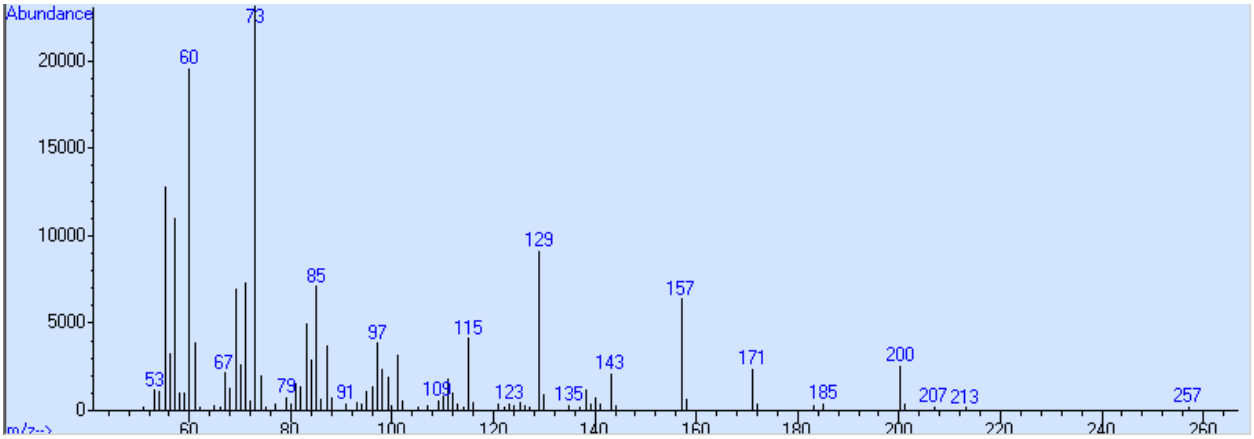
MS Spectrum 175: Dyed Caucasoid Extracted in Methanol – RT 10.411



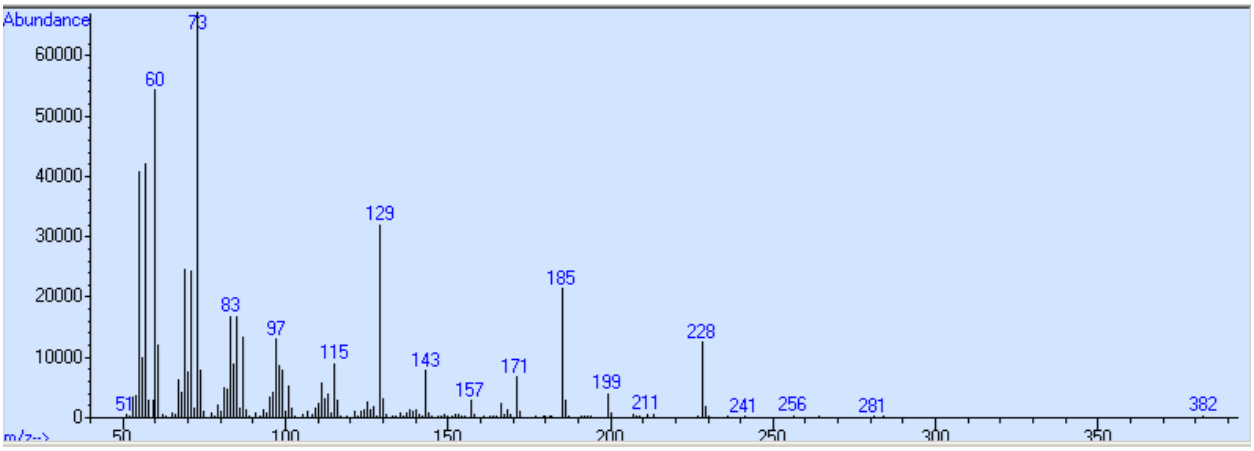
MS Spectrum 176: Dyed Caucasoid Extracted in Methanol – RT 11.956



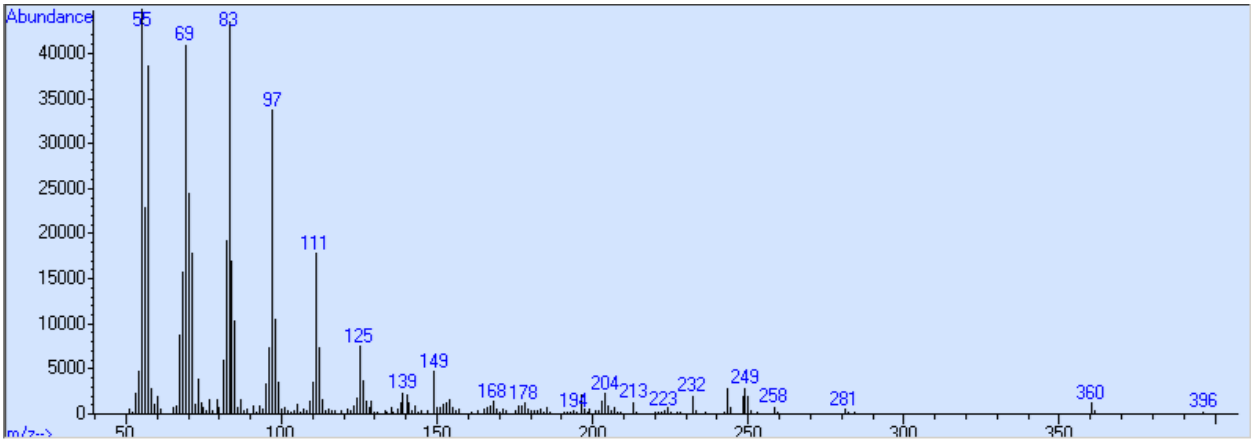
MS Spectrum 177: Dyed Caucasoid Extracted in Methanol – RT 12.963



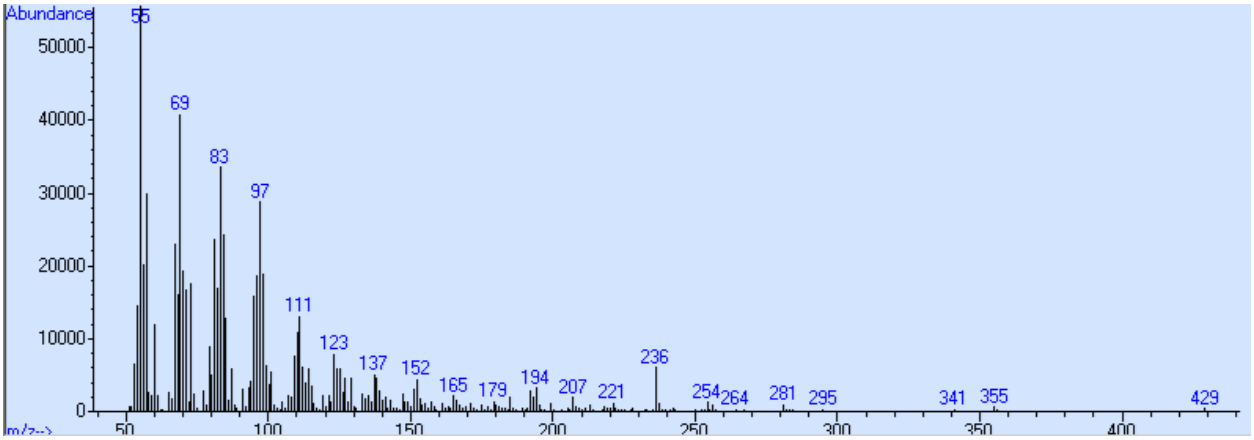
MS Spectrum 178: Dyed Caucasoid Extracted in Methanol – RT 15.269



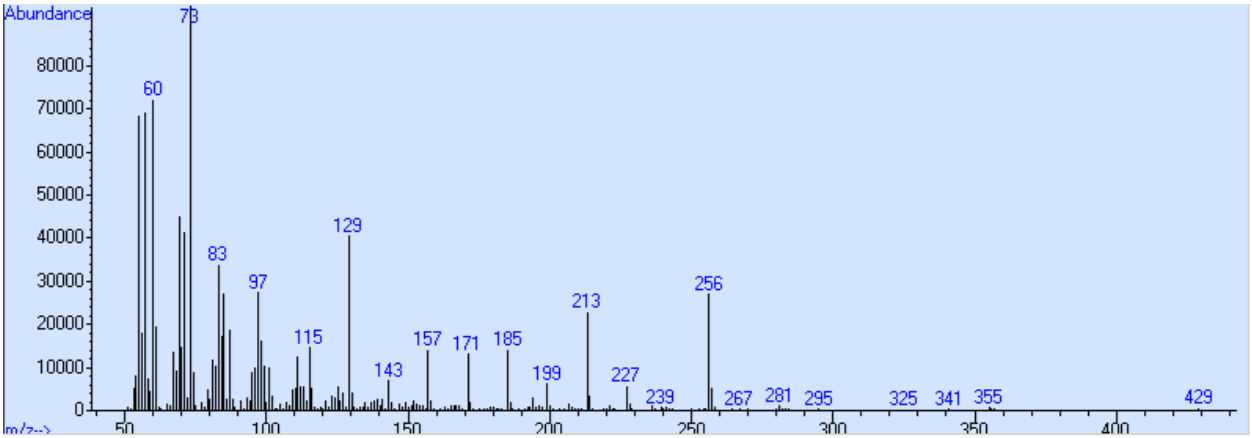
MS Spectrum 179: Dyed Caucasoid Extracted in Methanol – RT 16.596



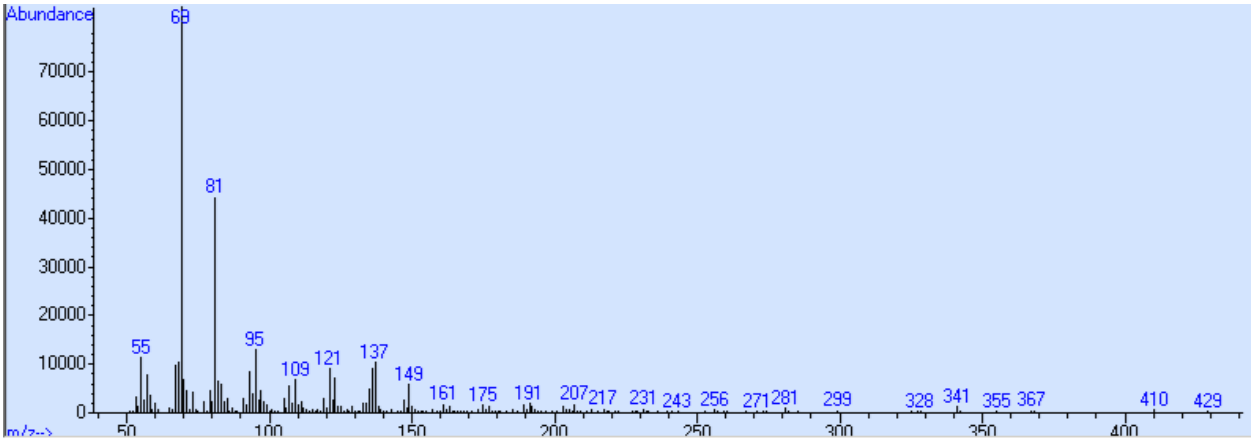
MS Spectrum 180: Dyed Caucasoid Extracted in Methanol – RT 17.186



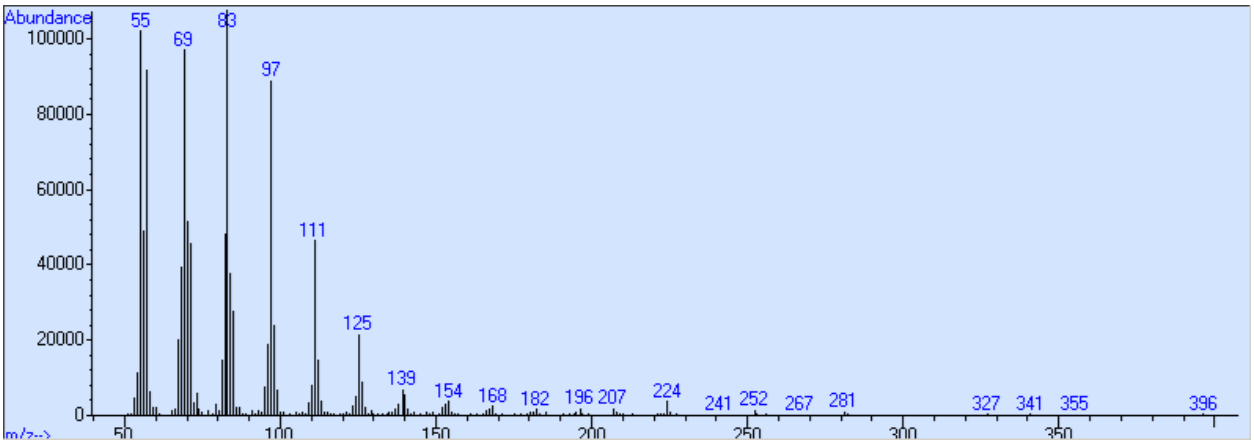
MS Spectrum 181: Dyed Caucasoid Extracted in Methanol – RT 17.380



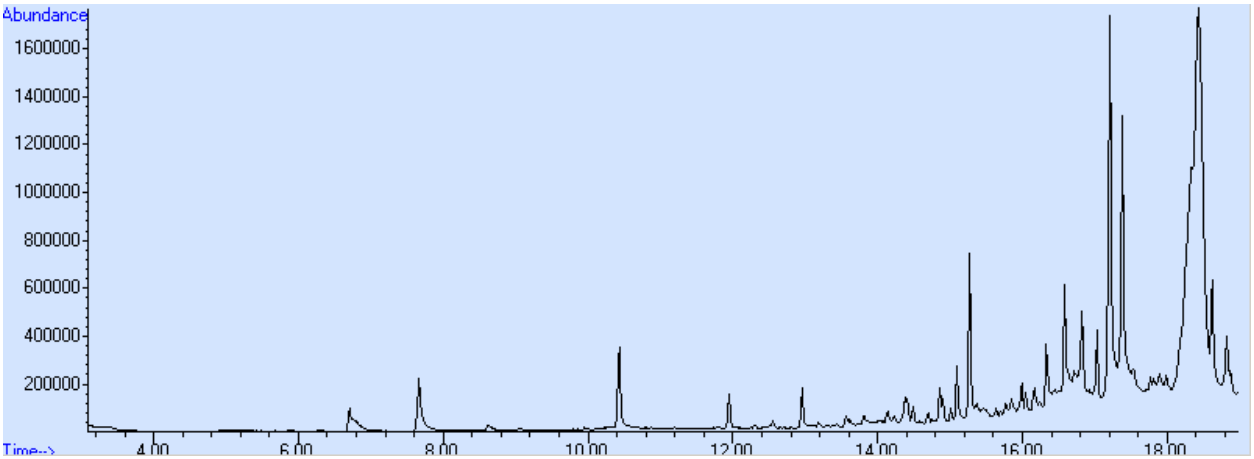
MS Spectrum 182: Dyed Caucasoid Extracted in Methanol – RT 18.296



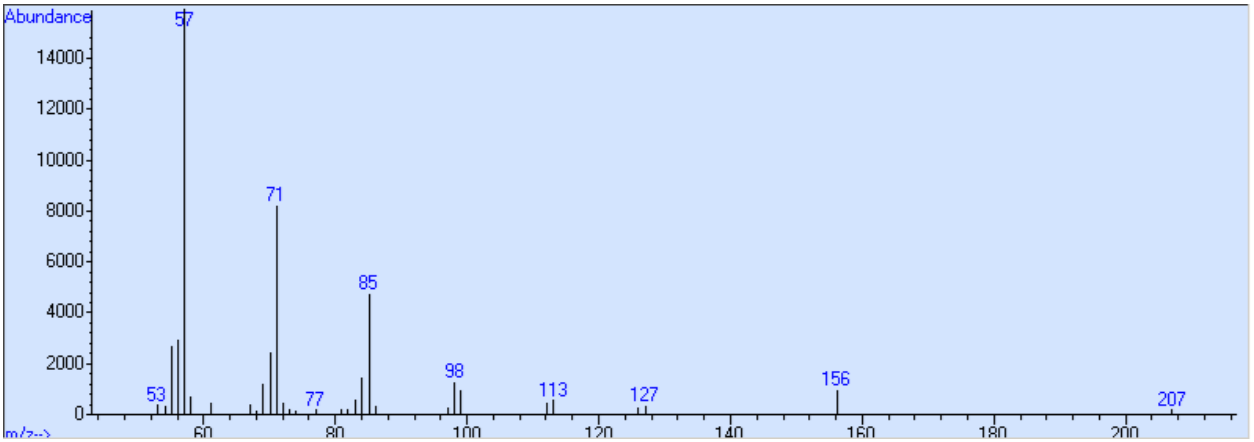
MS Spectrum 183: Dyed Caucasoid Extracted in Methanol – RT 18.622



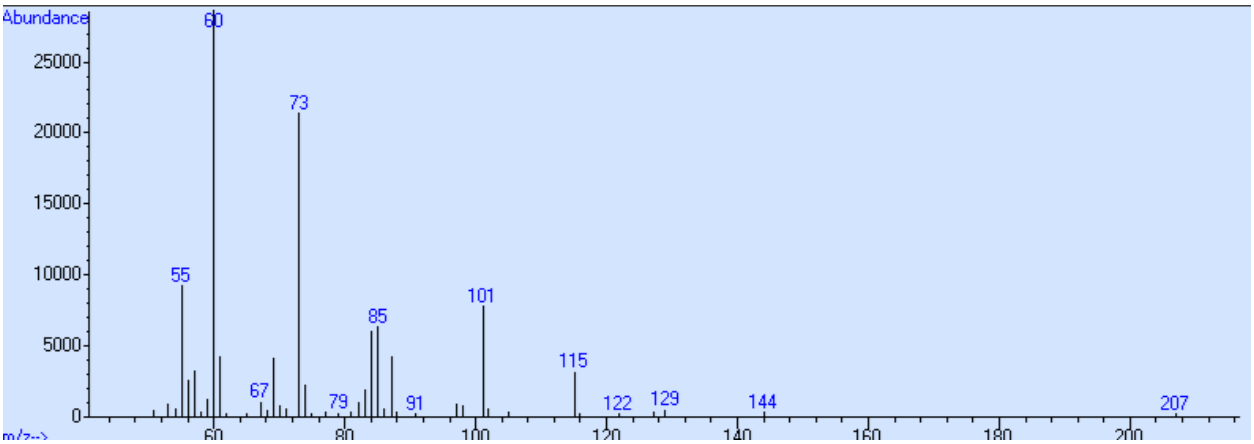
GC Spectrum 25: Undyed Mongoloid Extracted in Methanol



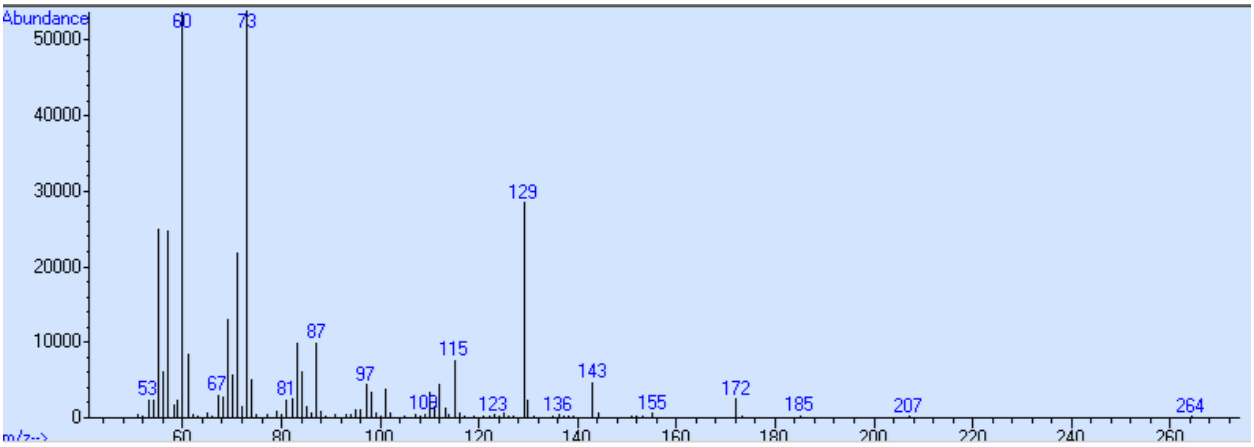
MS Spectrum 184: Undyed Mongoloid Extracted in Methanol – RT 6.772



