SEPARATION OF THREE EPHEDRA ALKALOIDS

USING GC-MS ANALYSIS

By

Kira Tokarz

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Chair:

Dr. James Girard, Ph.D.

Dr. William Hirzy, Ph.D.

Dean of the College of Arts and Sciences

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ABSTRACT

Ephedra, a plant extract, is known to contain six alkaloids—ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, methylephedrine, and methylpseudoephedrine. Of these six alkaloids, ephedrine and pseudoephedrine are present in the highest concentrations. When consumed, the effects of ephedrine include increased heart rate and blood pressure, stimulation of the central nervous system, and dilation of the bronchial tubes. The effects of pseudoephedrine include insomnia, excitability and anxiety.

Using GC-MS, this study is designed to analyze the alkaloids in Ephedra from two sources to determine if Ephedra alkaloids are present and if so, which ones.

ACKNOWLEDGMENTS

Many thanks to Dr. Girard. Your passionate teaching in Instrumental Analysis peaked my interest in analytical chemistry. It was your advising that got me through my undergraduate coursework and inspired me to pursue my Master's degree. There are no words to describe how much I appreciate your guidance on my path to my future success in my professional career.

Thank you Mom and Dad. You two are my greatest support in the world and have always been my biggest cheerleaders through all my triumphs, but more importantly, my failures, and because you were there for each of my failures, I have been able to pick myself up and transform each failure into my success.

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CHAPTER 1

INTRODUCTION

Ephedra sinica (Ma Huang) has been used for over 5,000 years in traditional Chinese medicine as a remedy for both nasal congestion and asthma. The medicinal herb is derived from the stems and branches of the plant. More than fifty species of Ephedra exist worldwide. Most common in the western hemisphere are E. Sinica, E. Intermedia, and E. Equisetina[1]. Western cultures have taken these strains and incorporated them in various dietary supplements as a stimulant to promote weight loss.

Ephedra contains alkaloids know as phenylalkylamines, which consist of a phenyl group, a carbon chain and an amine group. Ephedra plants contain between 0.02 percent to 3.4 percent of six optically active alkaloids[1]. They appear in three sets of diastereomers—(-)ephedrine and (+)pseudoephedrine, (-)methylephedrine and (+)methylpseudoephedrine, and (-)norephedrine and (+)norpseudoephedrine. Of these alkaloids, ephedrine comprises from 30-90 percent of the total alkaloids. These alkaloids have a wide range of physiologic responses including an increase in blood pressure and heart rate, as well as vasoconstriction and bronchodilation.

The use of Ephedra in America became popular in the 1920s with medical use as a nasal decongestant, central nervous system stimulant, and treatment for asthma. But the use of Ephedra declined in the following years because of safety concerns and the discovery of safer alternatives[2].

Ephedra returned to the market in a variety of dietary supplement, credited as the active ingredient aiding in weight loss. Because herbs are classified as dietary supplements in the United States, they can be sold without testing by the FDA. During the 1990s, when the dietary supplement industry expanded, science could not match the expansion with reliable methods for quality control. Many neutraceutical producers will purchase bulk material from an outside source without the ability to determine the composition[1]. A reliable means of analysis is necessary to ensure the quality of supplements on the market.

Without quality control, product misuse becomes a major issue. There are two major factors that contribute to product misuse: consumer abuse and manufacturer abuse. Consumer abuse encompasses taking more than the recommended dosage, as well as negative interactions with other prescriptions or supplements. When looking at the dietary value of a supplement, the relative value is determined by weighing the benefits of taking the drug against the health risks. A study based on data from the US poison control centers and sales information in 2001, showed that although products containing Ephedra represented less than one percent of total herbal supplement sales, 64 percent of all adverse effects reported in the year were attributed to Ephedra products. Of these adverse effects reported, hypertension was the most common, followed by palpitations, tachycardia, stroke and seizures[2]. Combining Ephedra with other stimulants can cause severe reactions. In fact, the FDA banned combinations of ephedrine, pseudoephedrine, and norephedrine with caffeine due to amphetamine like effects. Also, norpseudoephedrine and methylephedrine have potential for high abuse and are linked to

neurological disorders. Many studies have now shown that the potential for consumer misuse is very high.

The other serious factor contributing to product misuse is manufacturer abuse. This event comes in the form of false reporting of the active ingredients in supplements. The Dietary Supplement Health and Education Act (DSHEA) passed in 1994 states dietary supplements are exempt from FDA regulation. As a result, consumers must rely entirely on the neutraceutical industry for quality control. A study performed in 2000 analyzed the Ephedra alkaloid content of 20 Ephedra containing supplements using HPLC. Of the 20 products, total alkaloid content ranged from 0.0 to 18.5 mg per dosage unit. Specifically, ephedrine varied from 1.1 to 15.3 mg and pseudoephedrine ranged 0.2 to 9.5 mg. Analysis also proved variation in lot-to-lot for many products. Besides variation in alkaloid content, manufacturers are also guilty of false product labeling. Alkaloid content is normally expressed as standardized percentage of total herb content. For example, a label may read, “standardized to 6% Ephedra alkaloids”. This means total alkaloid content is calculated by taking the product of Ephedra content and standardized percentage, so if a capsule contains 300 mg, only 15 mg of this is Ephedra alkaloids. This can be confusing for a consumer to understand from the label. And even this value isn't necessarily valid. This same study found that alkaloid content varied from 0 to 154 percent of the label claim[3].

The presence of product misuse emphasizes the need for reliable forms of analysis. Many forms of analysis have been performed to determine the alkaloid content in Ephedra. These analyses include gas chromatography (GC), thin layer chromatography

(TLC), carbon 13-NMR, isotachophoresis (ITP), capillary electrophoresis (CE), and high performance liquid chromatography (HPLC). Although HPLC has been the most commonly used technique to analyze all six alkaloids[4], GC-MS is a simpler technique. Prior studies in GC methods required derivitization with N-methyl-N-trimethylsilyfrifluroacetamide (MSTFA) before analysis. The extra step adds to the time necessary for analysis, as well as increasing the overall cost. In this study, analysis was attempted with GC-MS to separate Ephedra alkaloids without derivitization.

Objectives of this Study Were:

1. Solvate samples with safe and readily available solvents
2. Design a concise GC temperature program
3. Determine which Ephedra alkaloids were present in samples, if any were present at all by GC-MS

CHAPTER 2

LITERATURE REVIEW

Since the introduction of nutritional supplements containing Ephedra, many analyses have been performed using a variety of techniques.

Analysis of Ephedra Alkaloids by FAIMS-MS

In 2003, researchers at the Institute for National Measurement Standards in Canada used field asymmetric waveform ion mobility mass spectrometry for the separation of diastereomers in Ephedra. The isomeric pairs were separated by their ion mobility differences in the gas phase. All data agreed with the conventional LC-UV method. Advantages of this method were shorter analysis time, achieved by eliminating the separation within the column, and there is no clean-up step required before sample analysis[5].

Compared Research in Standard Reference Materials

Combined research in 2005, facilitated by the National Institute of Standards and Technology, used five separate methods tested by four separate labs to analyze Standard Reference Materials (SRMs) containing Ephedra. The SRMs were prepared by NIST and analyzed through NIST and three participating labs. LC/UV proved to be lengthy because of extended run times required to elute all compounds in the matrix. LC/MS showed no interferences between compounds and was a much more sensitive approach to the

determination of the alkaloids. LC/MS/MS showed similar results to the LC/MS method, but with greater sensitivity and the ability to measure methylephedrine and methylpseudoephedrine. When using CE, only ephedrine and pseudoephedrine were targeted due to a lower sensitivity[6].

Analysis of Ephedra Alkaloids by ACPI-MS

In 2006, the FDA sponsored research on separation of both ephedrine and synephrine alkaloids using atmospheric pressure chemical ionization and mass spectrometric detection. Although this procedure proved to identify the alkaloids, it was concluded that there was potential for incomplete elution. This combined with the necessity of a multistep cleanup process shows that a simple filtration approach followed by MS/MS is a much easier procedure[7].

Analysis of Ephedra Alkaloids by HPLC

The FDA also performed research in this area using high performance liquid chromatography (HPLC). HPLC was used to determine all six alkaloids in Ephedra. A strong cation exchange (SCX) was used to further filter the aliquot and clean the column. The separated alkaloids were measured using UV and fluorescence detectors. Ephedrine and pseudoephedrine were accurately identified and compared to analysis by LC-MS[8].

Analysis of Ephedra Alkaloids by GC-MS

One of the earliest studies in gas chromatography to analyze Ephedra alkaloids was performed in Japan in the 1970s. By treating the samples with acetone, the

oxazolidine derivatives of ephedrine and pseudoephedrine were detected. The derivatives were formed through a reflux reaction carried out for more than 48 hours.

Joint efforts by two Chinese universities analyzed plant samples collected from several areas of China. Separation of all six Ephedra alkaloids was achieved by trimethylsilylation of the hydroxy and amino groups. The plant samples were treated with N-methyl-N-trimethylsilyltrifluroacetamide (MSTFA) and all six alkaloids were separated and identified by gas chromatography using a nitrogen specific detector[9].

Another analysis using MSTFA was performed in 2001. A two-step process was employed, where the sample was combined with HCl as an internal standard and dried using nitrogen gas at 50 degrees Celsius, and then titrated with MSTFA. This solution was analyzed using GC-MS with auto-quant software to target selected ions at specific retention times. A pierce-heating module was used to control evaporation and derivation temperatures. Five of the six alkaloids were resolved through this procedure[10].

These GC-MS studies identified five or six of the Ephedra alkaloids. Both were performed using MSTFA, which is a highly flammable liquid that must be stored between 2-8 degrees Celsius. MSTFA was used in a derivitization step prior to analysis by GC-MS. The aim of this research is to employ a straightforward technique, such as these studies did, but also to determine whether the alkaloids can be separated without the derivitization step, which would make this analysis faster and cheaper.

CHAPTER 3

METHODS AND MATERIALS

In this chapter, details will be given about the preparation of samples for analysis.

Glassware

All glassware was cleaned and rinsed with deionized water and then allowed to dry in an oven before being used for analysis.

Chemicals

Ephedra Powder

Ephedra powder was obtained from mountainroseherbs.com. The powder was weighed out to approximately 1 gram and transferred to a beaker with 100 mL of ethanol. It was then heated at 30 degrees Celsius and stirred for one hour. This solution was covered and refrigerated until used for analysis.

Ephedra Tea

Ephedra tea was ordered online and came as a liquid beverage. This source of Ephedra was prepared in three different ways. First, the sample was analyzed as is. In the second preparation, the tea was diluted 1:1 by measuring 50 mL of the liquid and combining it with 50 mL of ethanol. This solution was covered and refrigerated until used for analysis. In the third preparation, the liquid was dried and it yielded 0.5237

grams of solid that was then diluted in 50 mL of ethanol. This solution was covered and refrigerated until used for analysis.