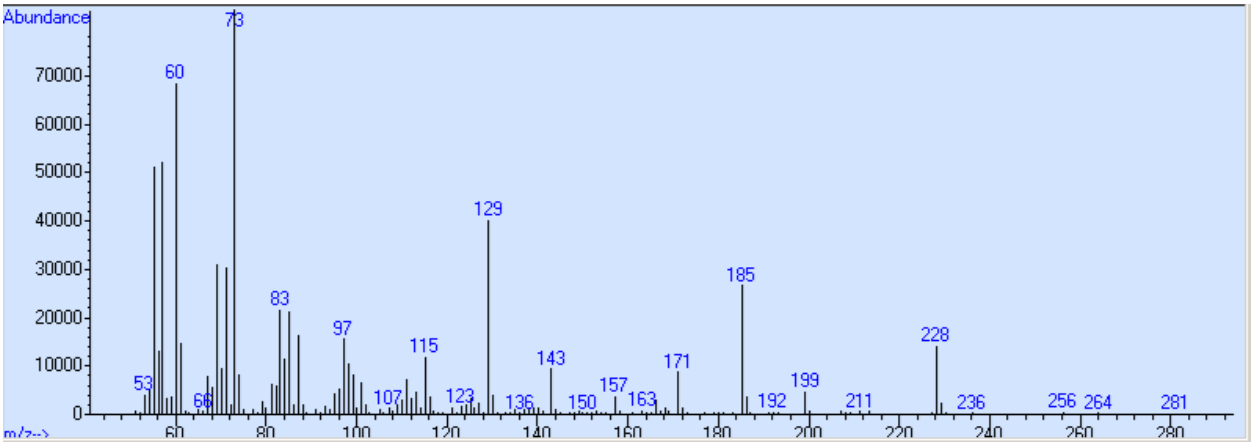
MS Spectrum 185: Undyed Mongoloid Extracted in Methanol – RT 7.687



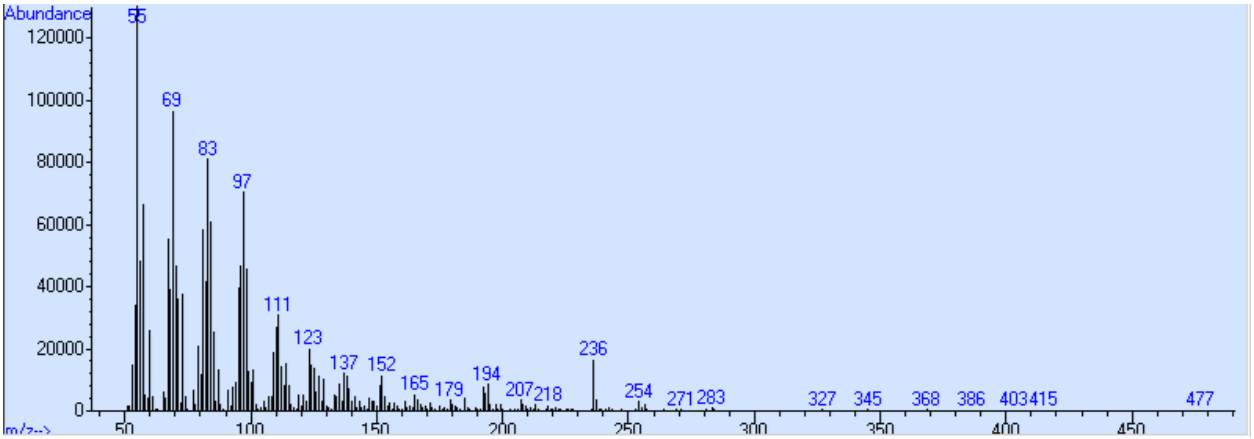
MS Spectrum 186: Undyed Mongoloid Extracted in Methanol – RT 10.434



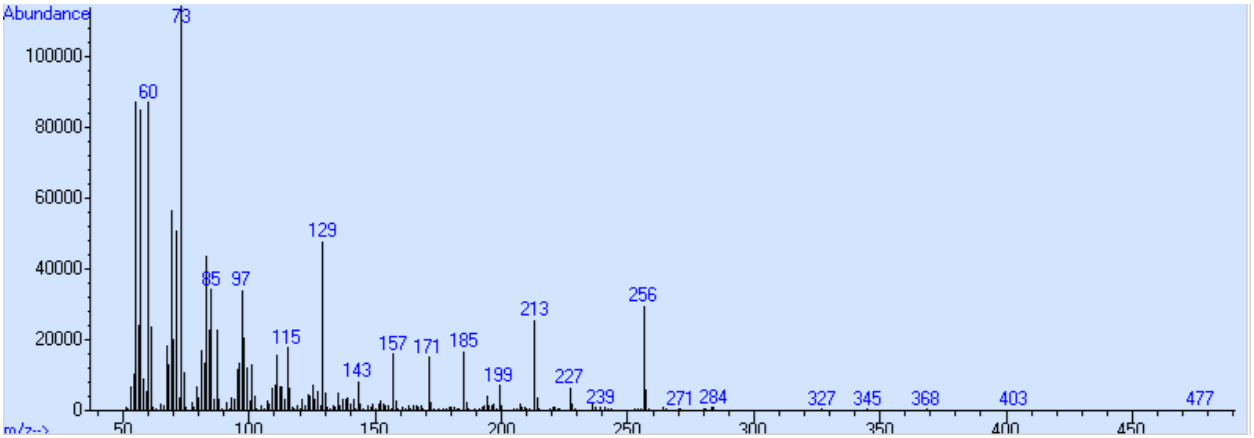
MS Spectrum 187: Undyed Mongoloid Extracted in Methanol – RT 15.269



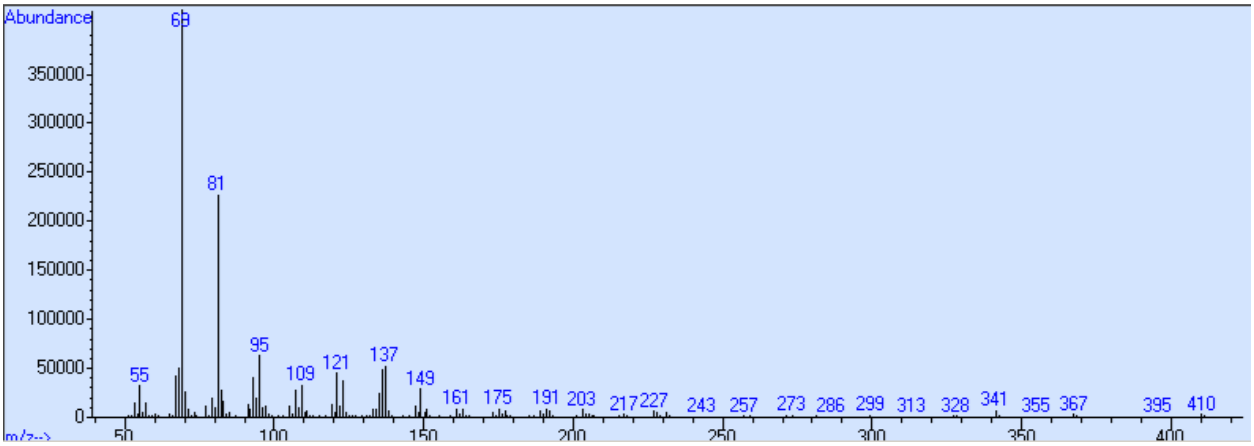
MS Spectrum 188: Undyed Mongoloid Extracted in Methanol – RT 17.209



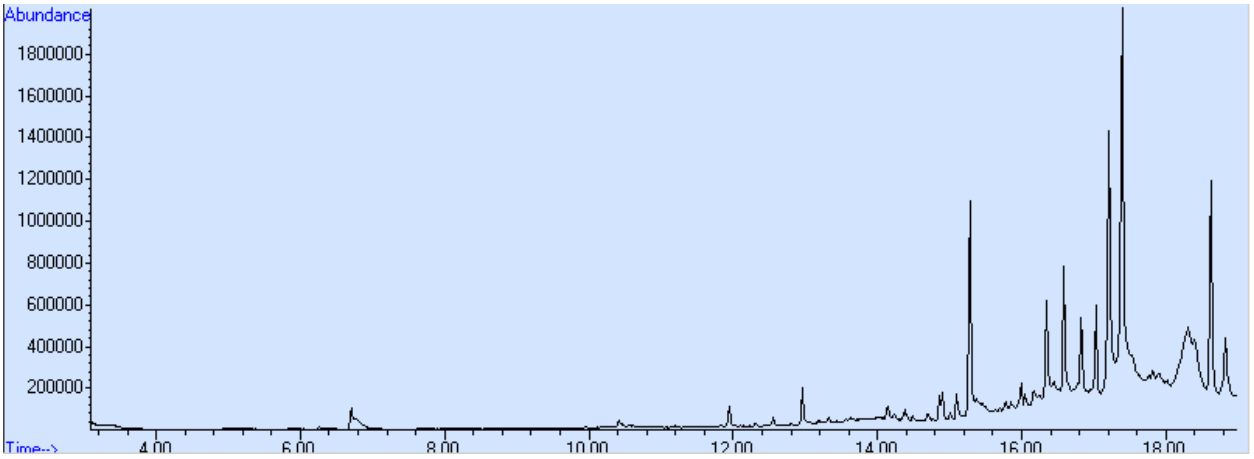
MS Spectrum 189: Undyed Mongoloid Extracted in Methanol – RT 17.380



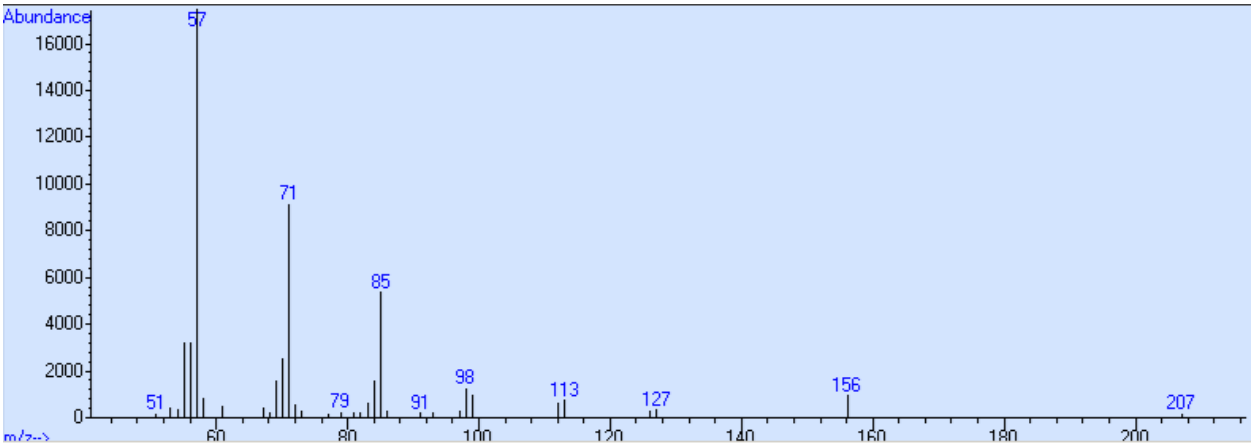
MS Spectrum 190: Undyed Mongoloid Extracted in Methanol – RT 18.427



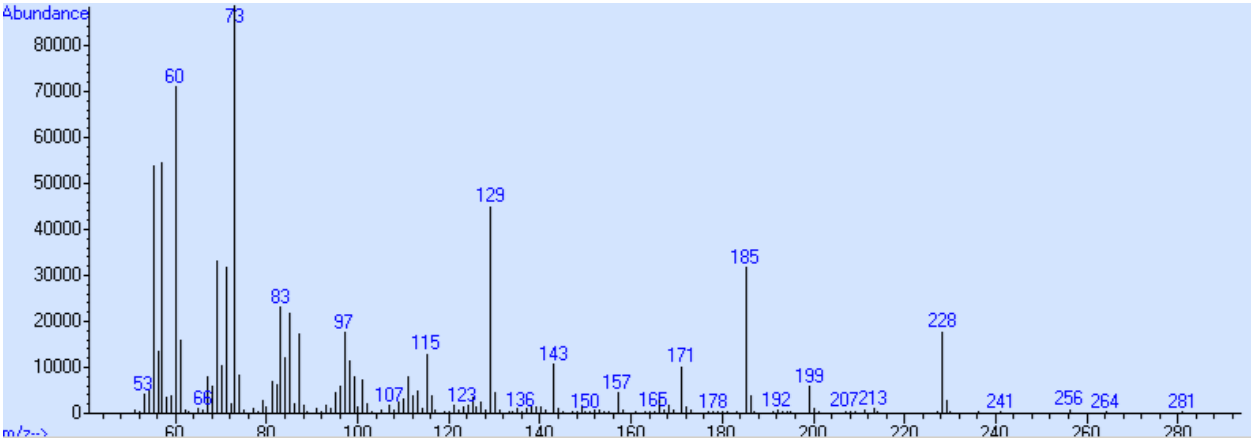
GC Spectrum 26: Dyed Mongoloid Extracted in Methanol



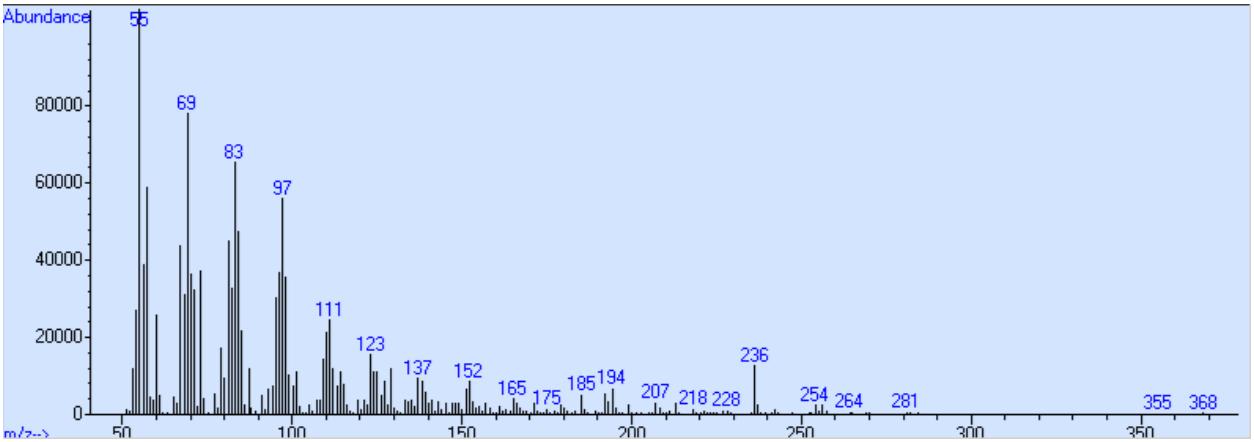
MS Spectrum 191: Dyed Mongoloid Extracted in Methanol – RT 6.755



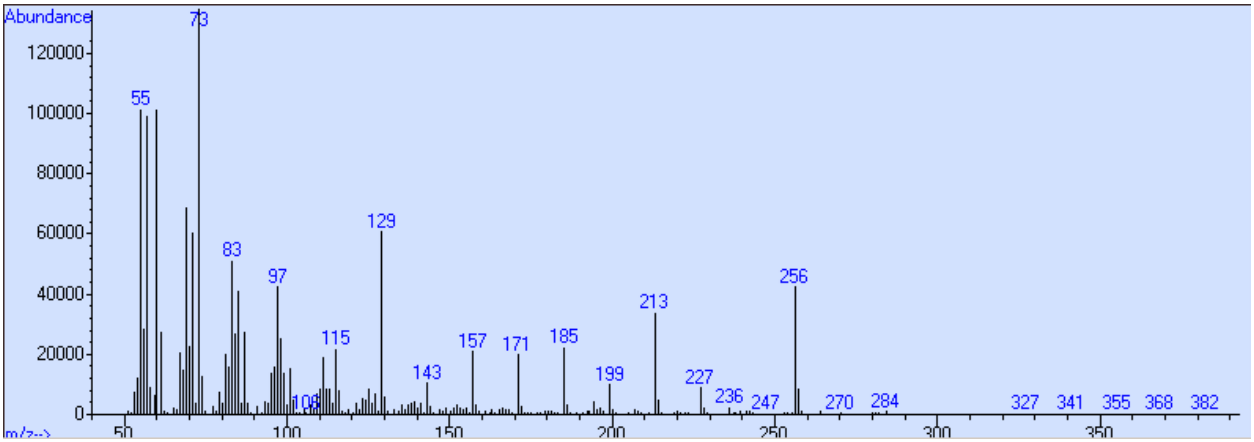
MS Spectrum 192: Dyed Mongoloid Extracted in Methanol – RT 15.292



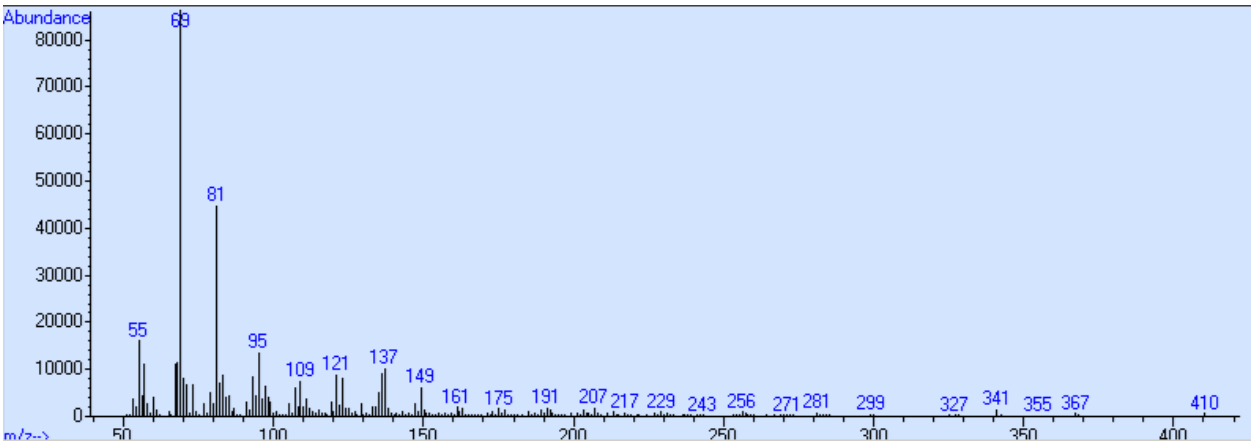
MS Spectrum 193: Dyed Mongoloid Extracted in Methanol – RT 17.209



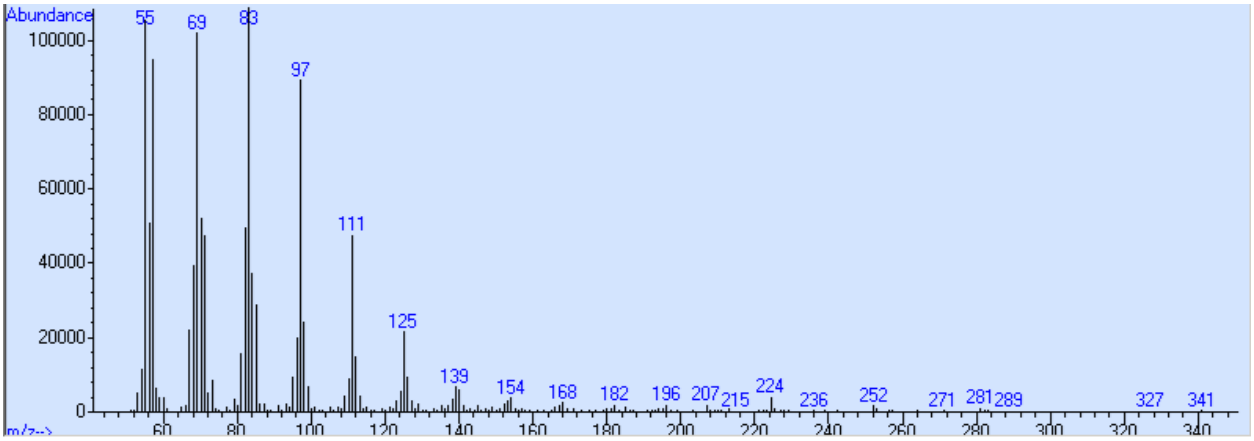
MS Spectrum 194: Dyed Mongoloid Extracted in Methanol – RT 17.403



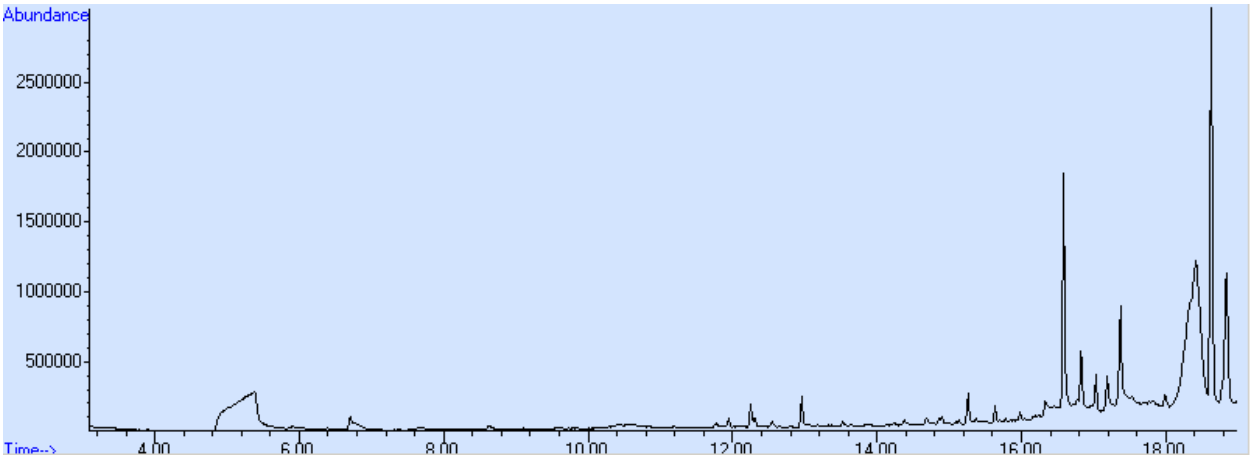
MS Spectrum 195: Dyed Mongoloid Extracted in Methanol – RT 18.296



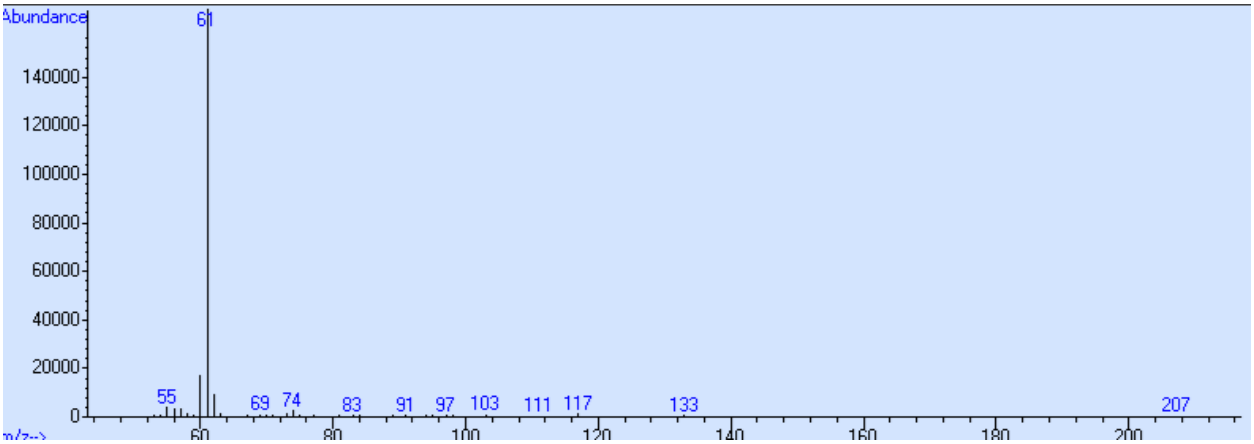
MS Spectrum 196: Dyed Mongoloid Extracted in Methanol – RT 18.622



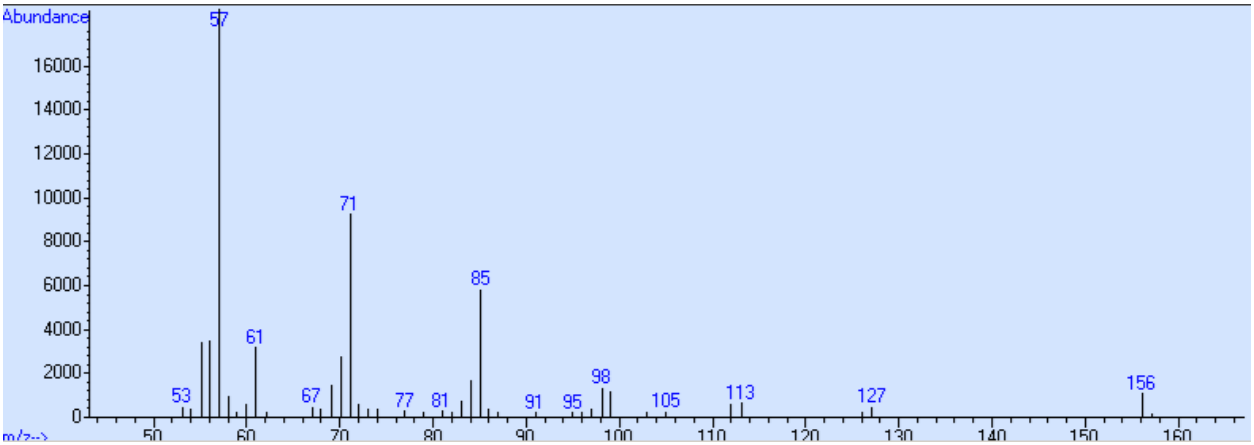
GC Spectrum 27: Undyed Negroid Extracted in Methanol



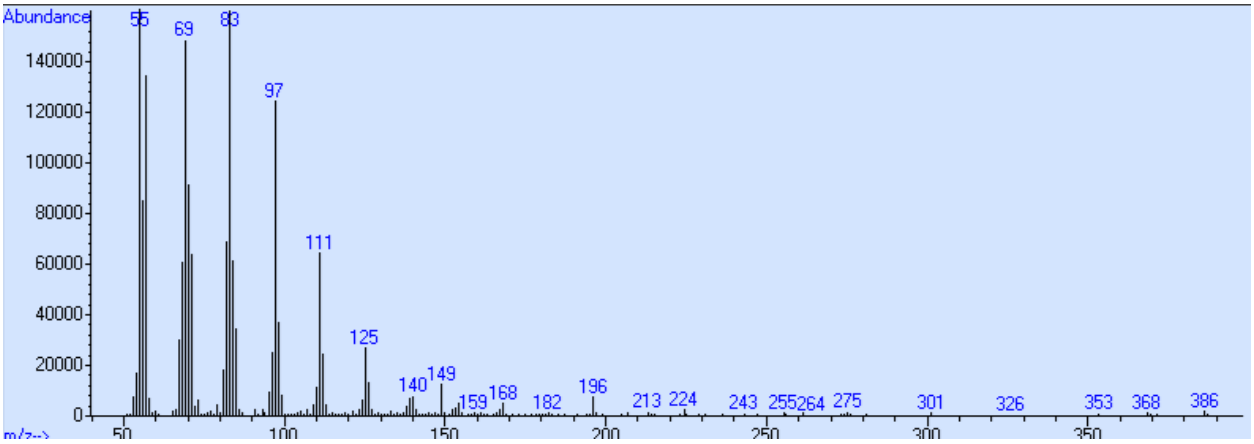
MS Spectrum 197: Undyed Negroid Extracted in Methanol – RT 5.204



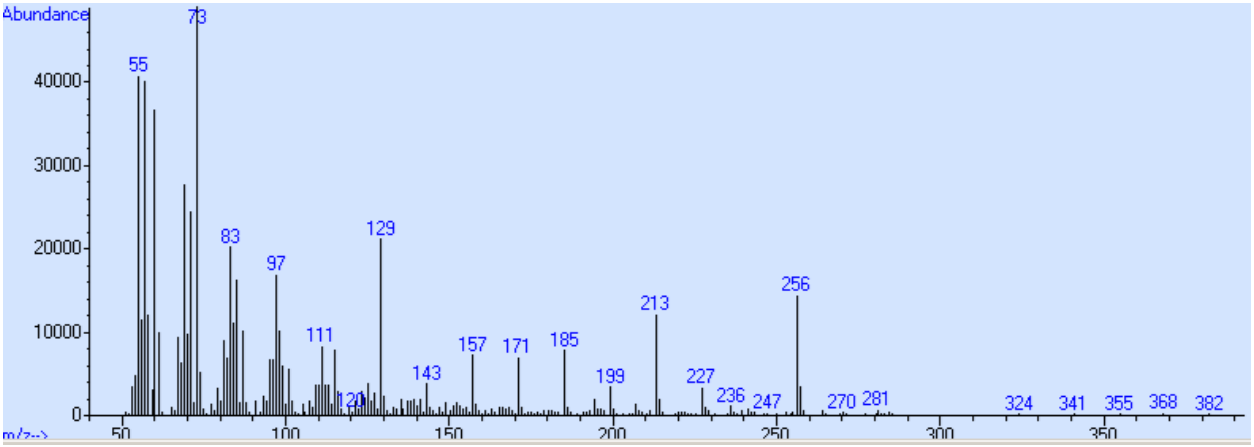
MS Spectrum 198: Undyed Negroid Extracted in Methanol – RT 6.755



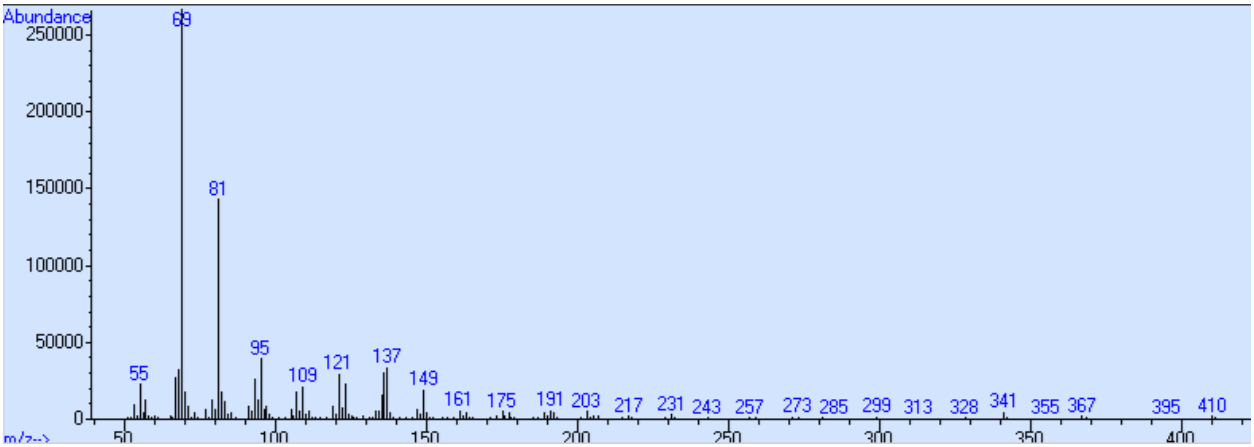
MS Spectrum 199: Undyed Negroid Extracted in Methanol – RT 16.579



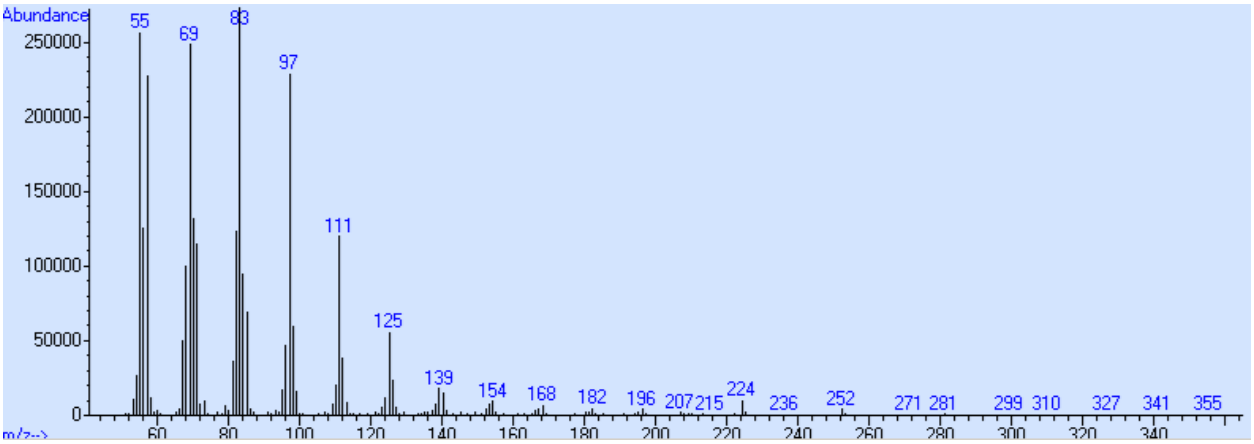
MS Spectrum 200: Undyed Negroid Extracted in Methanol – RT 17.380



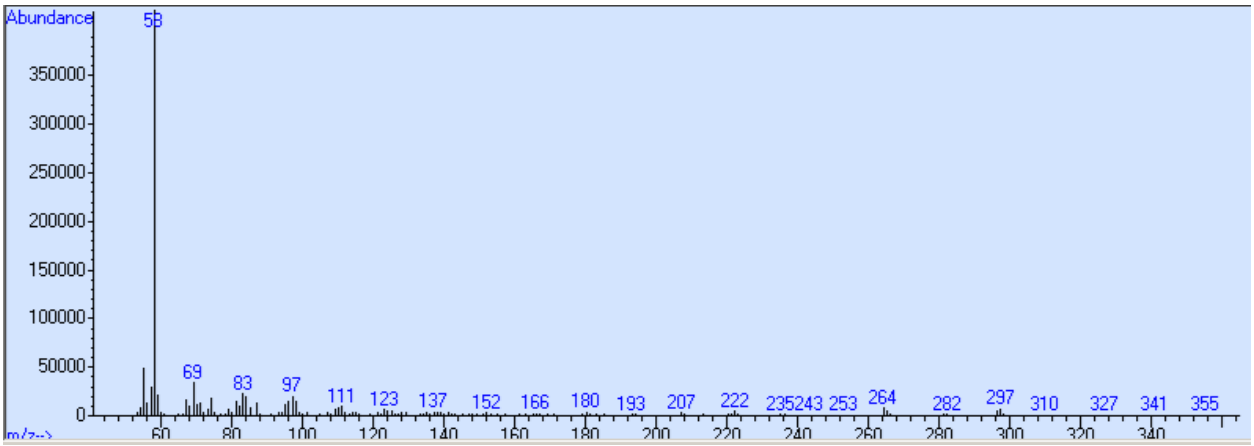
MS Spectrum 201: Undyed Negroid Extracted in Methanol – RT 18.387



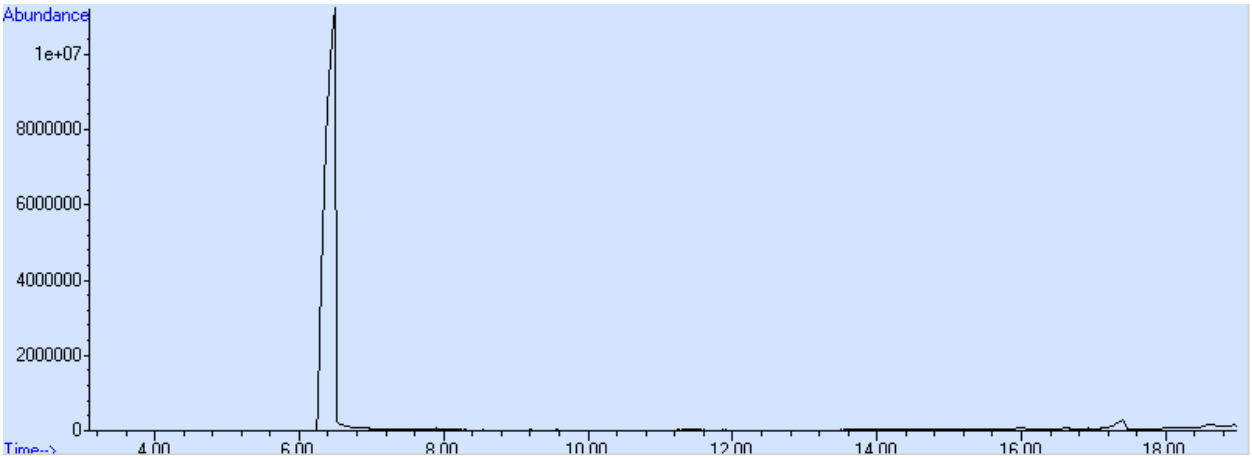
MS Spectrum 202: Undyed Negroid Extracted in Methanol – RT 18.622



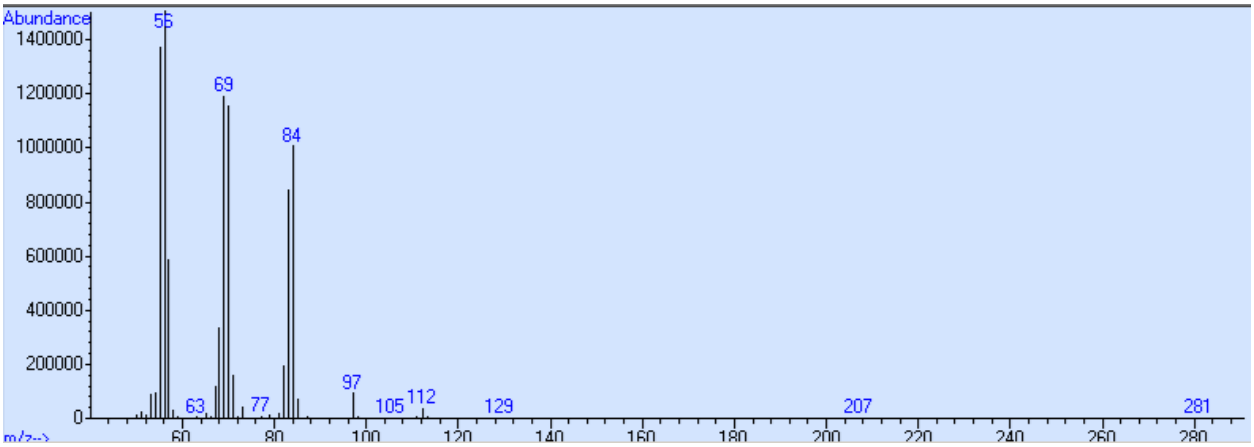
MS Spectrum 203: Undyed Negroid Extracted in Methanol – RT 18.845