Solvent

Ethanol (CAS # 64-17-5) was used for all preparations of Ephedra samples.

Instrument

The instrument used for all analysis was a Shimadzu GCMS-QP5050A Gas Chromatograph, Mass Spectrometer using Helium as the carrier gas.

GC Instrument Conditions

Control Mode	Split
Column Inlet Pressure	100 kPa
Column Flow	1.5 mL/minute
Linear Velocity	44.7 cm/second
Split Ratio	31
Total Flow	50 mL/minute

MS Instrument Conditions

Acquisition Mode	Scan
Detector Voltage	1.26 kV
Threshold	1000
Solvent Cut Time	2 minutes
Interval	0.3 seconds/scan

Experimental Design

Prior GC-MS analysis of Ephedra was used as a basis for this study. Specifically the GC temperature program was used as a template, but because of the difference in chemical solutions, the temperature program was modified through trial runs.

Analysis of Ephedra Powder

3 microliters was injected into the instrument and the GC temperature program for the solution is as follows:

Injection port temperature	225°C
Interface temperature	225°C
Initial column temperature	100°C
Initial column temperature held	3 minutes
Temperature ramp (100-160°C)	20°C/minute
Temperature ramp (160-200°C)	5°C/minute
Final temperature held	3 minutes

The total program time is 17 minutes.

Analysis of Ephedra Tea

For all three preparations of Ephedra tea, 3 microliters of the tea sample were injected. The GC temperature program used was:

Injection port temperature	225°C
Interface temperature	225°C

Initial column temperature	80°C
Initial column temperature held	3 minutes
Temperature ramp (100-160°C)	20°C/minute
Temperature ramp (160-200°C)	5°C/minute
Final temperature held	3 minutes

The total program time is 18 minutes.

Data Analysis

All data was analyzed with Shimadzu MS post run software.

CHAPTER 4

RESULTS

Ephedra Powder Samples

Approximately one gram of Ephedra powder was weighed and dissolved in 100 mL of ethanol. For the Ephedra powder samples, the injection and interface temperatures of the GC were held at 225 degrees Celsius. The initial column temperature was 100 degrees Celsius and this temperature was held for three minutes. It was then raised at a rate of 20 degrees Celsius per minute to 160 degrees Celsius. The column temperature was then raised at a rate of five degrees Celsius per minute to 200 degrees Celsius and held at this final temperature for three minutes. Using this temperature program to analyze the Ephedra solution gave consistent results over three trials. The GC chromatogram in Figure 1 shows these results:

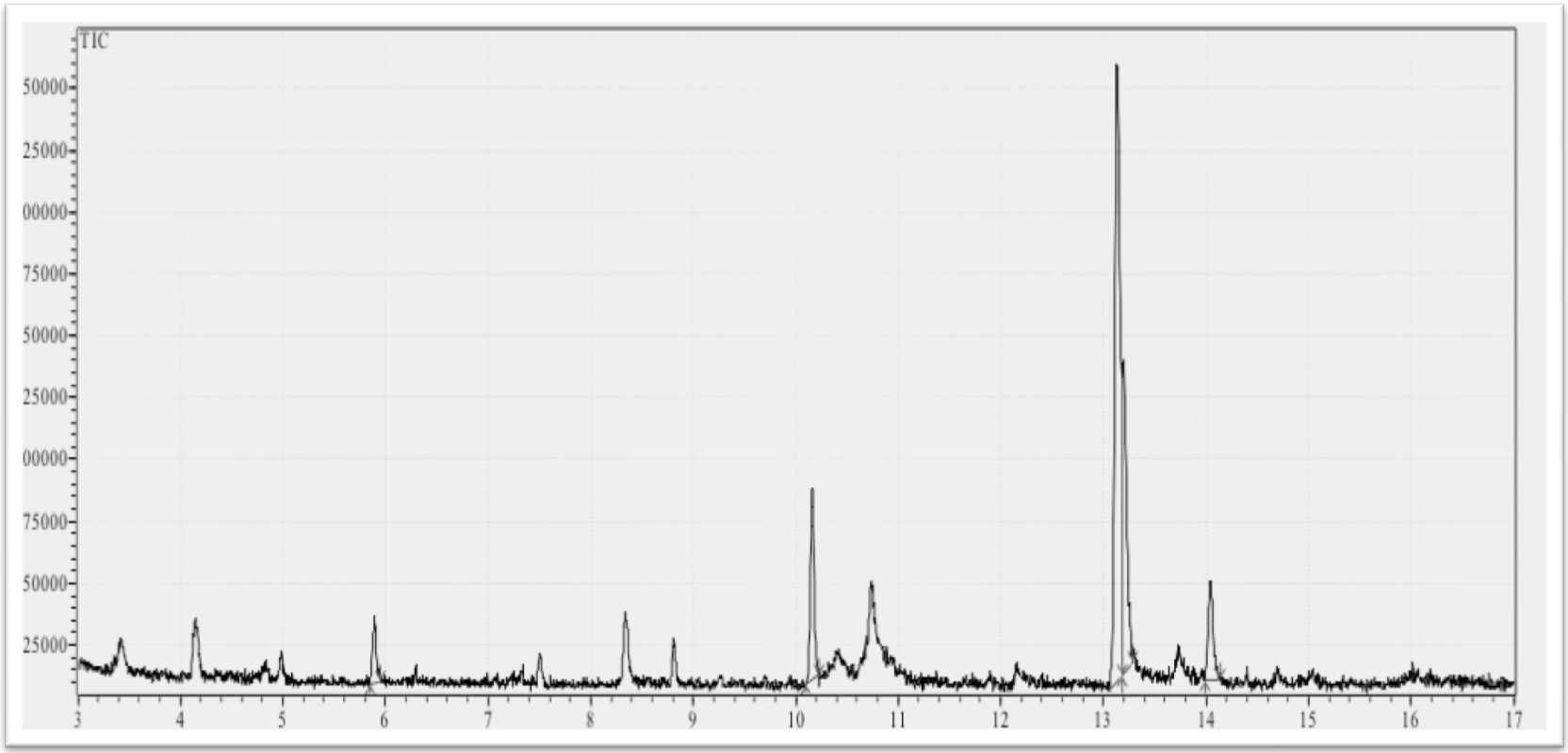

Figure 1: GC Chromatogram of Ephedra Powder

From the GC chromatograms, the four peaks listed in Table 1 were identified by their MS spectra. Tables 1 through 3 show that each of the three analyses produced identical compounds for the four peaks, as well as consistent retention times that were within one tenth of a minute of each other.

Table 1: Identified Peaks from First Run of Ephedra Powder

Compound	M/z	Retention time
1,2,3-Propanetriol	43.00	10.150
Ephedrine	58.00	13.130
Pseudoephedrine	58.00	13.185
(+)-N-Methylephedrine	72.00	14.035

Table 2: Identified Peaks from Second Run of Ephedra Powder

Compound	M/z	Retention time
1,2,3-Propanetriol	43.00	10.160
Ephedrine	58.00	13.130
Pseudoephedrine	58.00	13.200
(+)-N-Methylephedrine	72.00	14.040

Table 3: Identified Peaks from Third Run of Ephedra Powder

Compound	m/z	Retention time
1,2,3-Propanetriol	43.00	10.165
Ephedrine	58.00	13.140
Pseudoephedrine	58.00	13.205
(+)-N-Methylephedrine	72.00	14.045

To determine the identity of each peak, the mass spectra were recorded and compared through Shimadzu post-run software and indentified at a confidence interval above 80 percent. Each spectrum was compared to the spectra in the NIST database.

The mass spectrum of the peak at retention time 10.1 minutes can be seen in Figure 3. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 2, which is 1,2,3-Propanetriol.

The mass spectrum of the peak at retention time 13.13 minutes can be seen in Figure 5. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 4, which is Ephedrine.

The mass spectrum of the peak at retention time 13.18 minutes can be seen is Figure 7. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 6, which is Pseudoephedrine.

The mass spectrum of the peak at retention time 14.03 minutes can be seen in Figure 9. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 8, which is Methylephedrine.