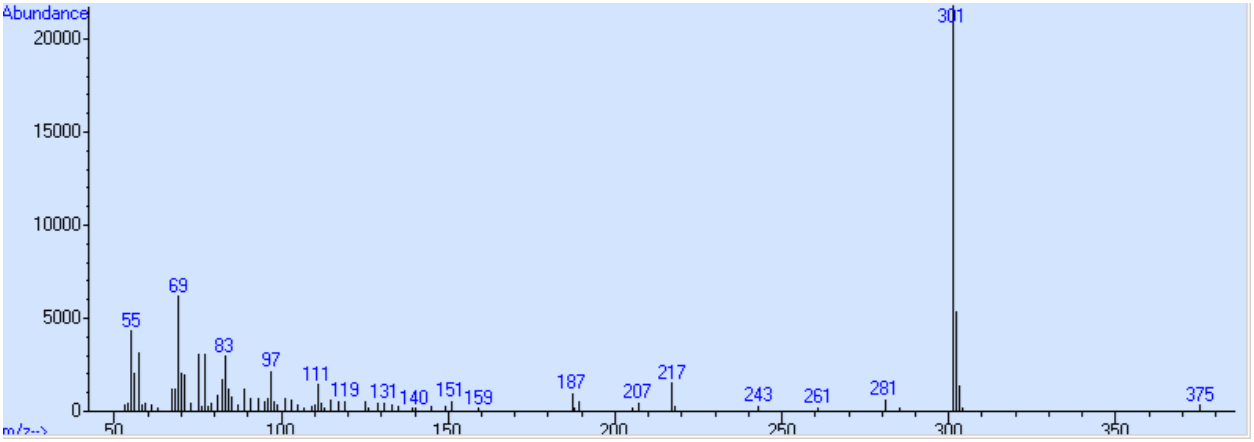
GC Spectrum 28: Solvent 1-Octanol



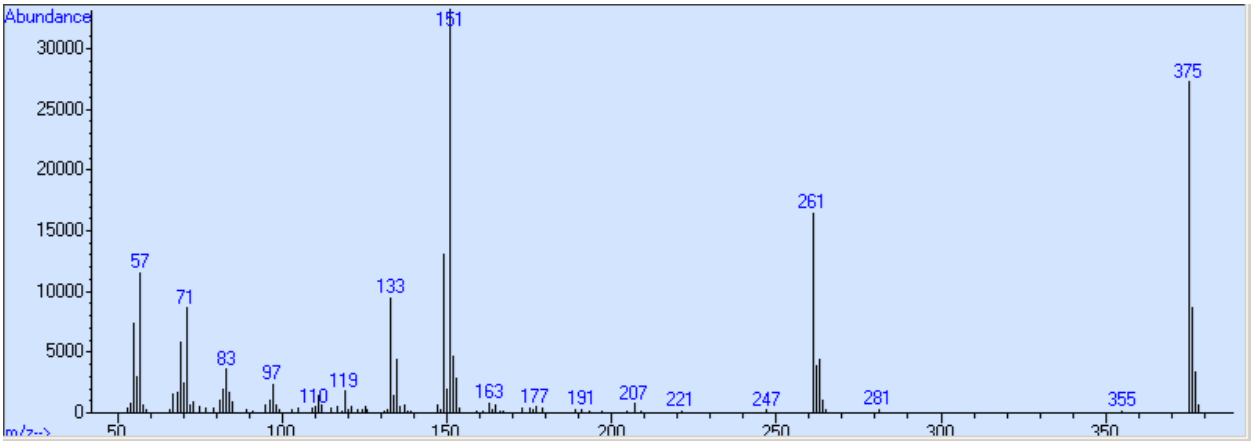
MS Spectrum 204: Solvent 1-Octanol – RT 6.406



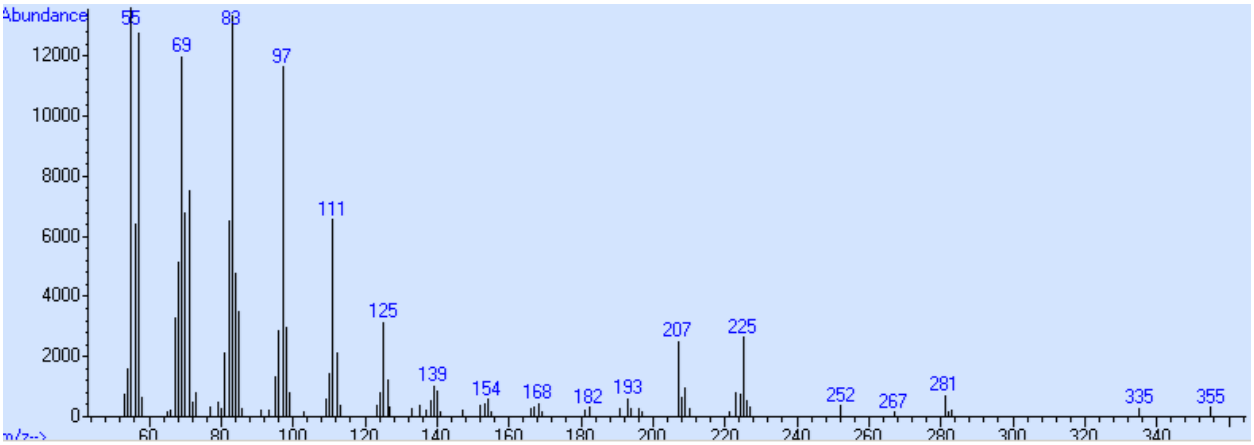
MS Spectrum 205: Solvent 1-Octanol – RT 15.990



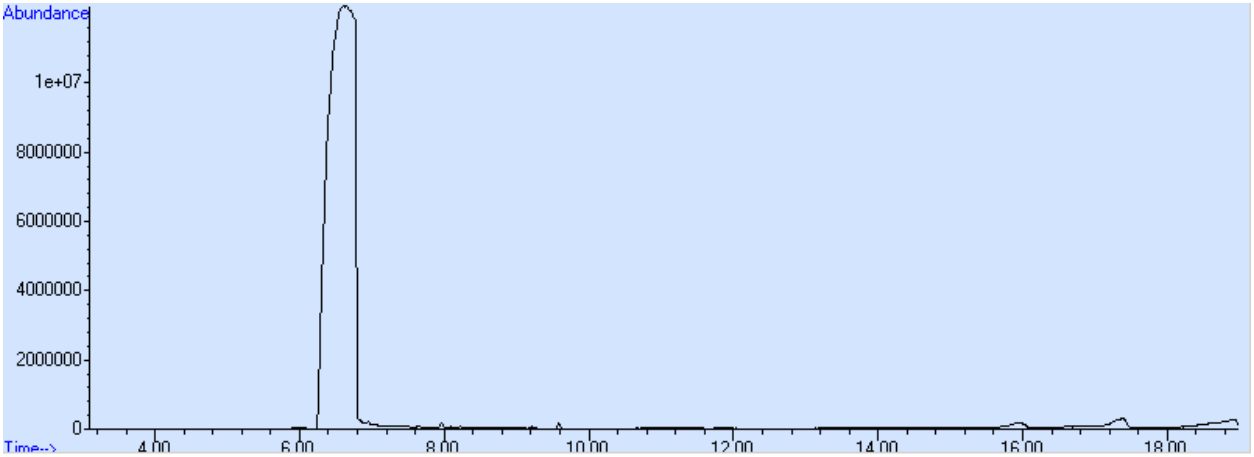
MS Spectrum 206: Solvent 1-Octanol – RT 17.340



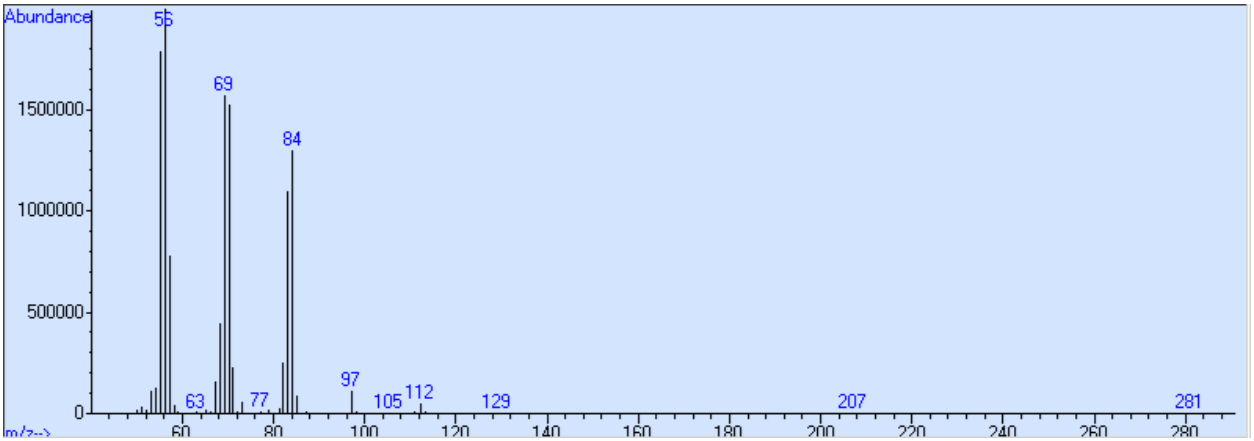
MS Spectrum 207: Solvent 1-Octanol – RT 18.582



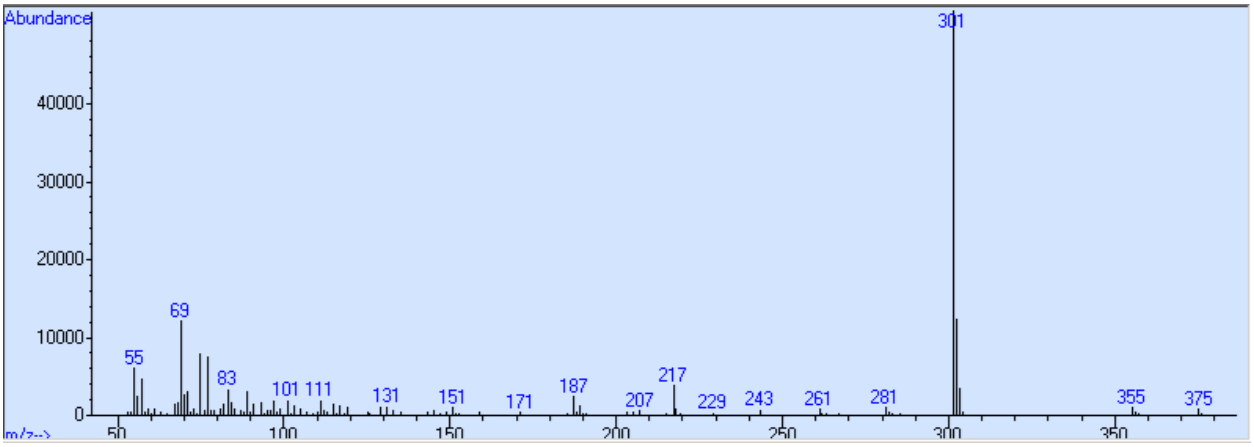
GC Spectrum 29: Remy XQ Extracted in 1-Octanol



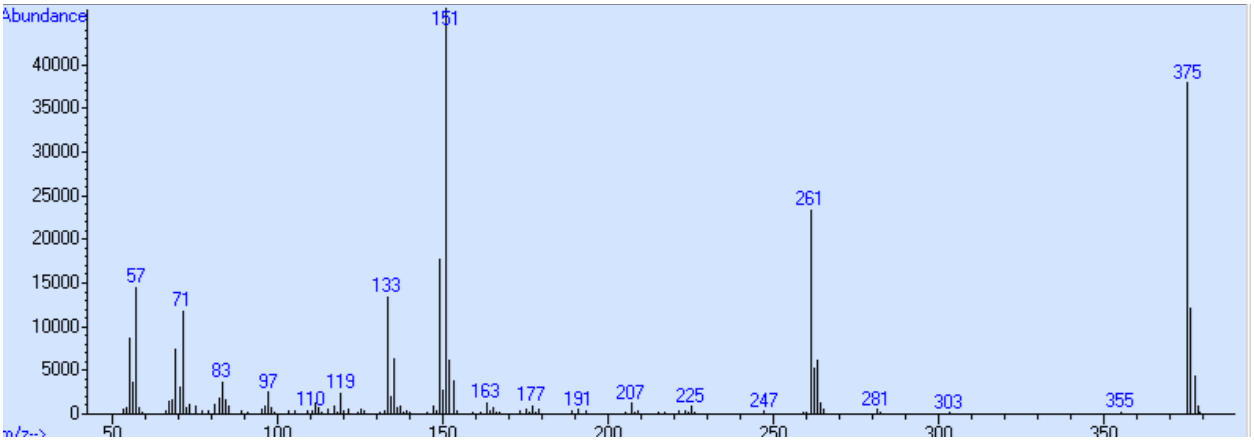
MS Spectrum 208: Remy XQ Extracted in 1-Octanol – RT 6.537



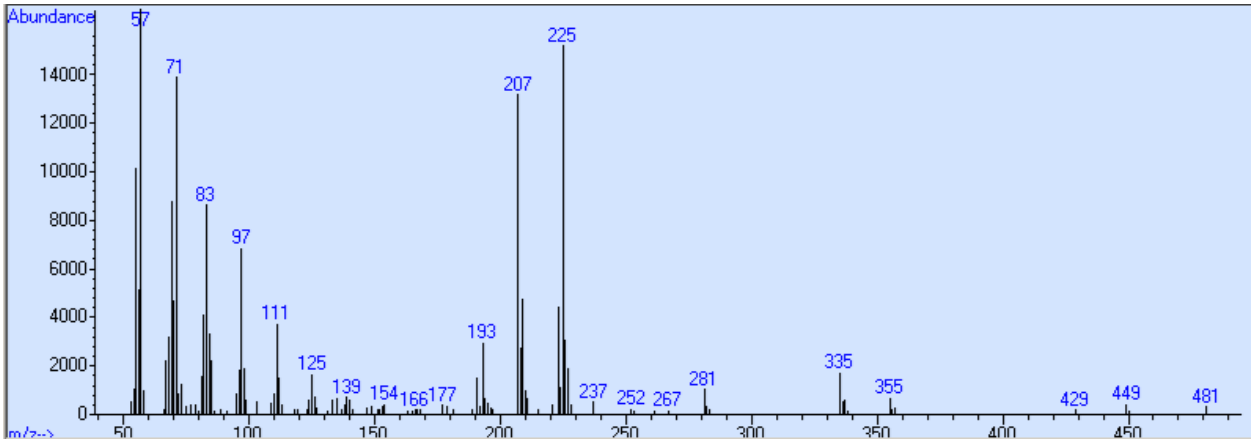
MS Spectrum 209: Remy XQ Extracted in 1-Octanol – RT 15.921



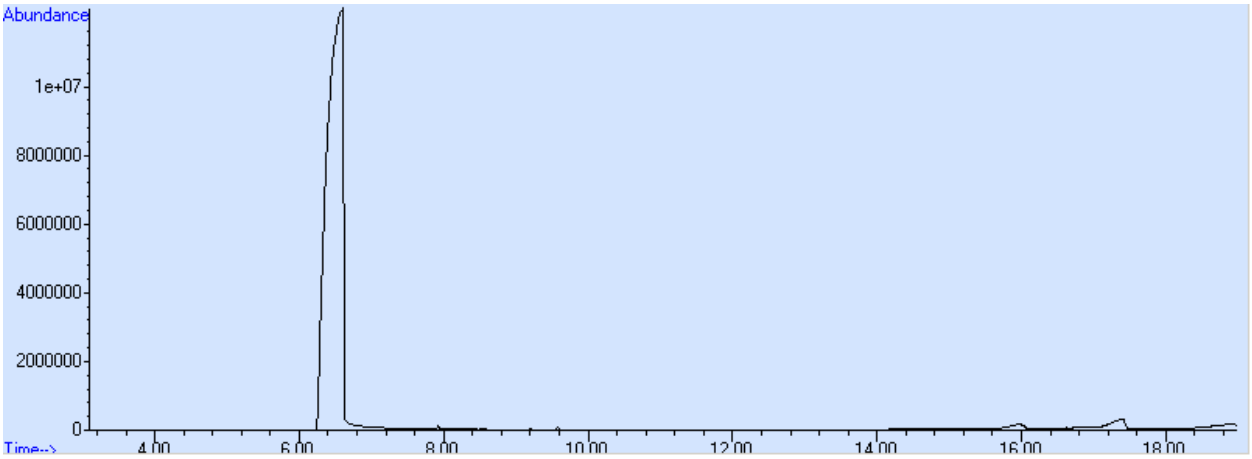
MS Spectrum 210: Remy XQ Extracted in 1-Octanol – RT 17.317



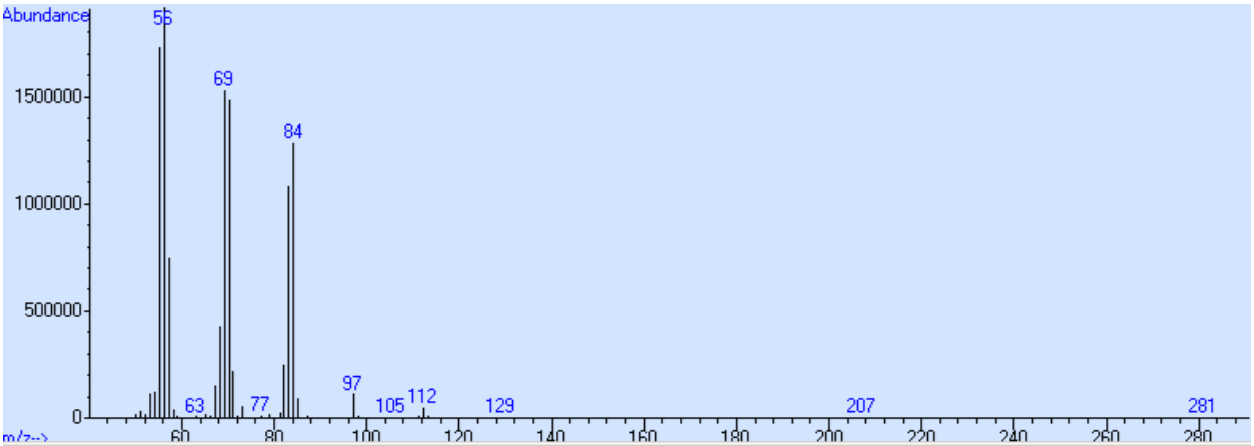
MS Spectrum 211: Remy XQ Extracted in 1-Octanol – RT 18.691



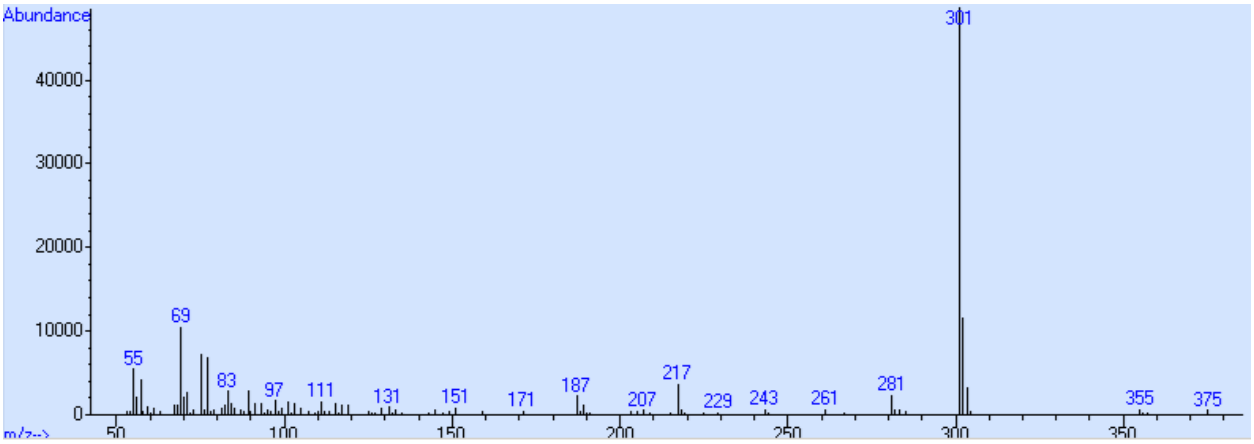
GC Spectrum 30: ECW Extracted in 1-Octanol



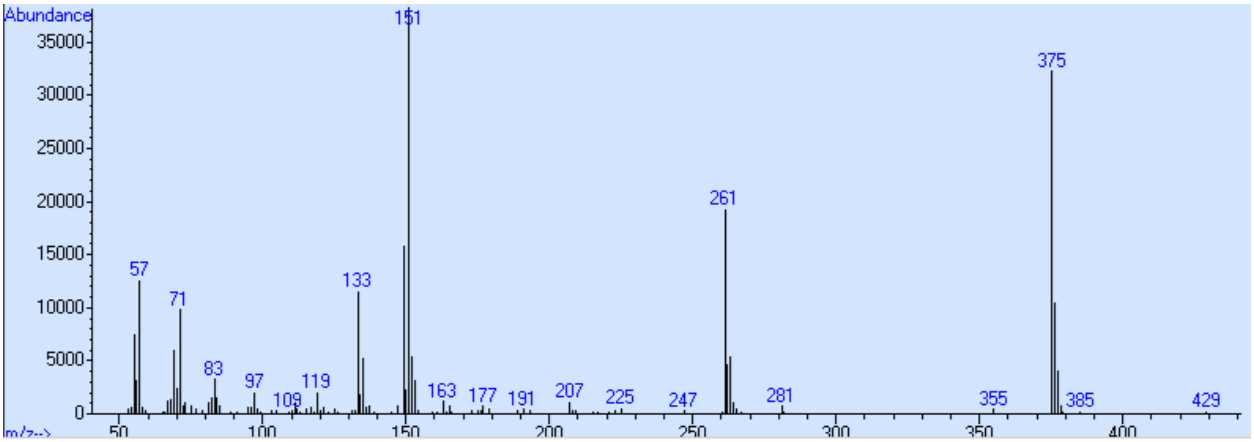
MS Spectrum 212: ECW Extracted in 1-Octanol – RT 6.514



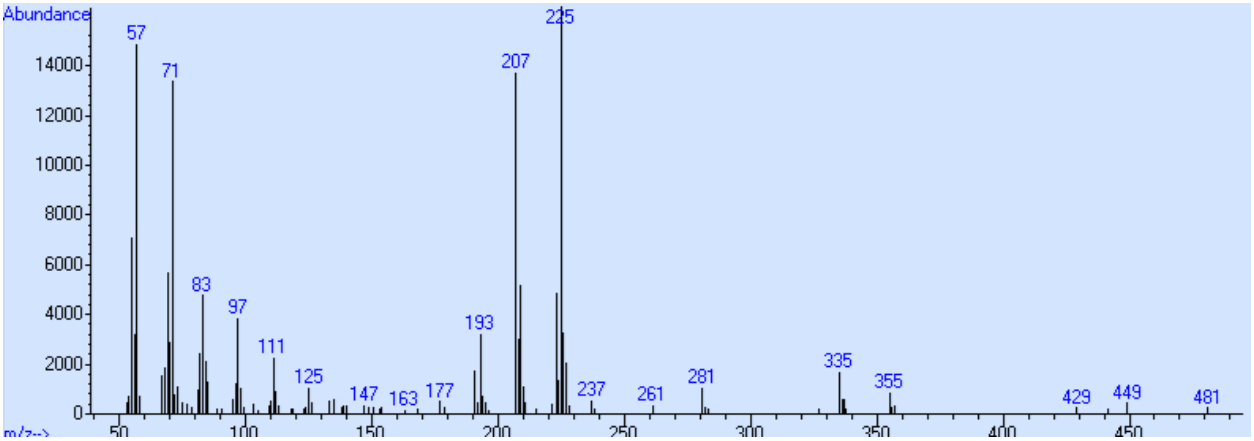
MS Spectrum 213: ECW Extracted in 1-Octanol – RT 15.921



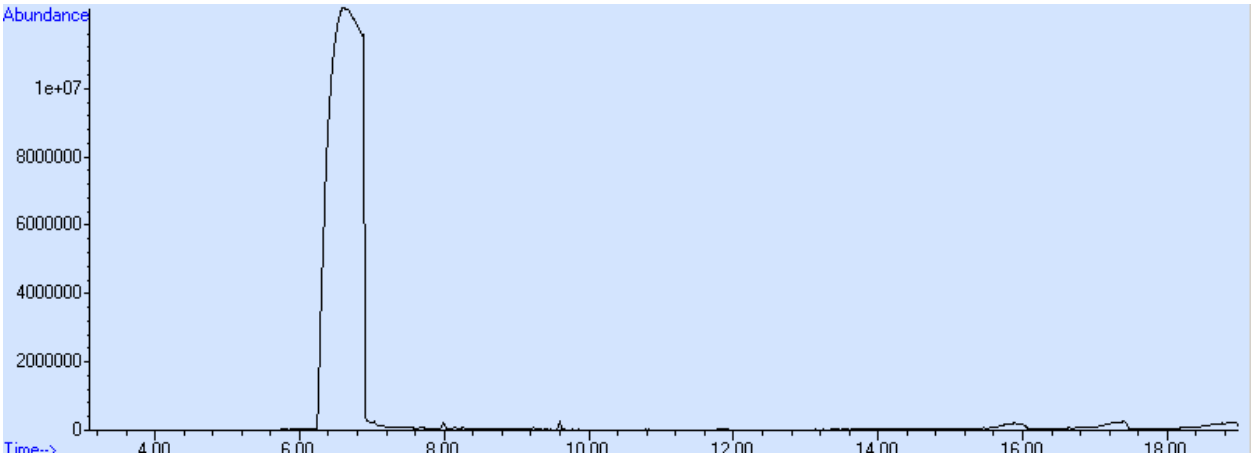
MS Spectrum 214: ECW Extracted in 1-Octanol – RT 17.272



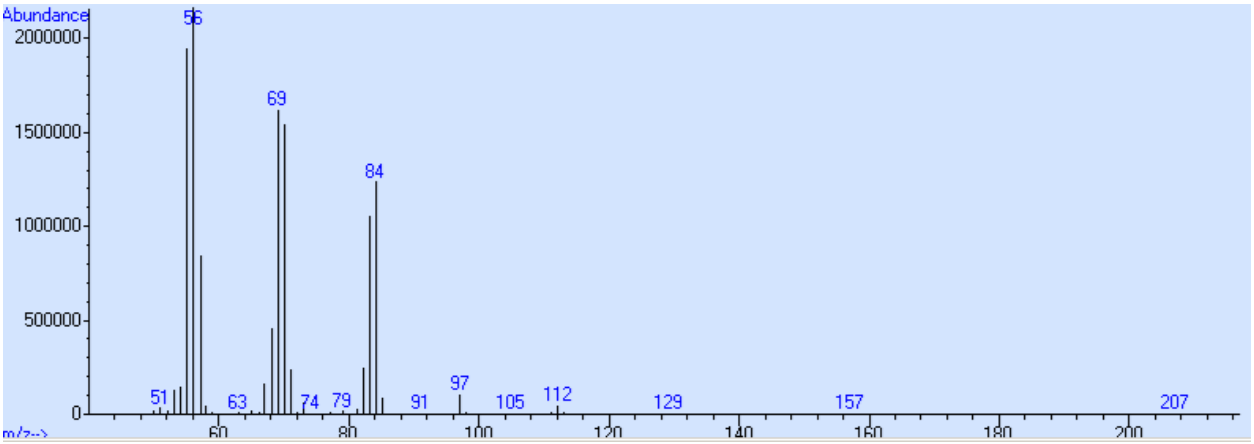
MS Spectrum 215: ECW Extracted in 1-Octanol – RT 18.776



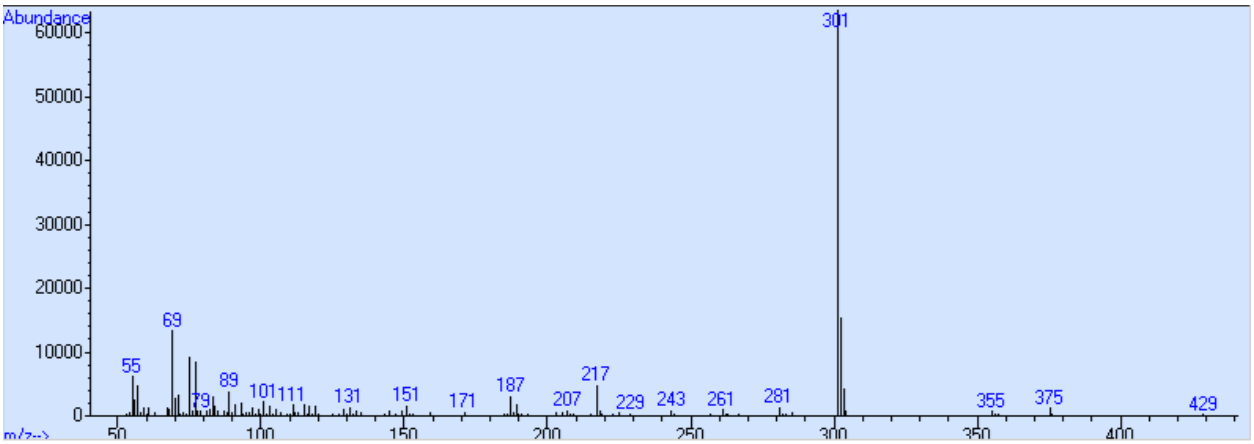
GC Spectrum 31: Que Extracted in 1-Octanol



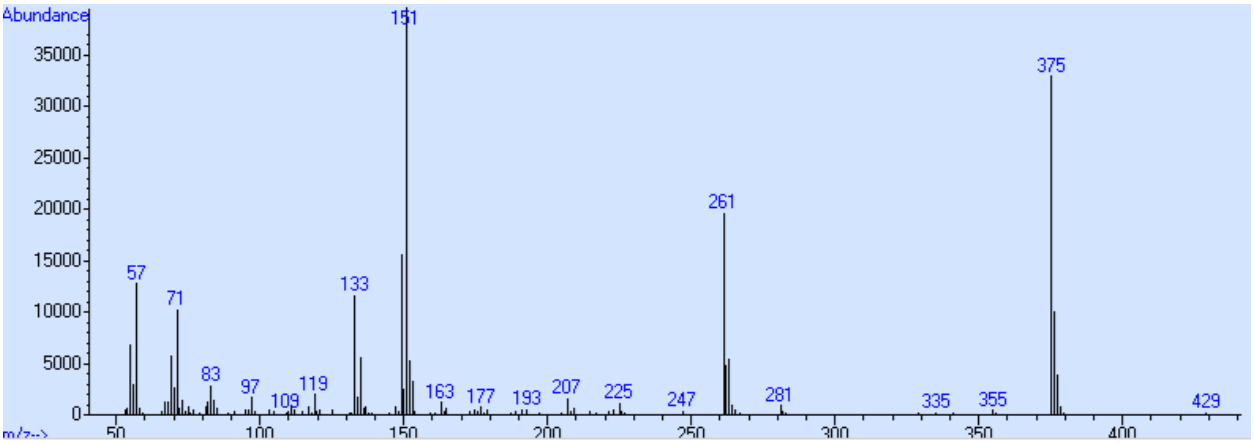
MS Spectrum 216: Que Extracted in 1-Octanol – RT 6.663



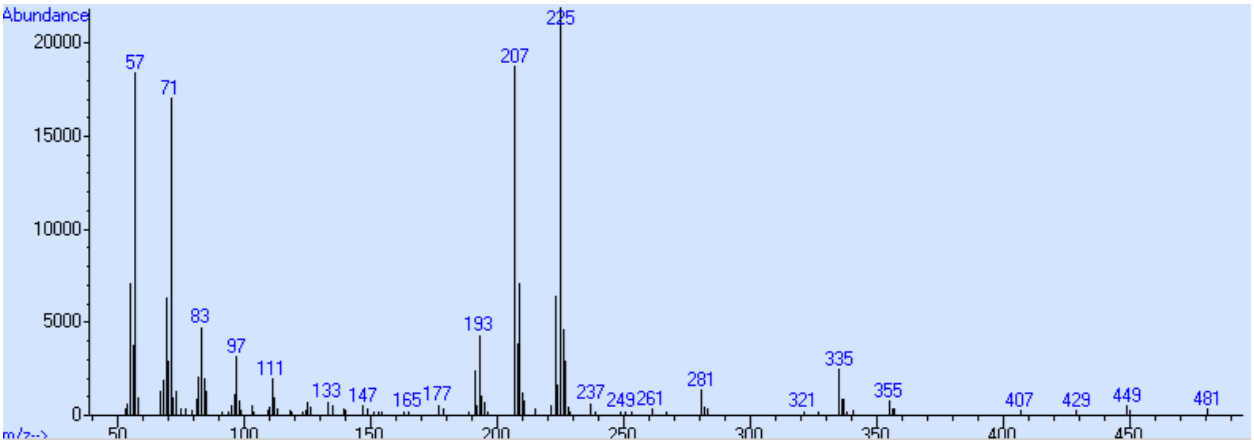
MS Spectrum 217: Que Extracted in 1-Octanol – RT 15.881



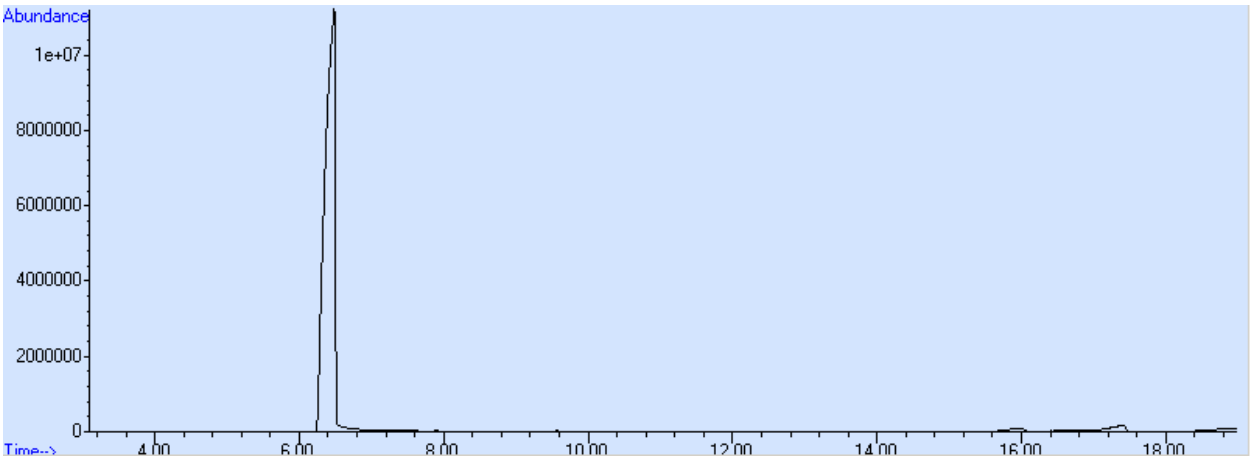
MS Spectrum 218: Que Extracted in 1-Octanol – RT 17.340



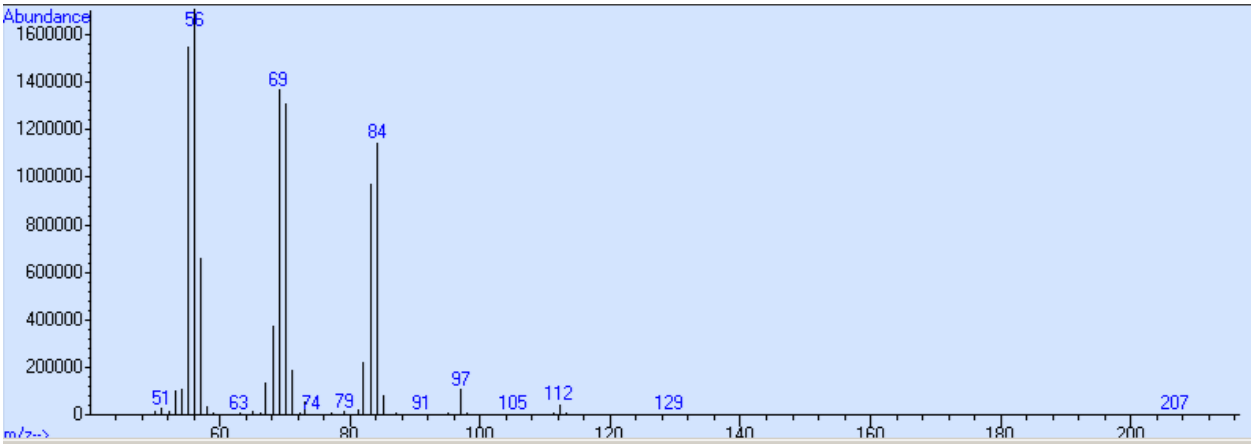
MS Spectrum 219: Que Extracted in 1-Octanol – RT 18.714



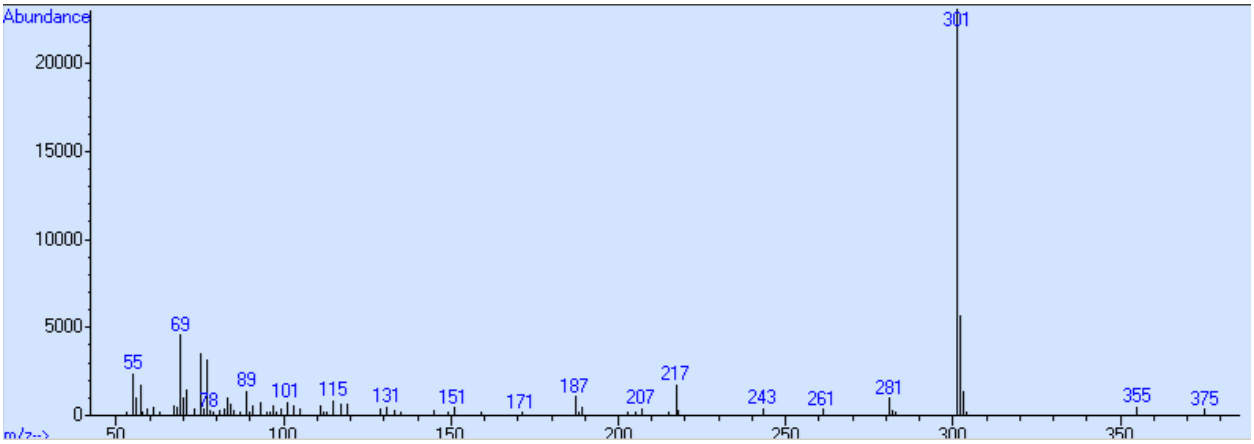
GC Spectrum 32: Undyed Caucasoid Extracted in 1-Octanol