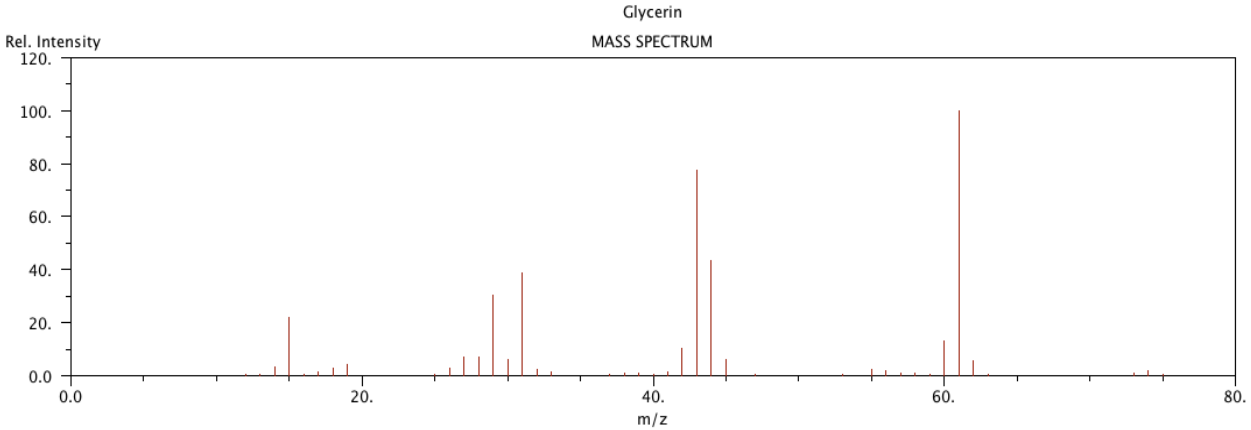

Figure 2: Mass Spectrum of Glycerin (1,2,3-Propanetriol) from NIST Database

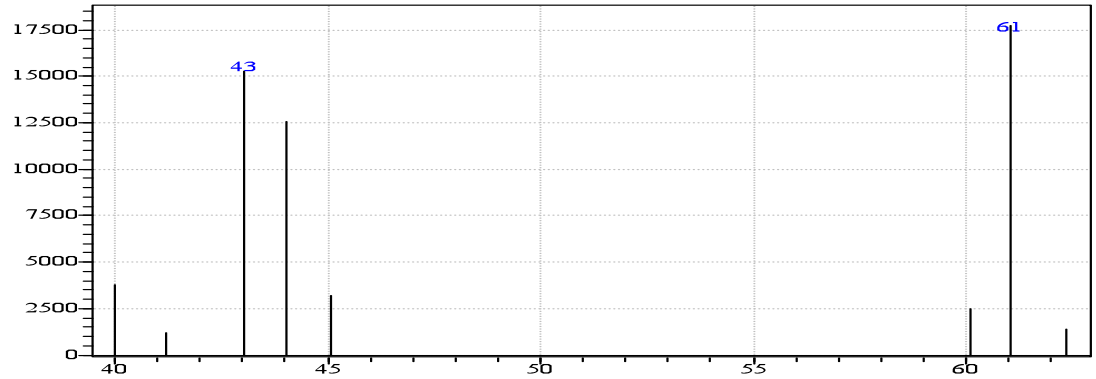

Figure 3: Mass Spectrum of Glycerin (1,2,3-Propanetriol) from Ephedra Powder

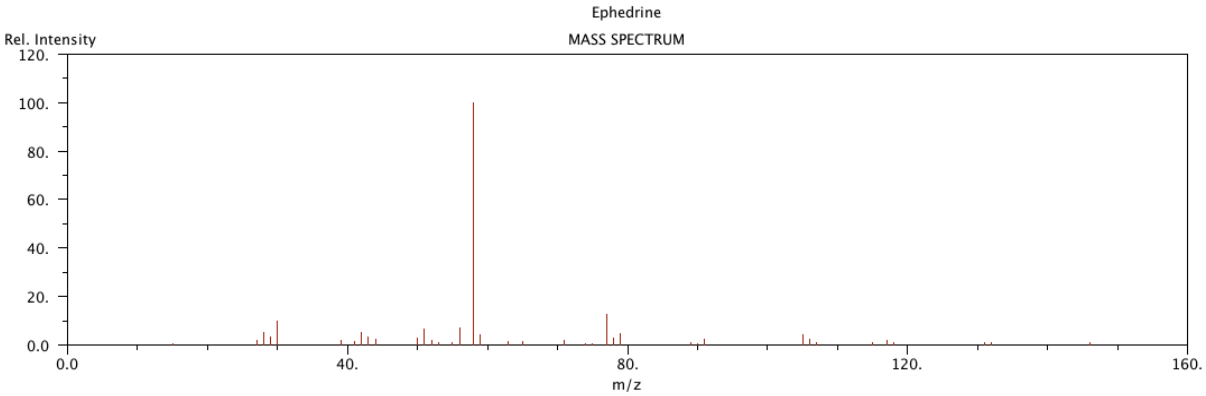

Figure 4: Mass Spectrum of Ephedrine from NIST Database

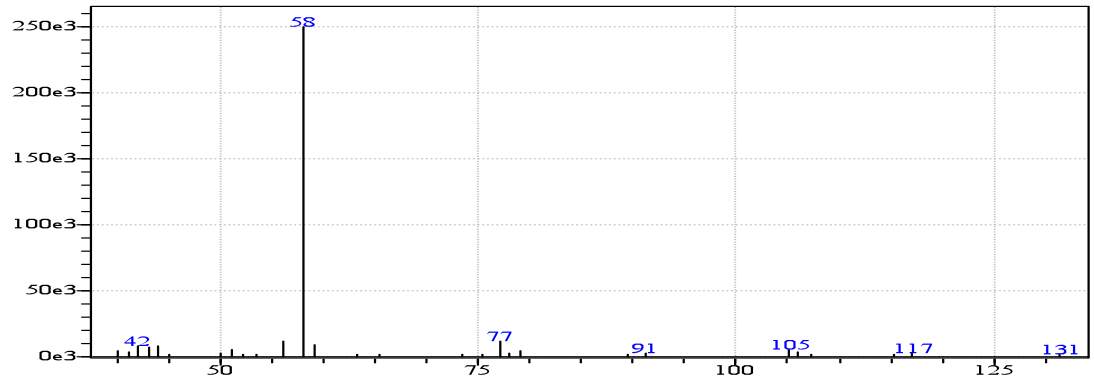

Figure 5: Mass Spectrum of Ephedrine from Ephedra Powder

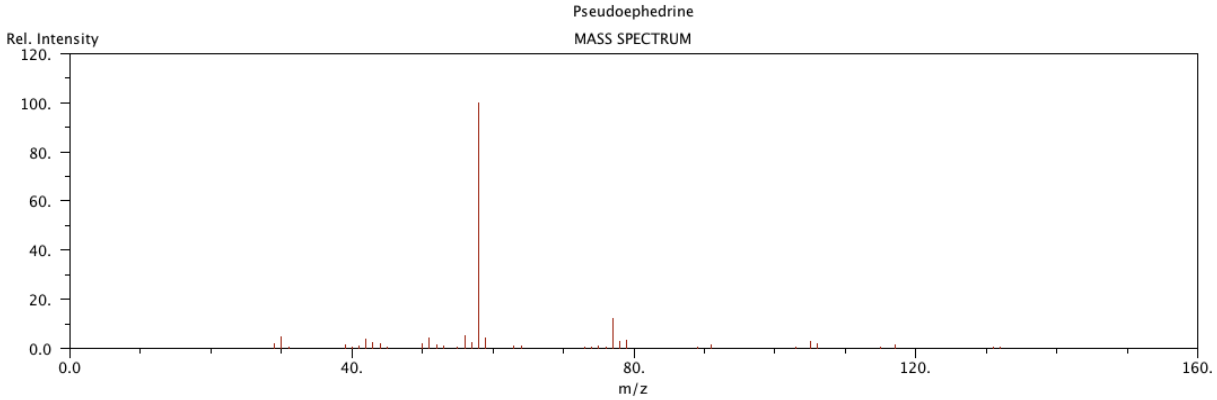

Figure 6: Mass Spectrum of Pseudoephedrine from NIST Database

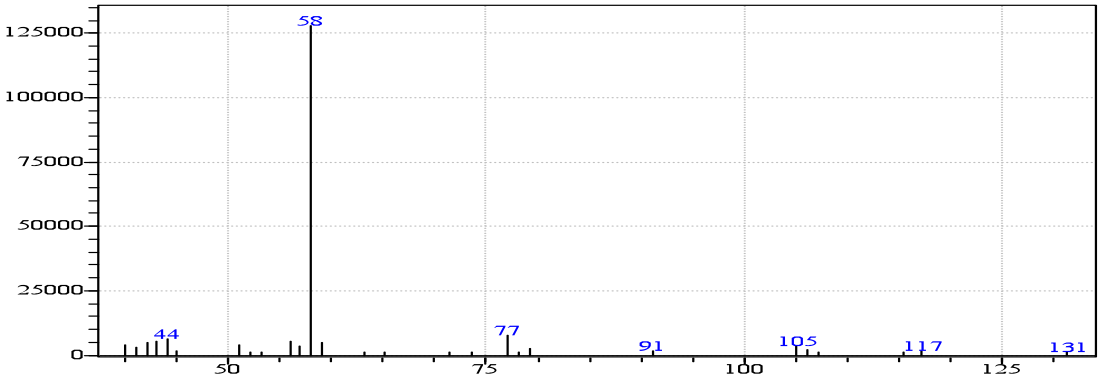

Figure 7: Mass Spectrum of Pseudoephedrine from Ephedra Powder

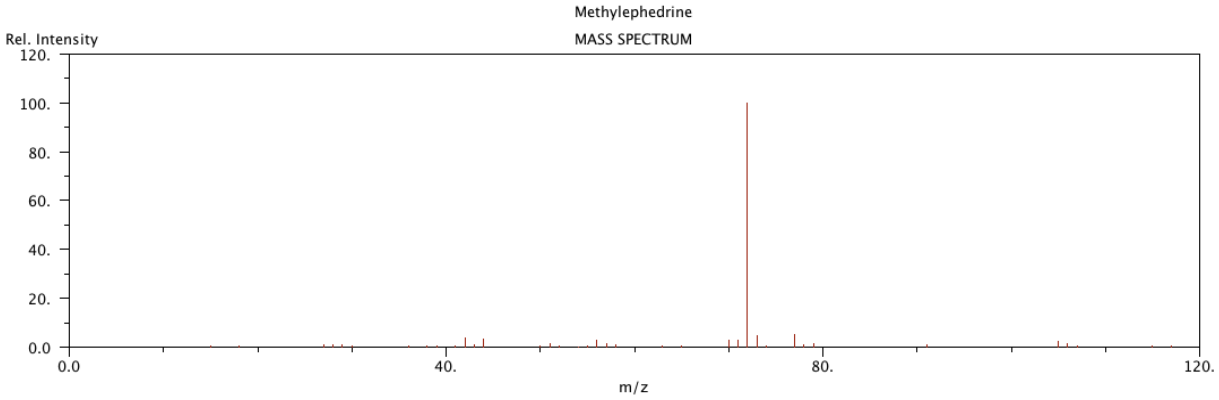

Figure 8: Mass Spectrum of Methylephedrine from NIST Database

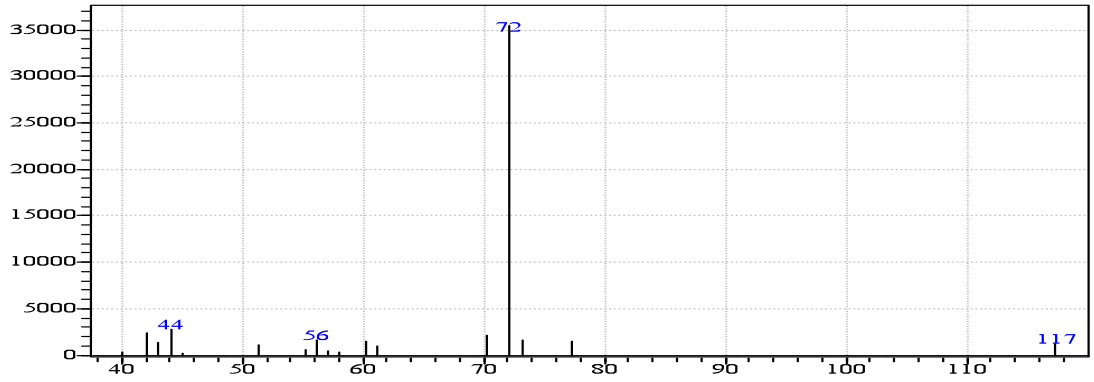

Figure 9: Mass Spectrum of Methylephedrine from Ephedra Powder

Tea Samples

When the tea samples were analyzed using the temperature program developed for the Ephedra powder, no peaks were found at the retention times found for Ephedrine (13.1 minutes), Pseudoephedrine (13.18 minutes), or Methylephedrine (14.0 minutes).