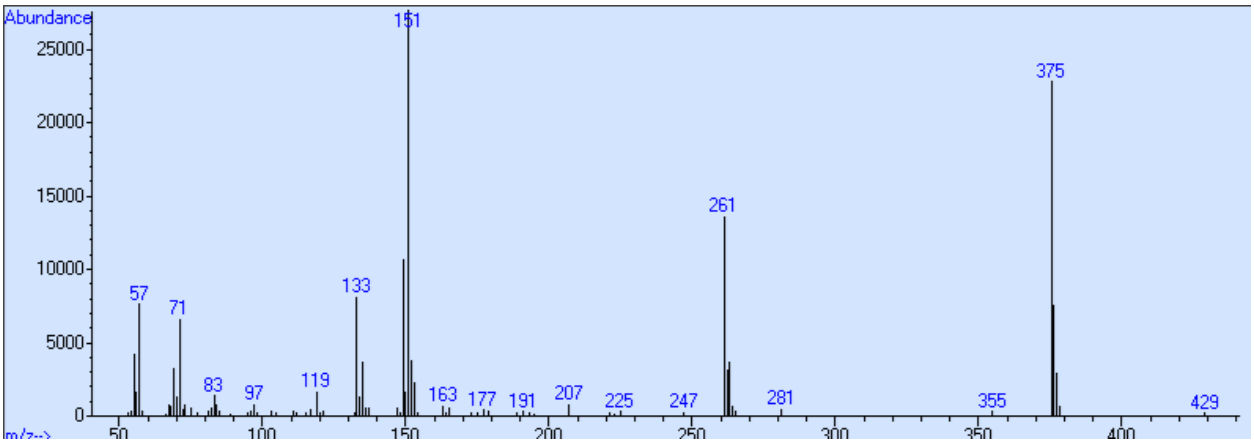
MS Spectrum 220: Undyed Caucasoid Extracted in 1-Octanol – RT 6.446



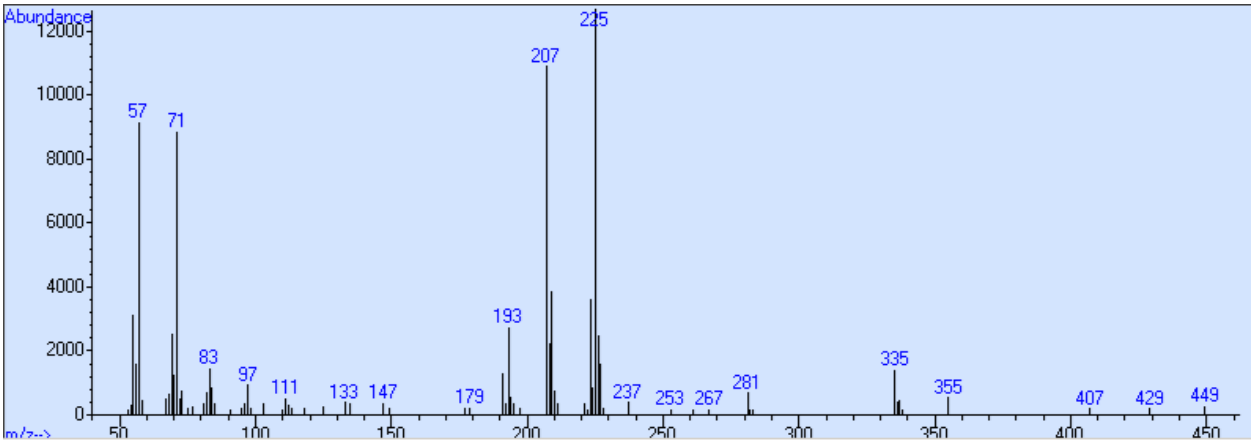
MS Spectrum 221: Undyed Caucasoid Extracted in 1-Octanol – RT 15.990



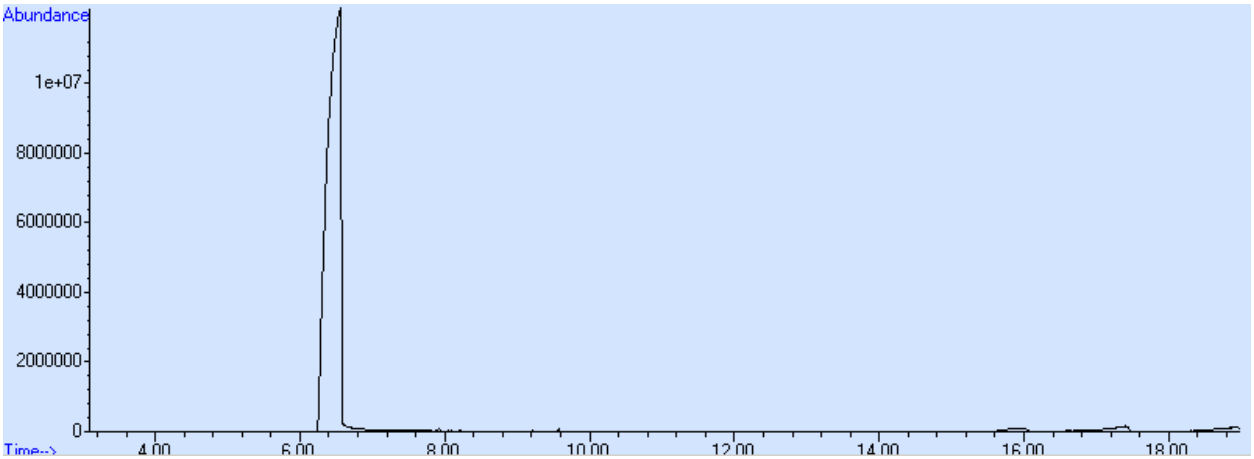
MS Spectrum 222: Undyed Caucasoid Extracted in 1-Octanol – RT 17.380



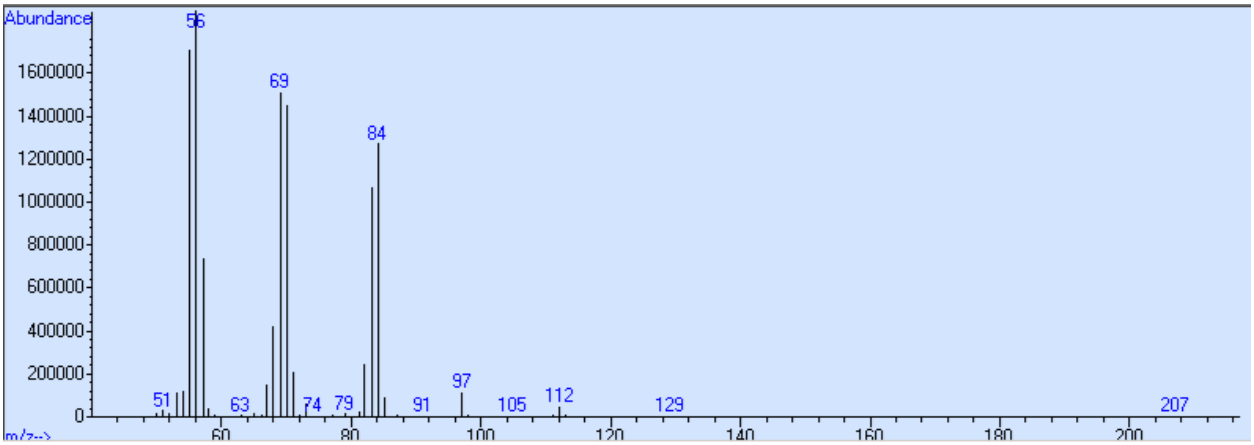
MS Spectrum 223: Undyed Caucasoid Extracted in 1-Octanol – RT 18.862



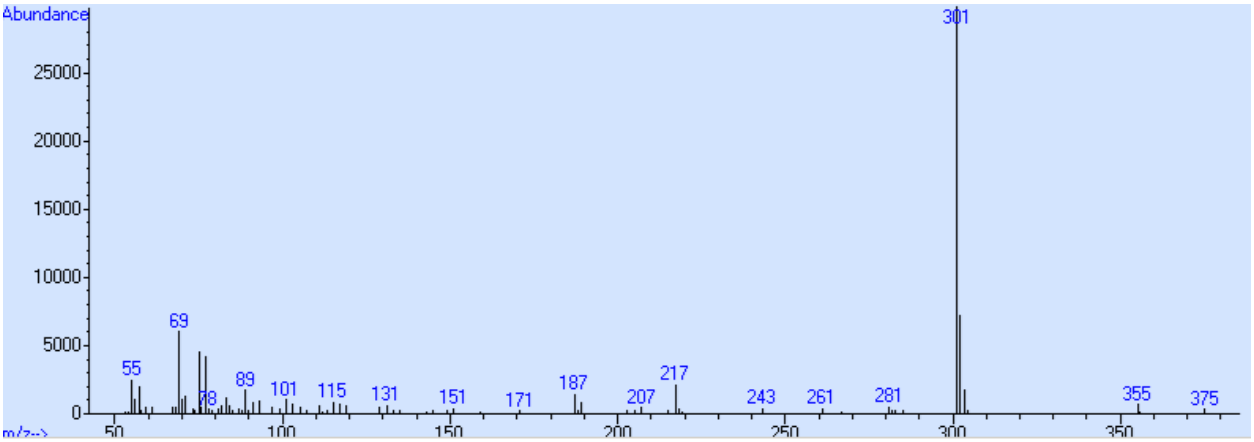
GC Spectrum 33: Dyed Caucasoid Extracted in 1-Octanol



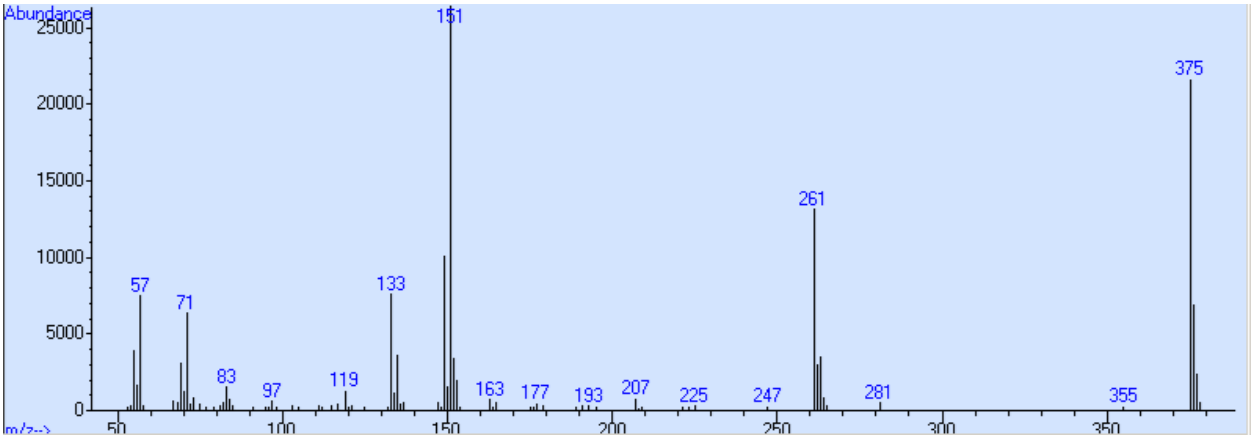
MS Spectrum 224: Dyed Caucasoid Extracted in 1-Octanol – RT 6.491



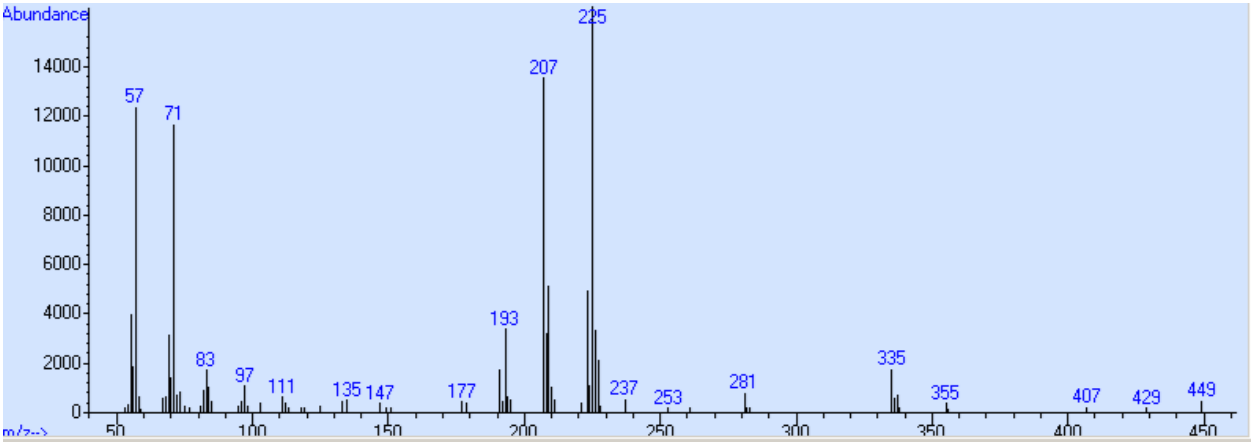
MS Spectrum 225: Dyed Caucasoid Extracted in 1-Octanol – RT 15.921



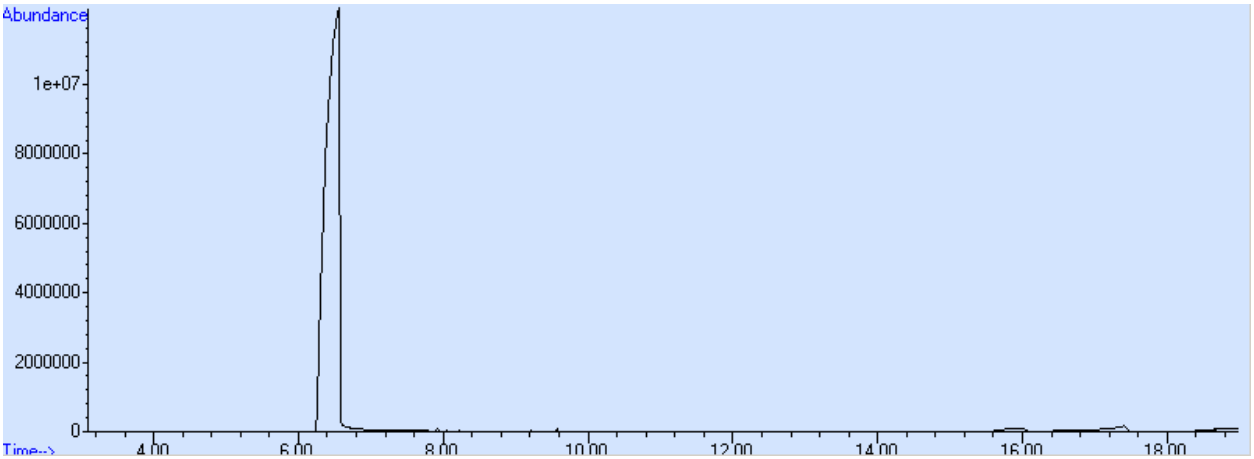
MS Spectrum 226: Dyed Caucasoid Extracted in 1-Octanol – RT 17.363



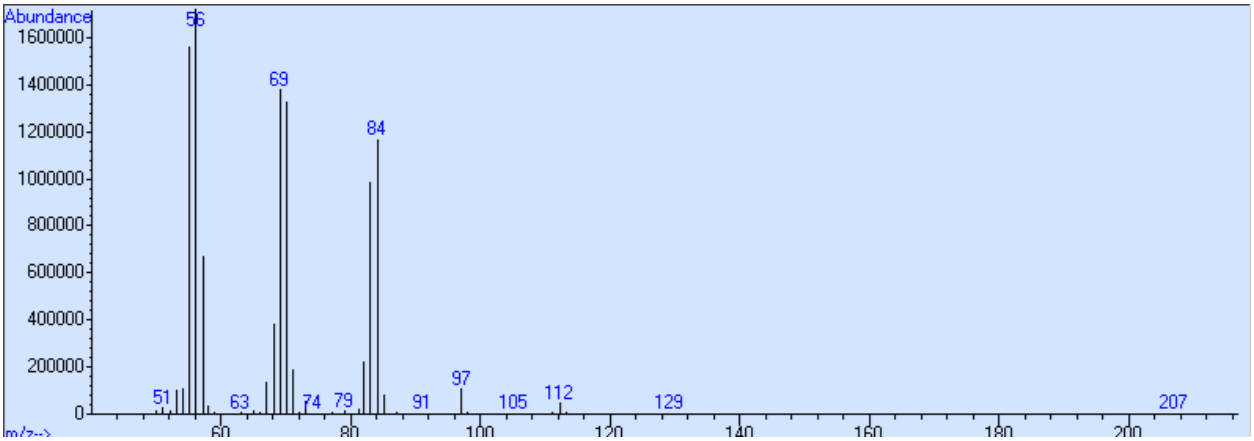
MS Spectrum 227: Dyed Caucasoid Extracted in 1-Octanol – RT 18.862



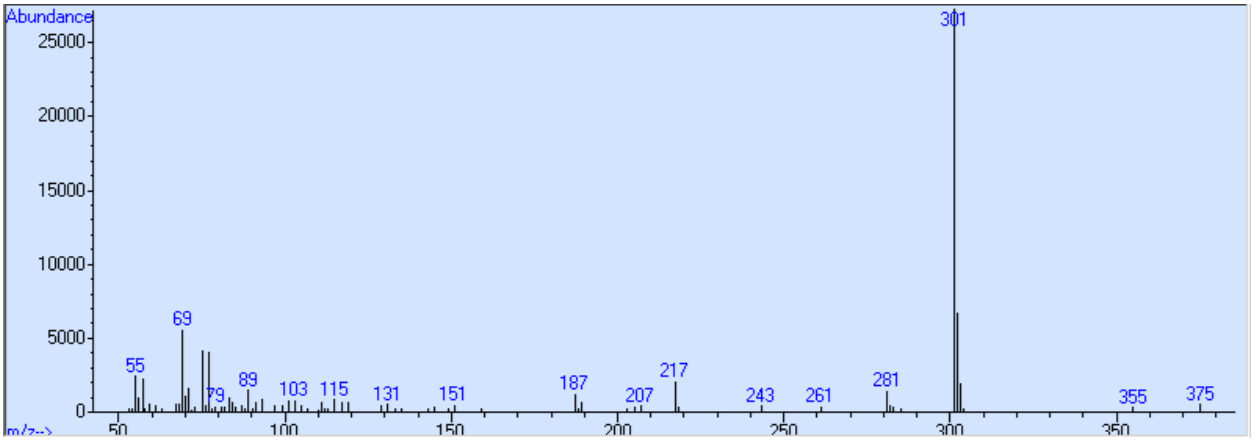
GC Spectrum 34: Undyed Mongoloid Extracted in 1-Octanol