The temperature program used for the tea samples was changed from that used for the Ephedra powder to separate the peaks in the tea sample. The temperature program was attempted with an initial column temperature of 100 degrees Celsius, but the peaks were not properly resolved. The injection and interface temperatures were still held at 225 degrees Celsius, however the initial column temperature was held at 80 degrees Celsius for three minutes. The remainder of the temperature program was the same. The temperature was raised at the rate of 20 degrees Celsius per minute to 160 degrees Celsius, and then raised at the rate of five degrees Celsius per minute to 200 degrees Celsius and held at this final temperature for three minutes. Using this temperature program for analyzing the tea, the samples that came from the bottle untreated gave similar results over three trial runs. The GC chromatogram for Ephedra tea is shown in Figure 10:

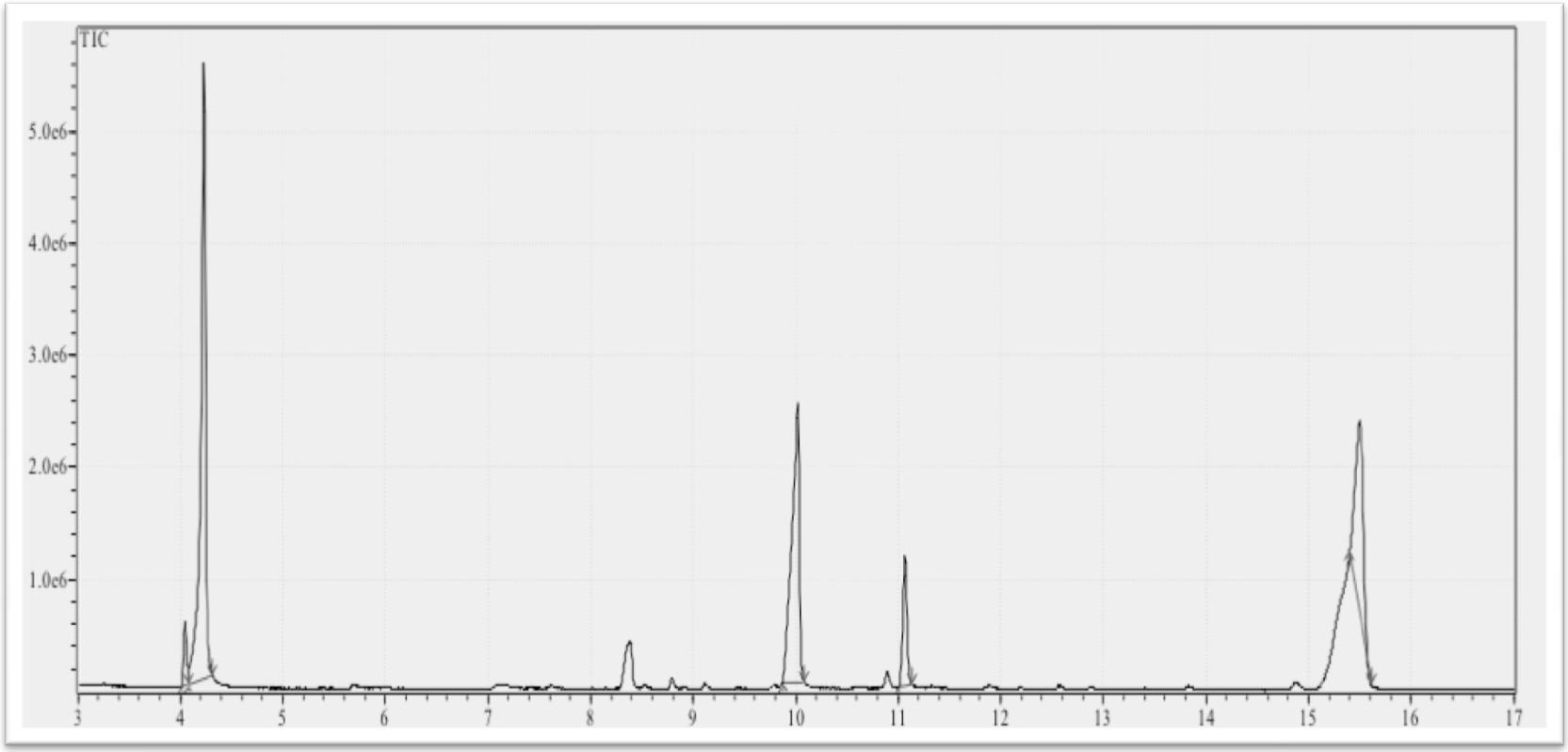

Figure 10: GC Chromatogram of Ephedra Tea

The mass spectra for the four major peaks were recorded and then analyzed through post run software. Their identities are shown in Tables 4 through 6. The tables show identical compounds for the four peaks, as well as retention times within one tenth of a minute of each other.

Table 4: Identified Peaks from First Run of Ephedra Tea

Compound	M/z	Retention time
Propylene Glycol	45.00	4.040
Benzoic Acid	105.00	10.020
2-Furancarboxaldehyde	41.00	11.065
Caffeine	194.00	15.500

Table 5: Identified Peaks from Second Run of Ephedra Tea

Compound	M/z	Retention time
Propylene Glycol	45.00	4.225
Benzoic Acid	105.00	10.005
2-Furancarboxaldehyde	41.00	11.045
Caffeine	194.00	15.435

Table 6: Identified Peaks from Third Run of Ephedra Tea

Compound	M/z	Retention time
Propylene Glycol	45.00	4.180
Benzoic Acid	105.00	10.010
2-Furancarboxaldehyde	41.00	11.050
Caffeine	194.00	16.240

To determine the identity of each peak represented, the mass spectra were compared through Shimadzu post-run software. Each compound was determined at a confidence interval above 80 percent.

The mass spectrum of the peak at retention time 4.1 minutes can be seen in Figure 12. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 11, which is Propylene glycol.

The mass spectrum of the peak at retention time 10.0 minutes can be seen in Figure 14. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 13, which is Benzoic acid.

The mass spectrum of the peak at retention time 11.0 minutes can be seen in Figure 16. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 15, which is 2-Furancarboxaldehyde.

The mass spectrum of the peak at retention time 15.4 minutes can be seen in Figure 18. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 17, which is Caffeine.