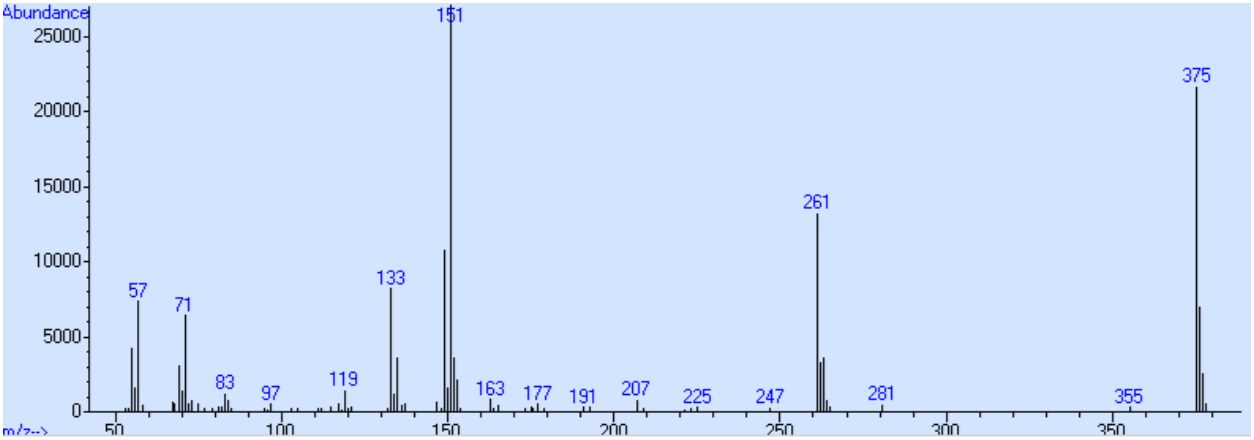
MS Spectrum 228: Undyed Mongoloid Extracted in 1-Octanol – RT 6.446



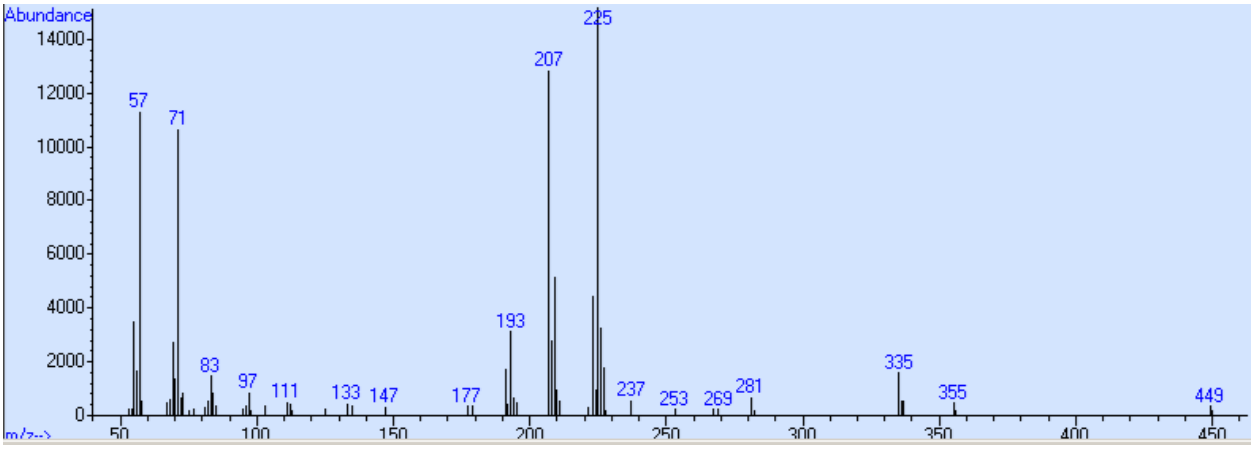
MS Spectrum 229: Undyed Mongoloid Extracted in 1-Octanol – RT 15.881



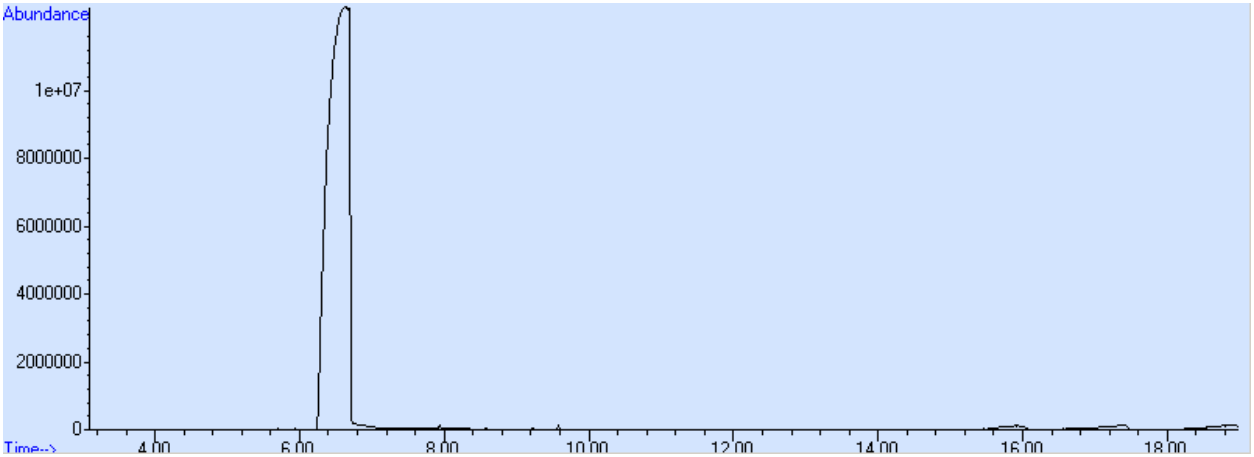
MS Spectrum 230: Undyed Mongoloid Extracted in 1-Octanol – RT 17.380



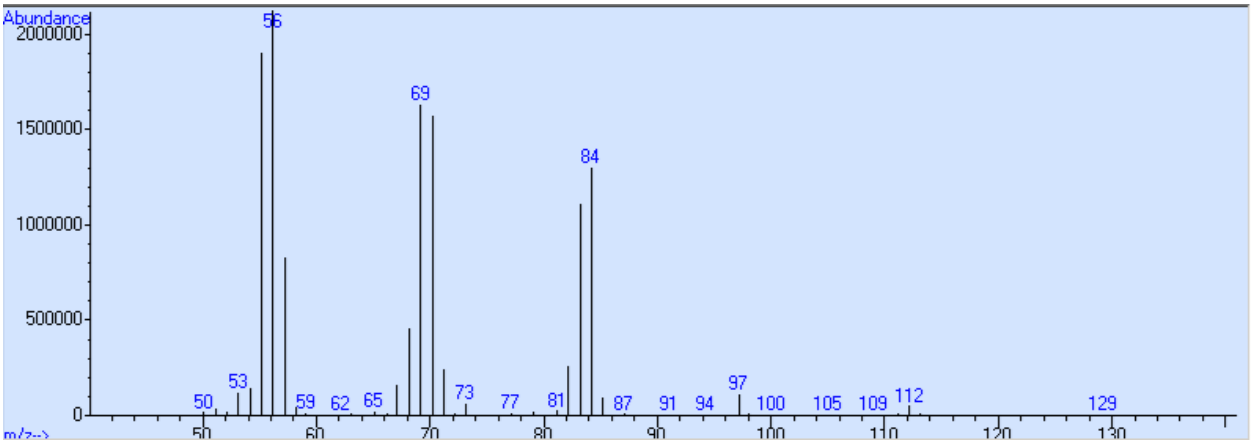
MS Spectrum 231: Undyed Mongoloid Extracted in 1-Octanol – RT 18.885



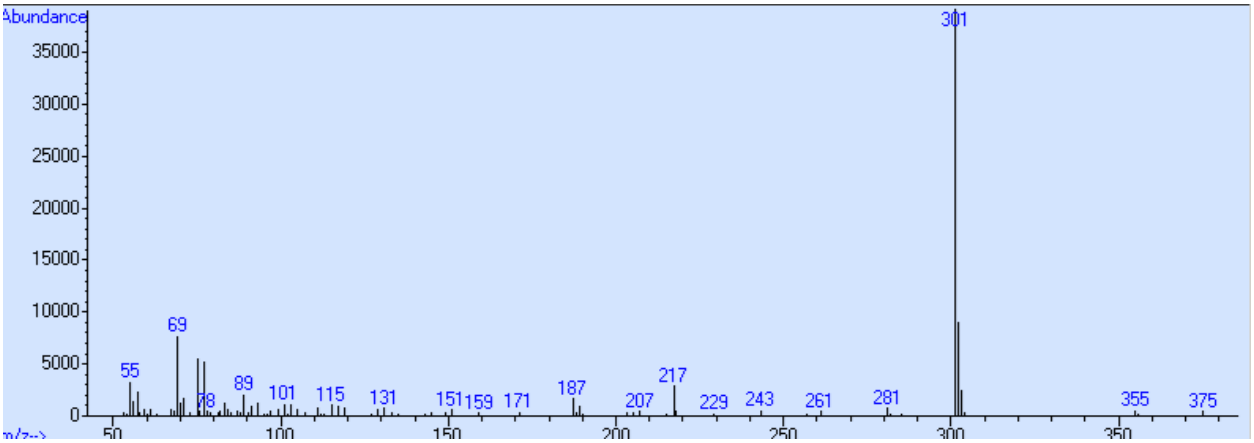
GC Spectrum 35: Dyed Mongoloid Extracted in 1-Octanol



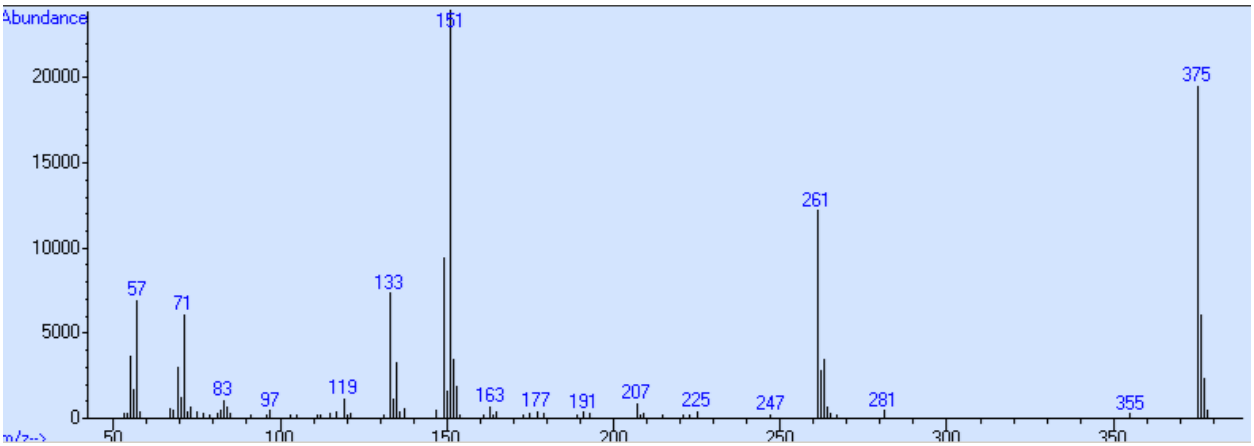
MS Spectrum 232: Dyed Mongoloid Extracted in 1-Octanol – RT 6.600



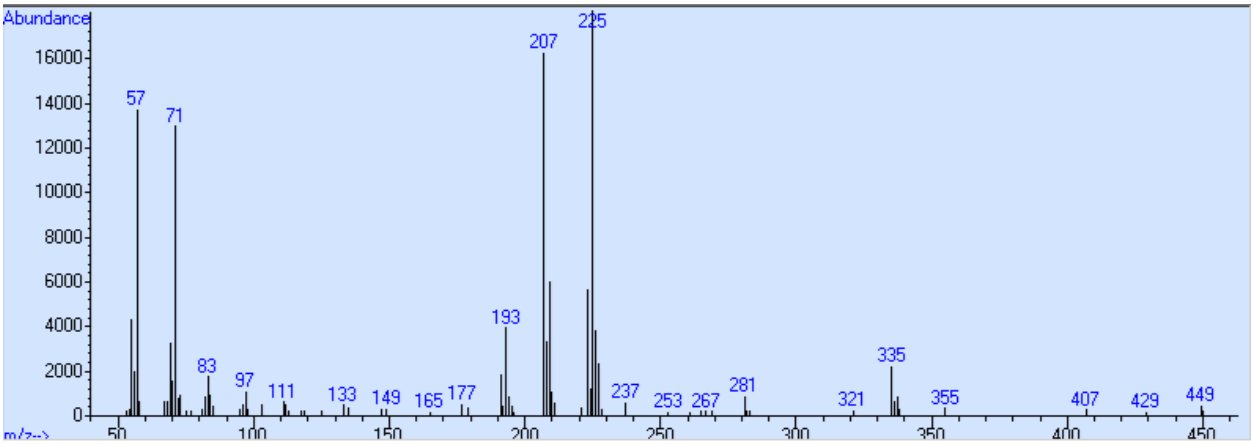
MS Spectrum 233: Dyed Mongoloid Extracted in 1-Octanol – RT 15.904



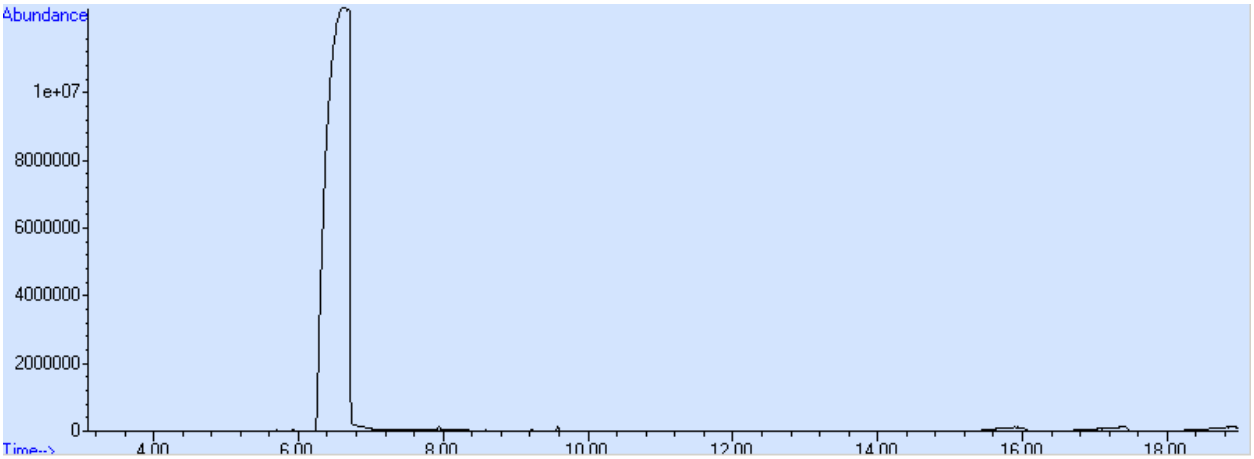
MS Spectrum 234: Dyed Mongoloid Extracted in 1-Octanol – RT 17.380



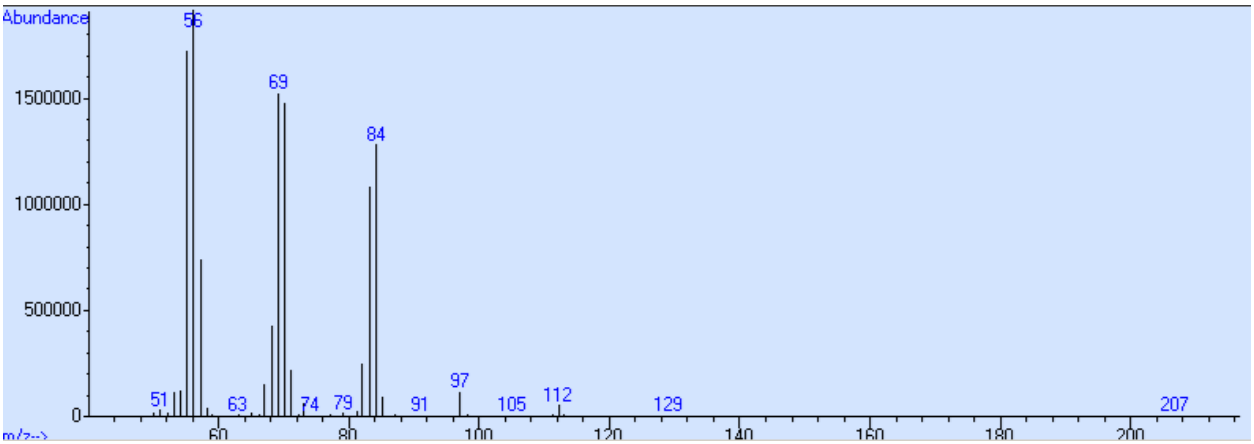
MS Spectrum 235: Dyed Mongoloid Extracted in 1-Octanol – RT 18.845



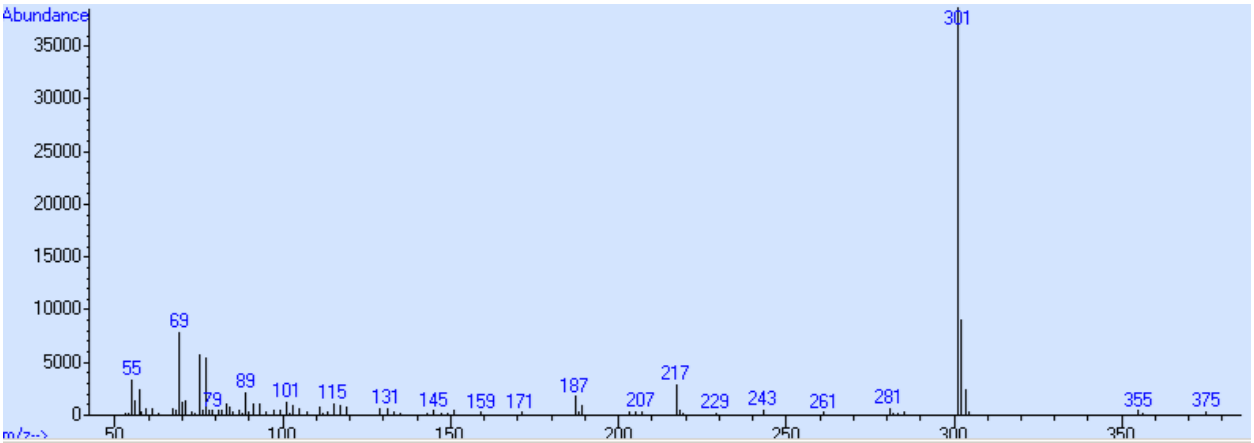
GC Spectrum 36: Undyed Negroid Extracted in 1-Octanol



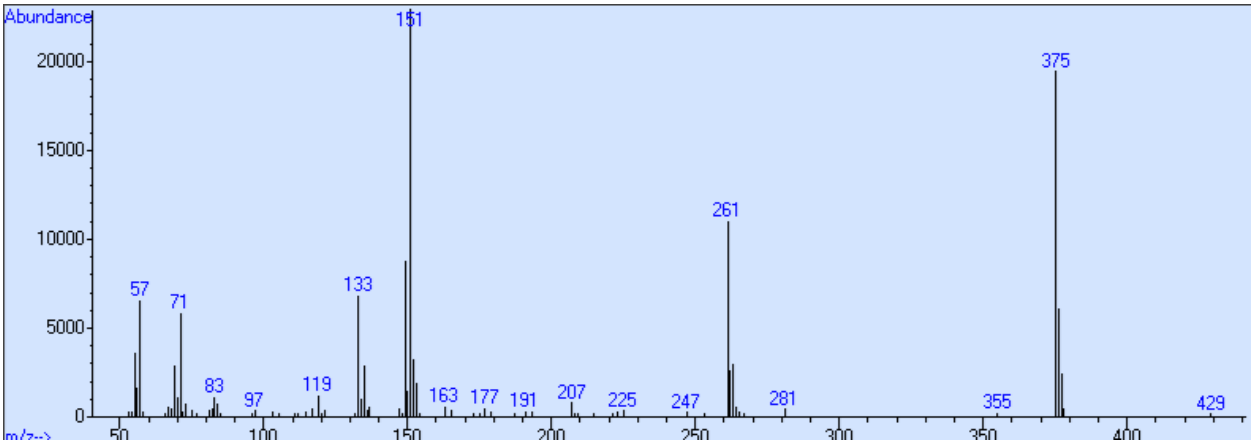
MS Spectrum 236: Undyed Negroid Extracted in 1-Octanol – RT 6.491