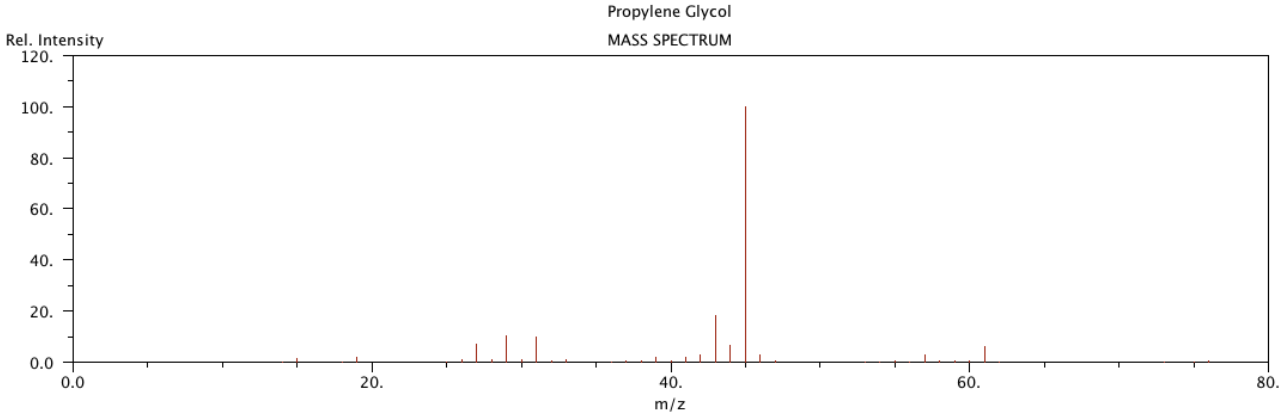

Figure 11: Mass Spectrum of Propylene Glycol from NIST Database

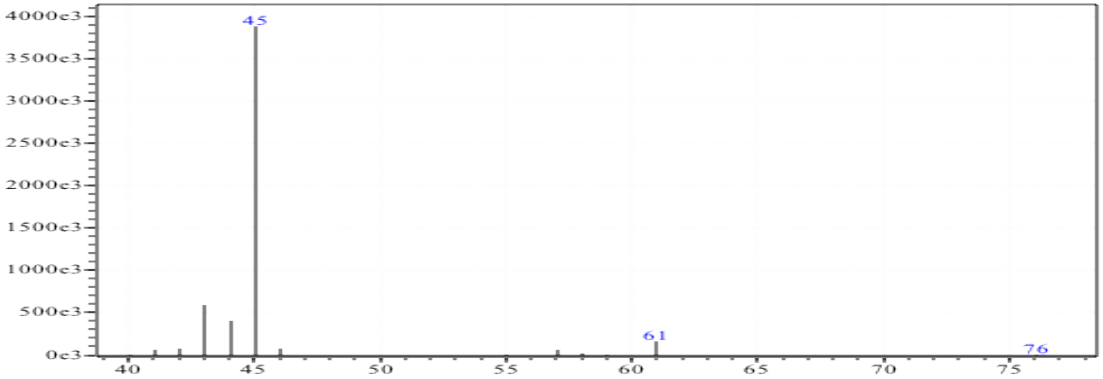

Figure 12: Mass Spectrum of Propylene Glycol from Ephedra Tea

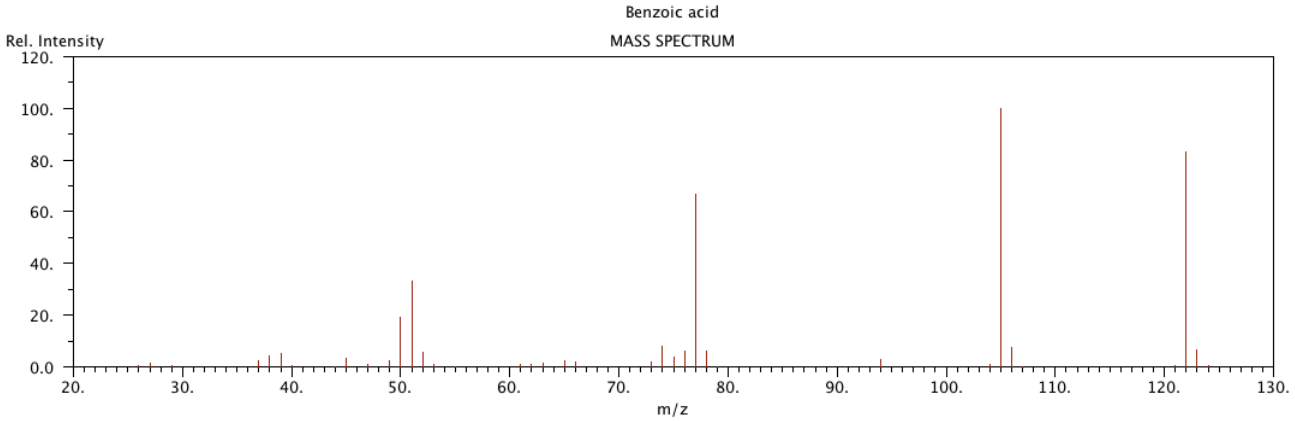

Figure 13: Mass Spectrum of Benzoic Acid from NIST Database

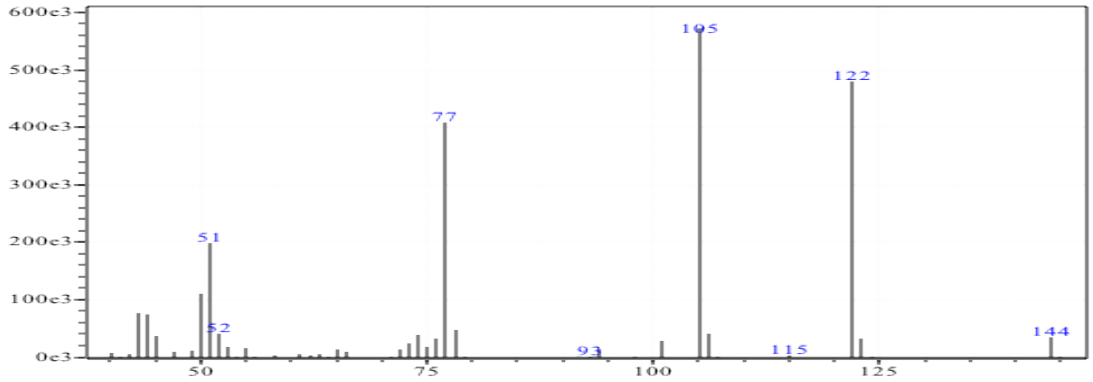

Figure 14: Mass Spectrum of Benzoic Acid from Ephedra Tea

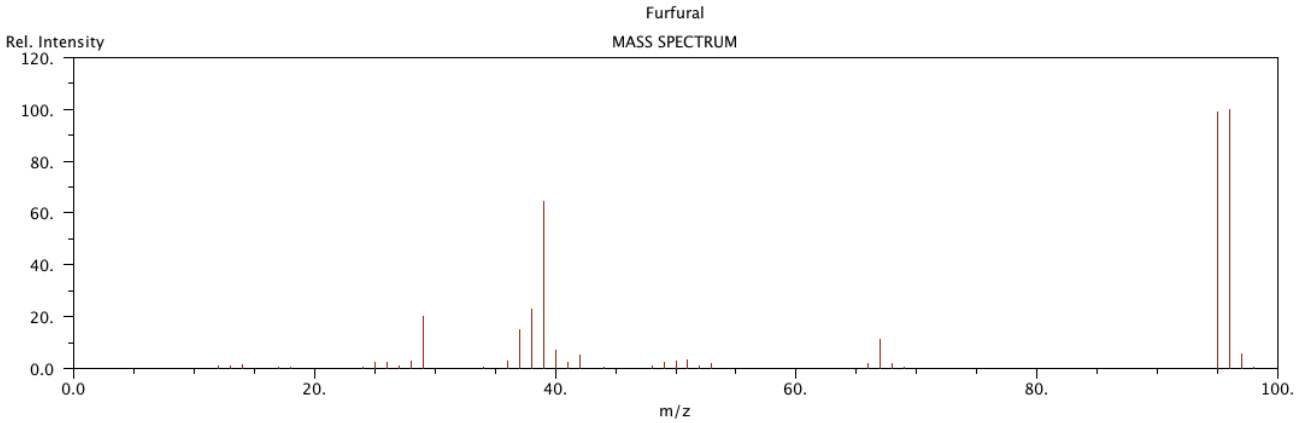

Figure 15: Mass Spectrum of Furfural (2-Furancarboxaldehyde) from NIST Database

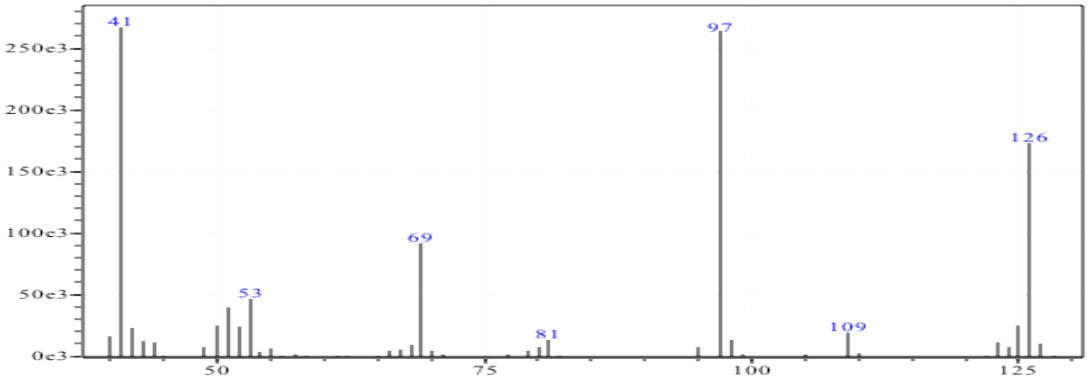

Figure 16: Mass Spectrum of Furfural (2-Furancarboxaldehyde) from Ephedra Tea

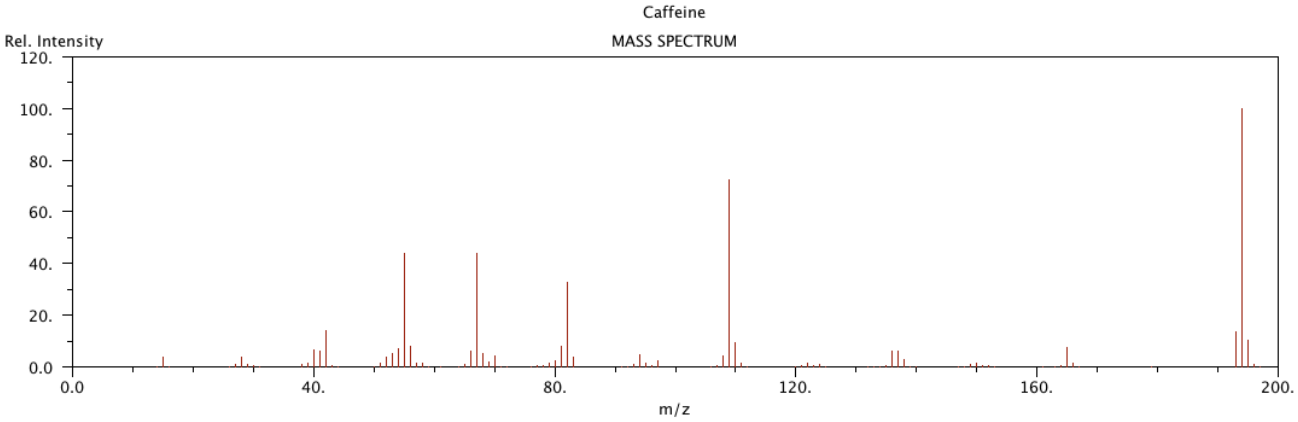

Figure 17: Mass Spectrum of Caffeine from NIST Database

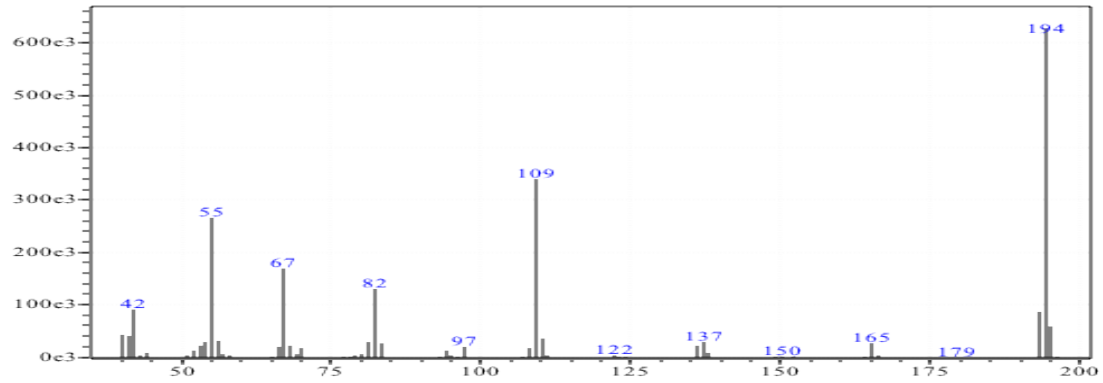

Figure 18: Mass Spectrum of Caffeine from Ephedra Tea

Diluted Ephedra Tea

The same temperature program that was used for the Ephedra tea was used with a diluted sample. The diluted sample was prepared by measuring 50 mL of the tea and then diluting it with 50 mL of ethanol. The diluted tea samples gave similar results over three trial runs. Figure 19 shows the GC chromatogram of the diluted tea:

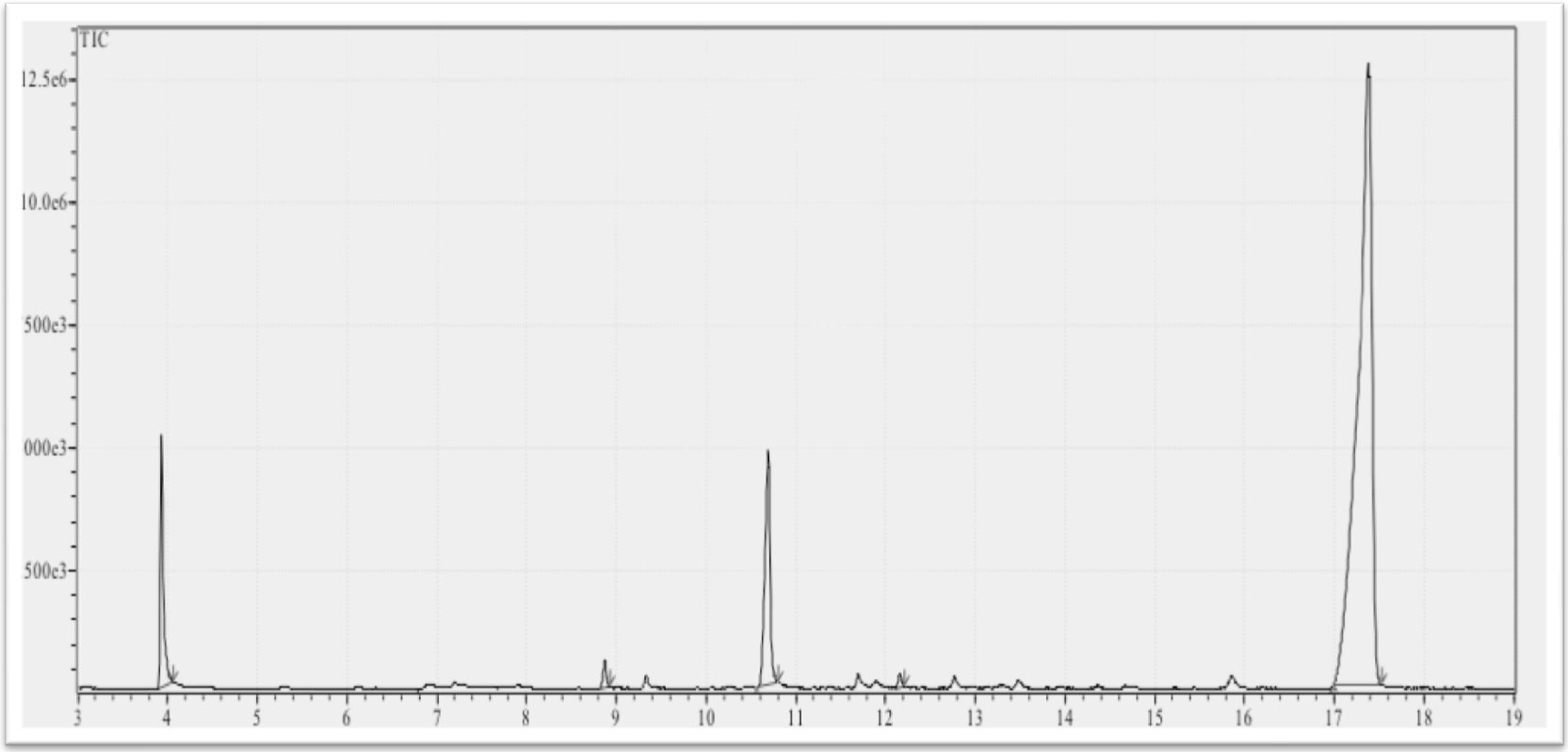

Figure 19: GC Chromatogram of Diluted Ephedra Tea

When determining the identity of each peak, the mass spectra were recorded and compared through Shimadzu post run software. Tables 7 through 9 show identical compounds for the four peaks, as well as retention times within one tenth of minute of each other.

Table 7: Identified Peaks from First Run of Diluted Ephedra Tea

Compound	**M/z**	**Retention time**
Propylene Glycol	45.00	3.933
Sorbic Acid	97.00	8.867
Benzoic Acid	105.00	10.692
Caffeine	194.00	17.383

Table 8: Identified Peaks from Second Run of Diluted Ephedra Tea

Compound	**M/z**	**Retention time**
Propylene Glycol	45.00	3.925
Sorbic Acid	97.00	8.883
Benzoic Acid	105.00	10.717
Caffeine	194.00	17.425

Table 9: Identified Peaks from Third Run of Diluted Ephedra Tea

Compound	M/z	Retention time
Propylene Glycol	45.00	3.917
Sorbic Acid	97.00	8.875
Benzoic Acid	105.00	10.708
Caffeine	194.00	17.358

To determine the identity of each peak, the mass spectra were compared through Shimadzu post-run software and identified at a confidence interval above 80 percent.

The mass spectrum of the peak at retention time 3.9 minutes can be seen in Figure 21. The Shimadzu post run software found the spectrum for this peak most closely matched the spectrum in Figure 20, which is Propylene glycol.

The mass spectrum of the peak at retention time 8.8 minutes can be seen in Figure 23. The Shimadzu post run software found the spectrum for this peak most closely matched the spectrum in Figure 22, which is Sorbic acid.

The mass spectrum of the peak at retention time 10.7 minutes can be seen in Figure 25. The Shimadzu post run software found the spectrum for this peak most closely matched the spectrum in Figure 24, which is Benzoic acid.

The mass spectrum of the peak at retention time 17.3 minutes can be seen in Figure 27. The Shimadzu post run software found the spectrum for this peak most closely matched the spectrum in Figure 26, which is Caffeine.