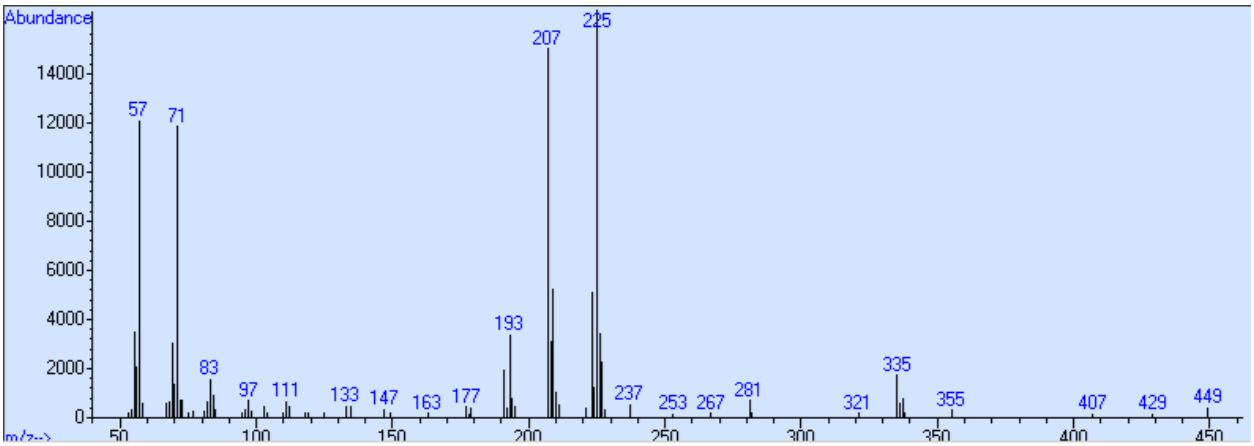
MS Spectrum 237: Undyed Negroid Extracted in 1-Octanol – RT 15.904



MS Spectrum 238: Undyed Negroid Extracted in 1-Octanol – RT 17.403



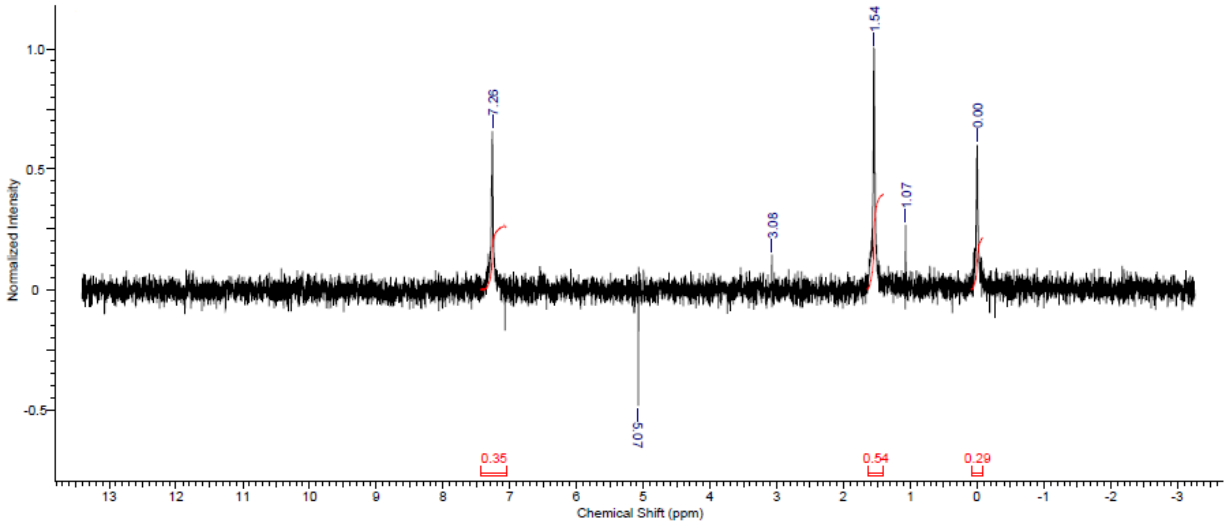
MS Spectrum 239: Undyed Negroid Extracted in 1-Octanol – RT 18.845



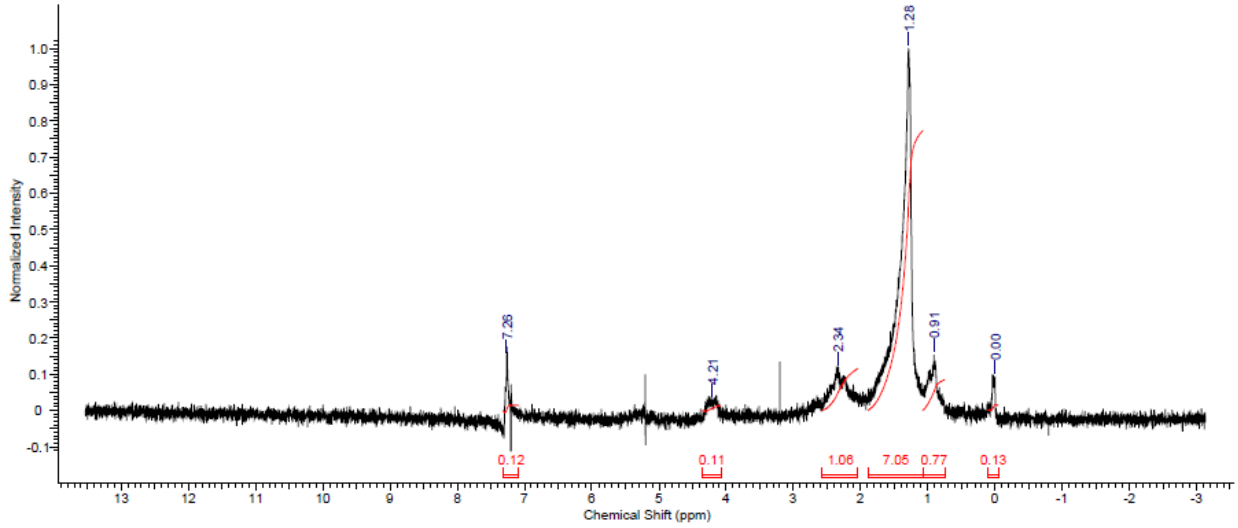
Appendix D

Nuclear Magnetic Resonance Spectroscopy Spectra

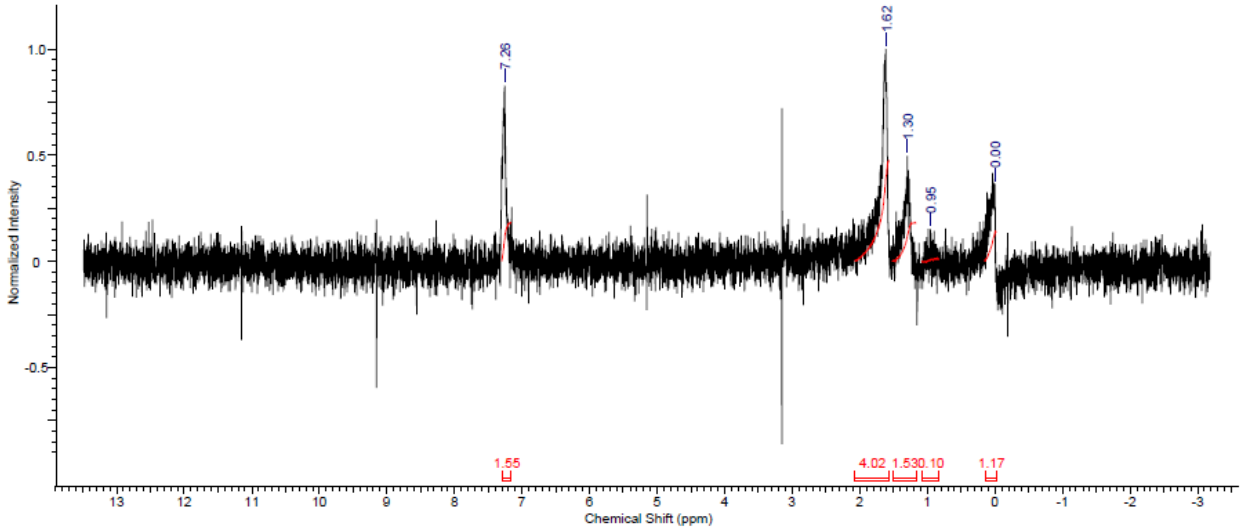
Spectrum 1: Solvent Chloroform-D



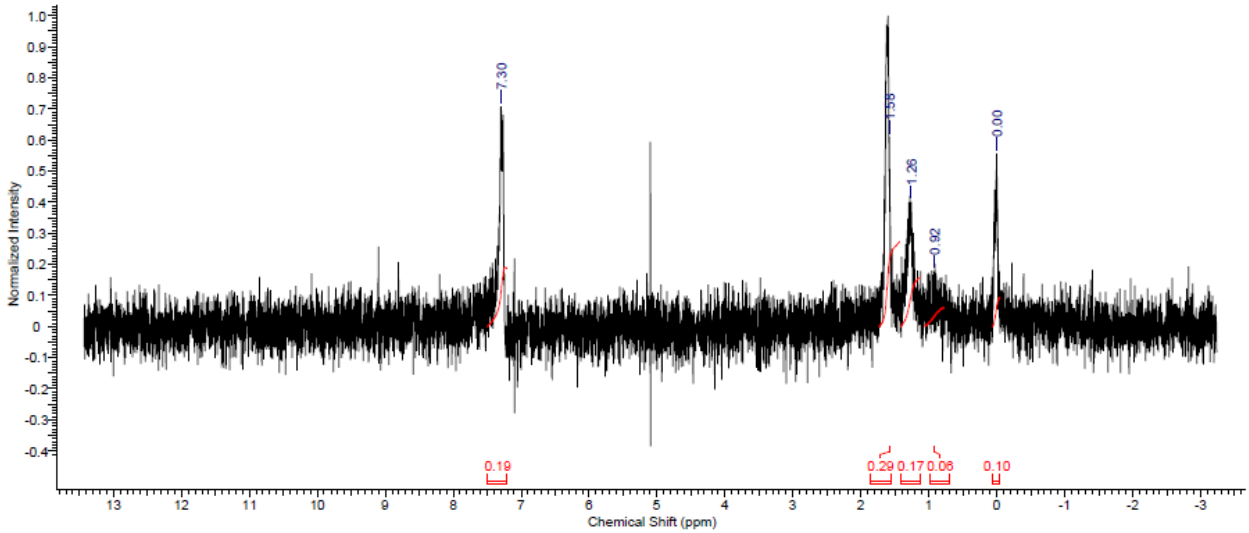
Spectrum 2: Remy XQ Extracted in Chloroform-D



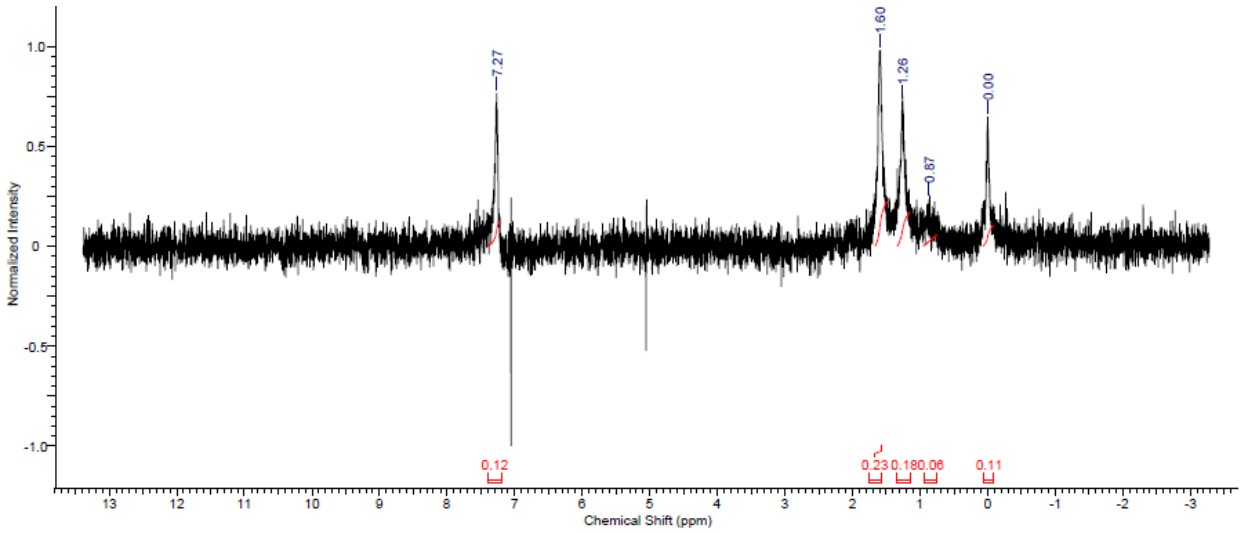
Spectrum 3: ECW Extracted in Chloroform-D



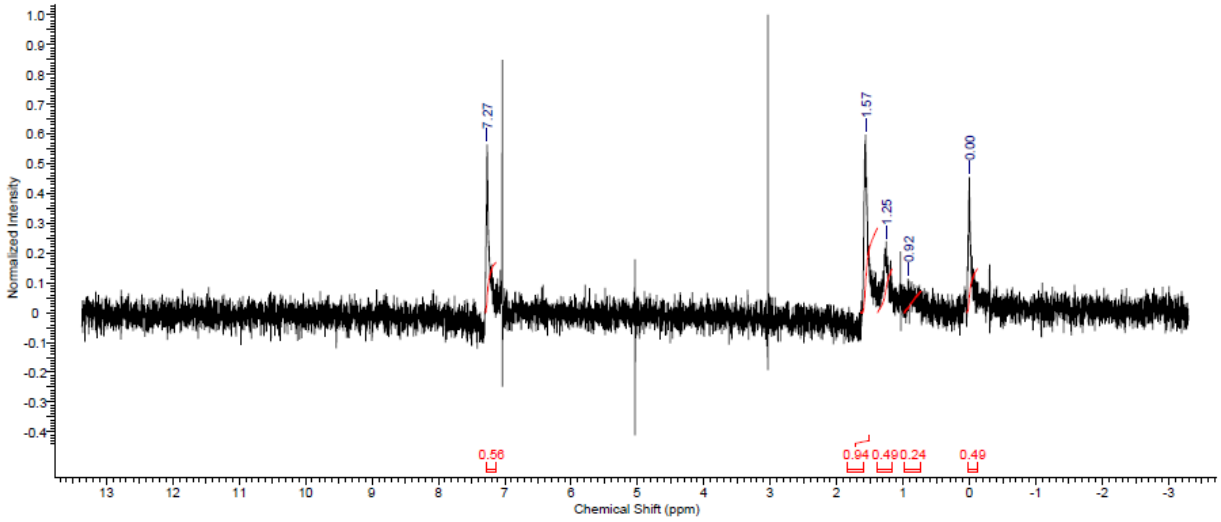
Spectrum 4: Que Extracted in Chloroform-D



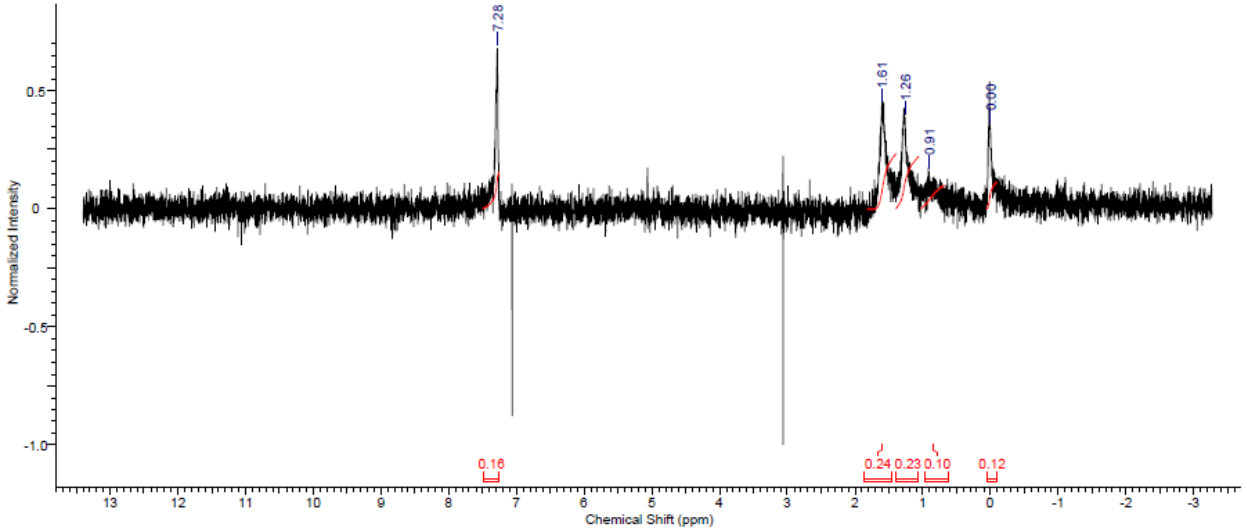
Spectrum 5: Undyed Caucasoid Extracted in Chloroform-D



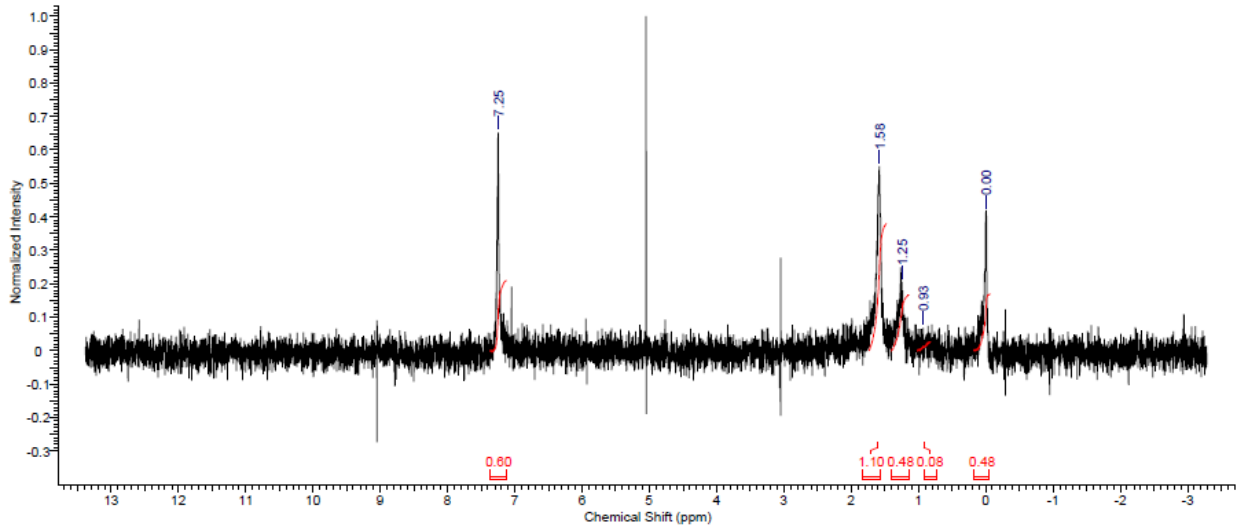
Spectrum 6: Dyed Caucasoid Extracted in Chloroform-D



Spectrum 7: Undyed Mongoloid Extracted in Chloroform-D



Spectrum 8: Dyed Mongoloid Extracted in Chloroform-D



Spectrum 9: Undyed Negroid Extracted in Chloroform-D