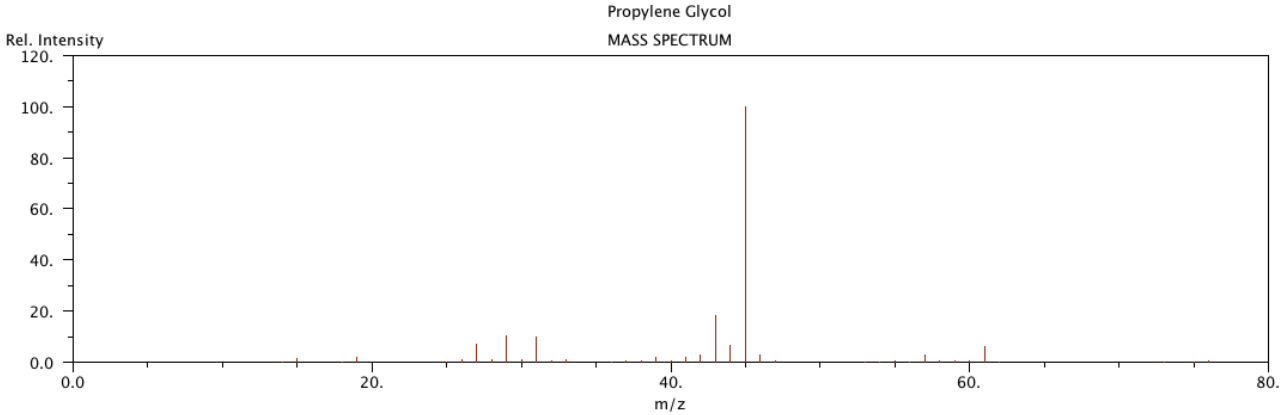

Figure 20: Mass Spectrum of Propylene Glycol from NIST Database

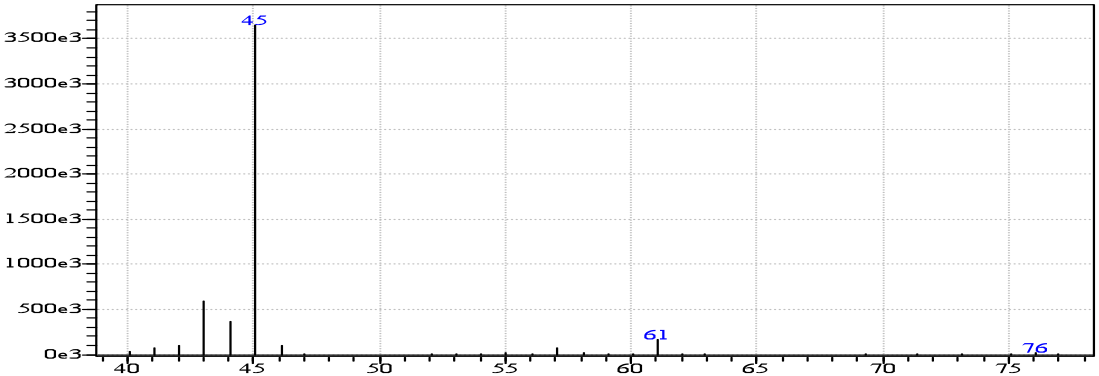

Figure 21: Mass Spectrum of Propylene Glycol from Diluted Ephedra Tea

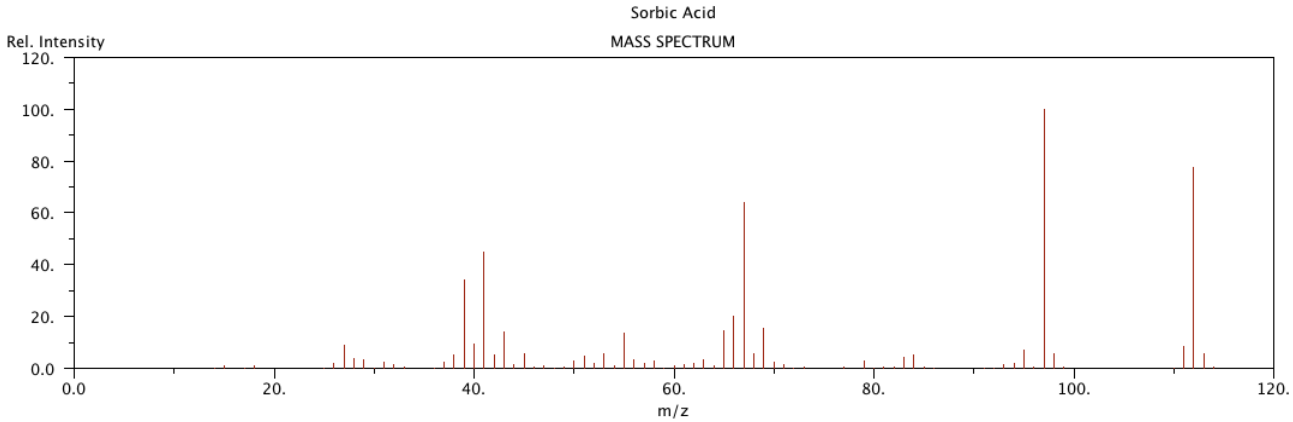

Figure 22: Mass Spectrum of Sorbic Acid from NIST Database

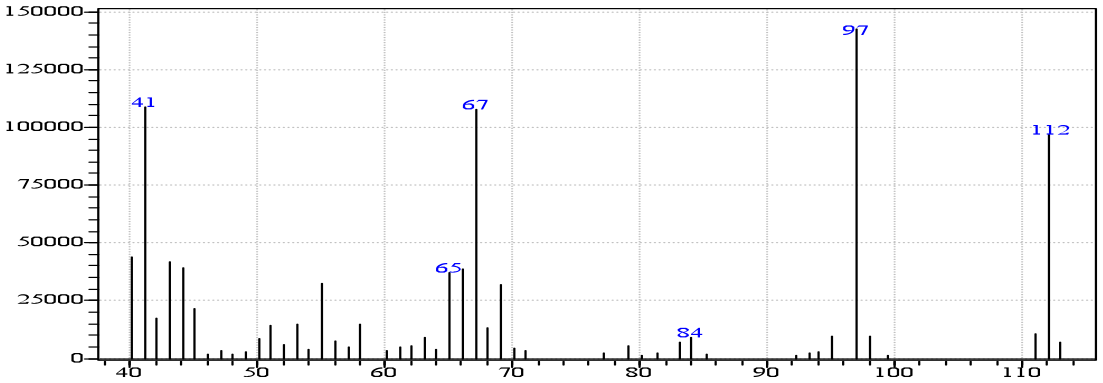

Figure 23: Mass Spectrum of Sorbic Acid from Diluted Ephedra Tea

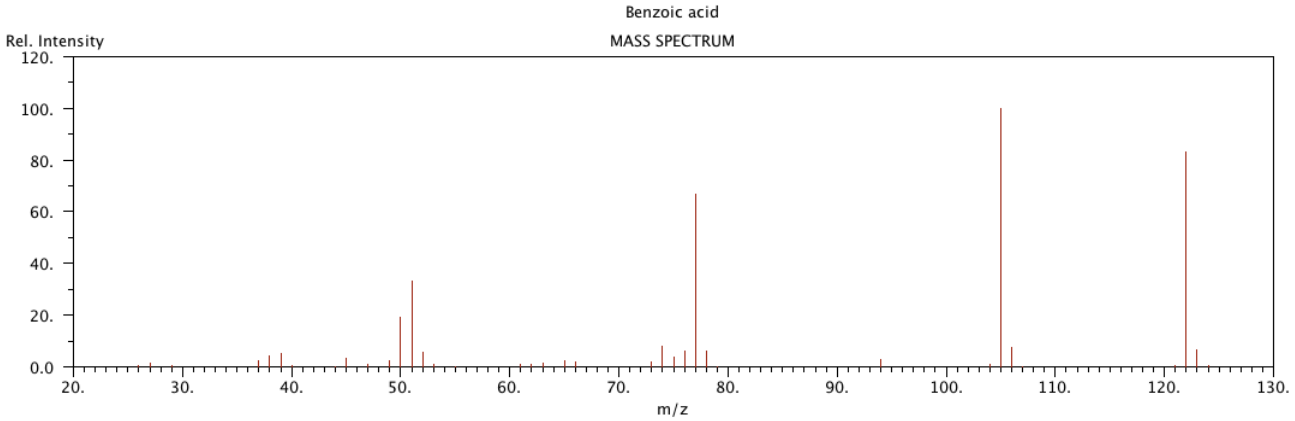

Figure 24: Mass Spectrum of Benzoic Acid from NIST Database

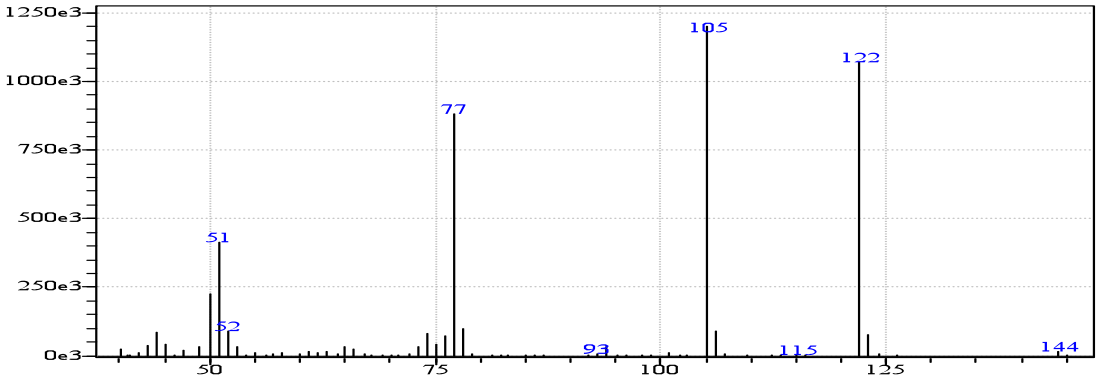

Figure 25: Mass Spectrum of Benzoic Acid from Diluted Ephedra Tea

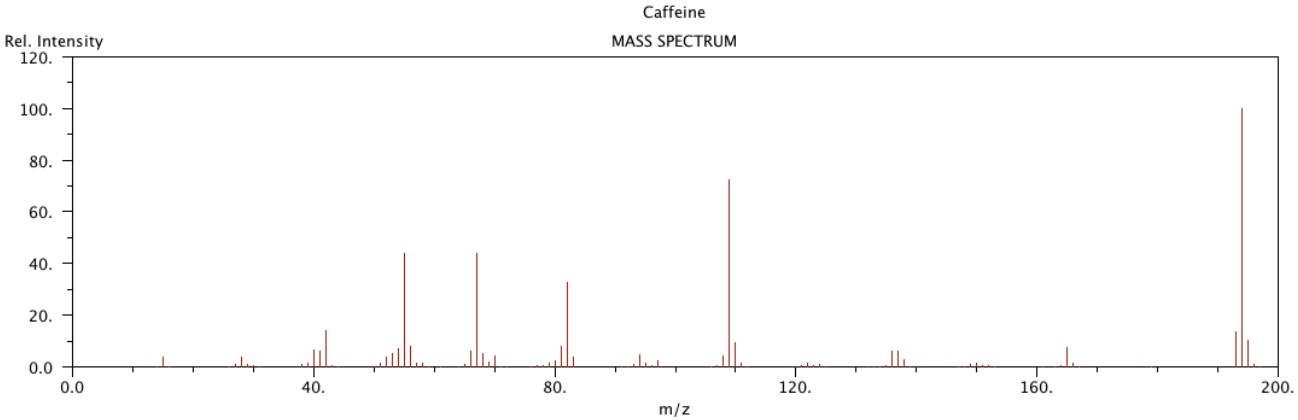

Figure 26: Mass Spectrum of Caffeine from NIST Database

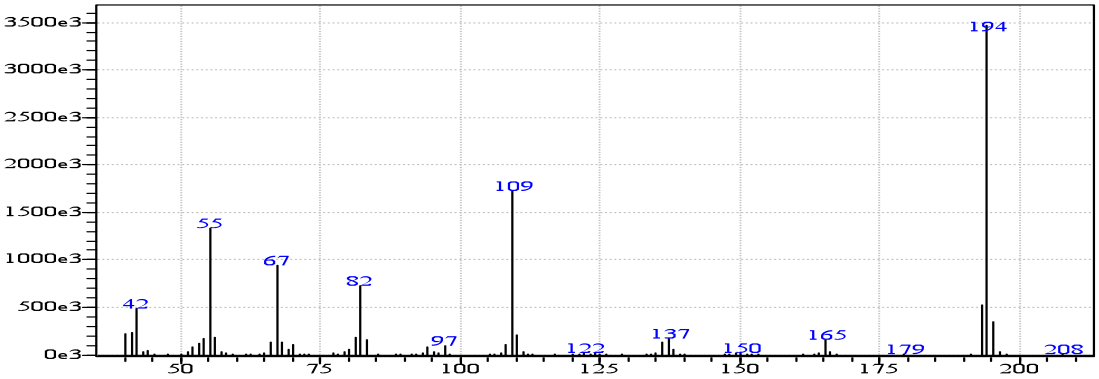

Figure 27: Mass Spectrum of Caffeine from Diluted Ephedra Tea

Concentrated Ephedra Tea

The same temperature program used for the Ephedra tea and diluted Ephedra tea was used to analyze the concentrated Ephedra tea. The sample was prepared by drying the tea to a solid and this product was diluted with 50 mL of ethanol. The concentrated samples gave similar results over two trial runs. Figure 28 shows the GC chromatogram of the concentrated tea:

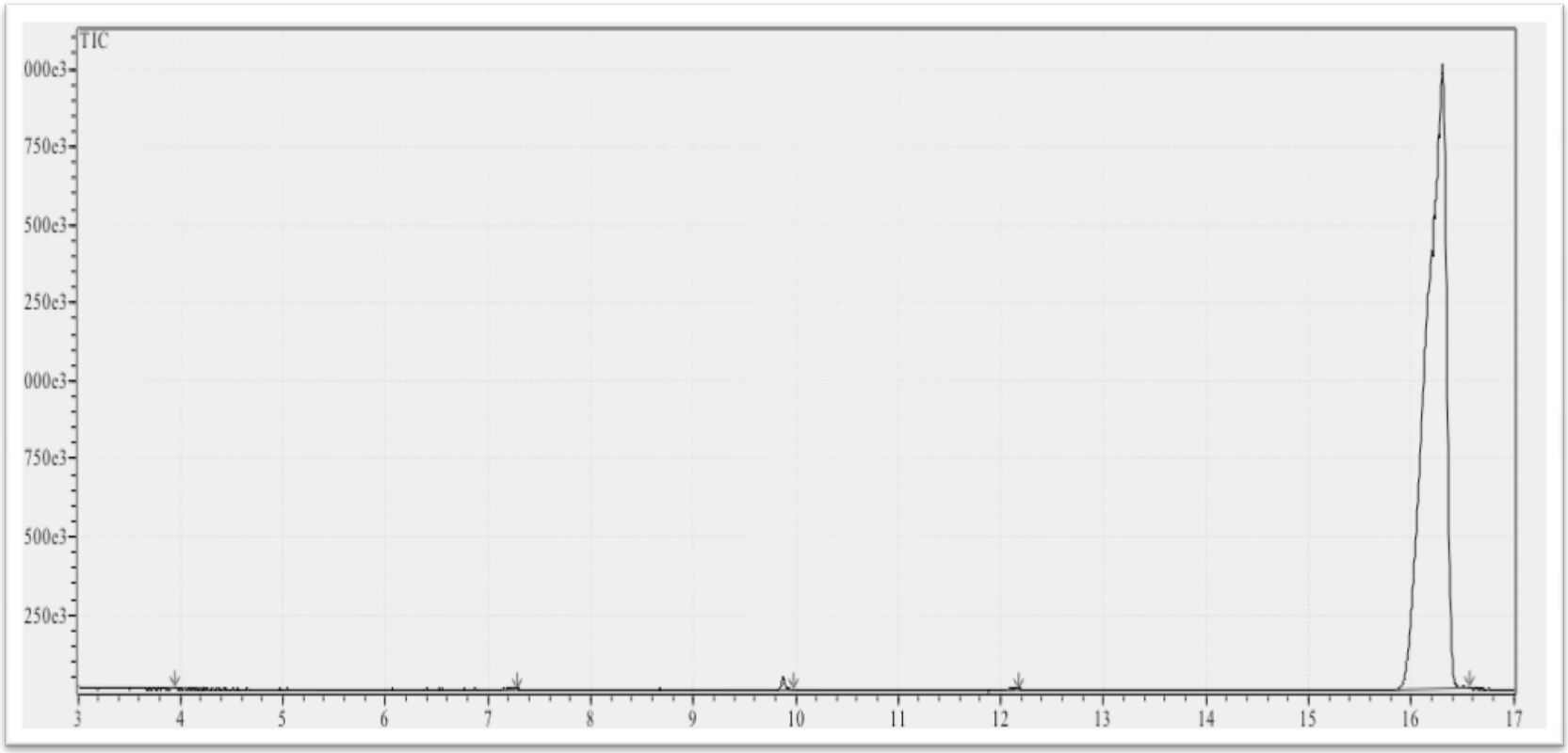

Figure 28: GC Chromatogram of Concentrated Ephedra Tea

The mass spectra for the two major peaks were recorded and analyzed. Their identities are shown in Tables 10 and 11. The tables show identical compounds for the two peaks, as well as retention times within one tenth of a minute of each other.

Table 10: Identified Peaks from First Run of Concentrated Ephedra Tea

Compound	M/z	Retention time
Benzoic Acid	105.00	9.865
Caffeine	194.00	16.245

Table 11: Identified Peaks from Second Run of Concentrated Ephedra Tea

Compound	M/z	Retention time
Benzoic Acid	105.00	9.875
Caffeine	194.00	16.305

To determine the identity of each peak, the mass spectra were compared through Shimadzu post-run software and identified at a confidence interval above 80 percent.

The mass spectrum of the peak at retention time 9.8 minutes can be seen in Figure 29. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 28, which is Benzoic acid.

The mass spectrum of the peak at retention time 16.3 minutes can be seen in Figure 31. The Shimadzu post run software found the spectrum of this peak most closely matched the spectrum in Figure 30, which is Caffeine.

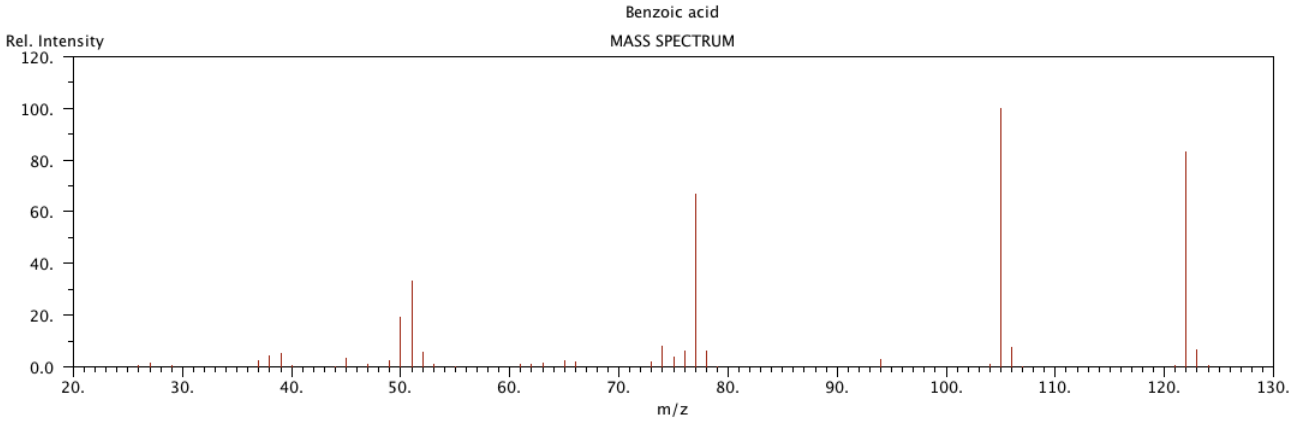

Figure 29: Mass Spectrum of Benzoic Acid from NIST Database

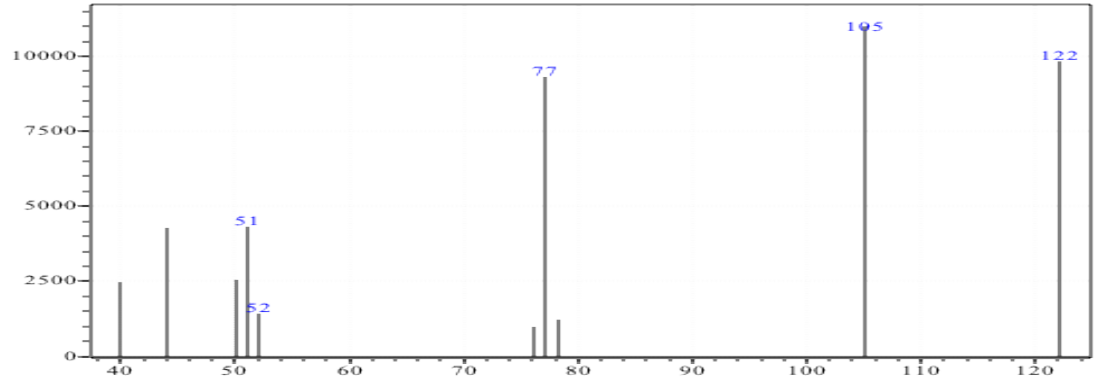

Figure 30: Mass Spectrum of Benzoic Acid from Concentrated Ephedra Tea

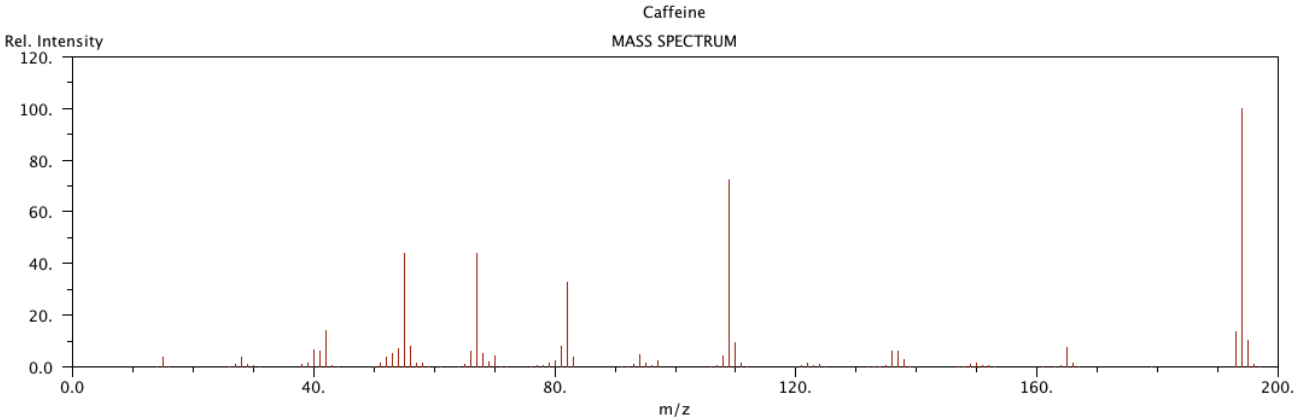

Figure 31: Mass Spectrum of Caffeine from NIST Database

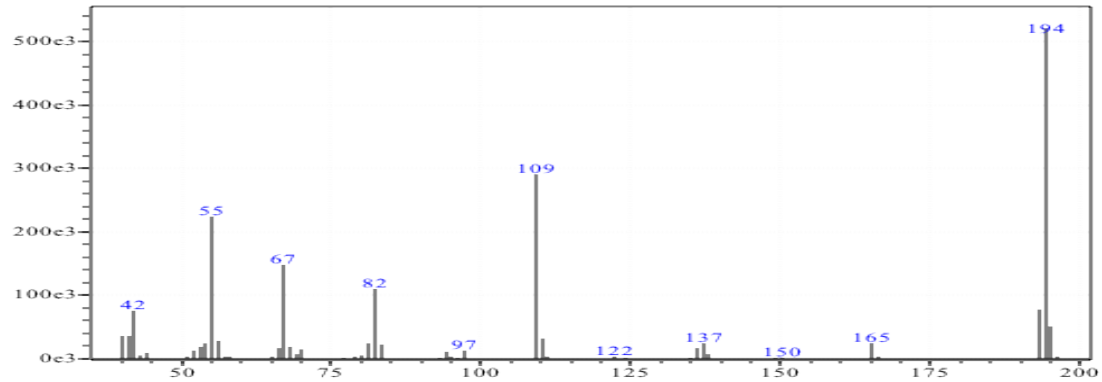

Figure 32: Mass Spectrum of Caffeine from Concentrated Ephedra Tea

CHAPTER 5

DISCUSSION

Ephedra powder

Three replicate data sets of the Ephedra powder were obtained. Each spectrum shows three peaks for the separation of Ephedra alkaloids. The compounds separated were Ephedrine, Pseudoephedrine and Methylephedrine. These compounds were determined through mass spectrometry analysis and the structure of these compounds were identified by matching their mass spectra to the NIST library with a greater than 80 percent confidence interval.

Ephedra Tea

Analysis of all three Ephedra tea preparations showed no traces of Ephedra. The tea contained four compounds: Propylene glycol, Benzoic acid, 2-Furancarboxaldehyde and Caffeine. 2-Furancarboxaldehyde is a compound that is occurs naturally in food and beverages, but can also be used as a flavoring agent. Propylene glycol is also used in food and beverage production and benzoic acid is commonly used as a preservative in this same process. Caffeine is a natural stimulant that has a similar effect to the active Ephedra alkaloids—Ephedrine and Pseudoephedrine.

Through the dilution of Ephedra tea, four consistent peaks were separated: Propylene glycol, Sorbic acid, Benzoic acid and Caffeine. The appearance of Sorbic acid is not unusual because similar to benzoic acid, it is also used as a food preservative.

Despite the fact that only two data sets of the concentrated tea were replicated, there was still no evidence of Ephedra in the sample. The compounds separated were Benzoic acid and Caffeine.

CHAPTER 6

CONCLUSIONS

It is relevant to point out that despite being advertised as containing Ephedra, the Ephedra tea does not contain any Ephedra. All three preparations of Ephedra tea showed large quantities of caffeine in the product. Caffeine in such high concentrations is most likely a substitute for the Ephedra alkaloids with enegizing properties. Caffeine and ephedrine are both stimulants that when ingested can cause similar effects.

The analysis of Ephedra powder shows to be a safe, efficient and straightforward method for GC-MS analysis for the separation of Ephedra alkaloids. Most importantly, the analysis was performed without a pretreatment step of the sample. The total program time was 17 minutes, making it a quicker means of separation than many other methods of analysis. When comparing the method of diluting samples to previous analysis in GC-MS, there is no use of MSTFA. MSTFA is used as a derivitization agent for the Ephedra. Derivitization is used to improve chromatographic properies and give higher sensitivity of results. In this mechanism, the active alkyl group is replaced with a silyl group. MSTFA is useful as a silylation reagent and is also the most volatile of the available TMS acetamide reagents, so no byproducts are produced on the GC chromatogram. However, the amine group in the alkaloids being treated is one of the harder groups to be silanized. This analysis has shown that it is possible to determine Ephedra alkaloids without the use of a silyation reagent. This makes the analysis more effective, both in time and cost.

Prior GC-MS analysis has achieved separation of all six Ephedra alkaloids, however not every sample of Ephedra is guaranteed to contain measurable amounts of all six alkaloids. Alkaloids make up less than five percent of the total Ephedra plant, and of this five percent, anywhere from 30-90 percent is ephedrine. This variation is dependent on the species of plant harvested and the parts of the plant used. Generally, in sets of diastereomeric pairs, ephedrine and pseudoephedrine are the most abundant, followed by norephedrine and norpseudoephedrine, followed by methylephedrine and methylpseudoephedrine. In this experiment, ephedrine, pseudoephedrine and methylephedrine were separated. It is unusual that methylephedrine was separated over norephedrine or norpseudoephedrine, but again the alkaloid content is dependent on the species of the plant.

Most importantly, ephedrine and pseudoephedrine were identified through this analysis. These two alkaloids are the active ingredients in the plant, and the reason why the FDA bans Ephedra consumption. Using the method developed here to treat and analyze Ephedra containing samples would be helpful in determining if ephedrine or pseudoephedrine are present and therefore should be treated appropriately, as these compounds are illegal.

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