Simultaneous Analysis of 1,8-Cineole and Menthol in Vicks VapoRub via Static Headspace Gas Chromatography-Mass Spectrometry

Emily Dagasso

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Abstract

Topical ointments, such as Vicks VapoRub, are widely utilized cough suppressants in North America containing many organic active ingredients. For quality control and assurance, this project focused on developing a method for simultaneous identification and quantification of two common monoterpenoids within a sample of Vicks VapoRub, 1,8-cineole and menthol. The instrument and technique utilized was static headspace gas chromatography-mass spectrometry, GC-MS, due to its proficiency in separating complex mixtures and analyzing volatile analytes. A GC-MS method, including temperature program, was developed specifically for analyzing the analytes 1,8-cineole and menthol. The employed method of quantification included both external standards and a standard addition series, with solely the standard addition method producing viable results. Utilizing the optimized GC-MS method both analytes were identified within the sample and data analysis was performed. The concentration and % W/W of 1,8-cineole was determined to be 4.09×10^{-3} g/mL and 1.66 respectively, whereas menthol demonstrated a concentration of 1.58×10^{-3} g/mL and % W/W of 0.640. When comparing these values to those on the manufacturer label of Vicks VapoRub, there is a 38 % difference between the values for 1,8-cineole and 75 % difference between the values for menthol.

Introduction

The coronavirus pandemic, COVID-19, infected and tragically killed millions of people across the globe as well as emphasized the importance of functioning healthcare systems. Of COVID-19's top five reoccurring symptoms is a persistent cough, a symptom that can be treated at home with over-the-counter and topical medications.[1] Vicks VapoRub, Figure. 1, is a topical cough suppressant commonly utilized in North America that can be applied on the chest to relieve respiratory infections. For consumer satisfaction, this ointment is available in various scents, such as regular, lavender and lemon.[2] As listed on the company's website, the active ingredients within a sample of Vicks VapoRub include camphor, eucalyptus oil, and menthol.

Figure. 1 Packaging of Vicks VapoRub, lemon scented.[3]

Eucalyptus essential oil is frequently utilized as a natural cold remedy and is derived from the leaves of a eucalyptus tree, native to Australia. There are many inexpensive and efficient techniques focused on the extraction of eucalyptus oil, such as hydrodistillation, steam distillation and supercritical fluid extraction.[4] Within the chemical composition of Eucalyptus oil, the major constituent is 1,8-cineole, a monoterpenoid depicted in Figure. 2.[5] 1,8-Cineole, otherwise known as Eucalyptol, is characterized as a colorless liquid that is insoluble with water and has a multifaceted odor that is described as minty, with sweet hints.[6]

Figure. 2 Chemical structure of 1,8-cineole.

Menthol, as shown in Figure. 3, is also a monoterpenoid that can be extracted from the essential oils of the *Mentha* genus, including spearmint and peppermint.[7] Similar to eucalyptus

oil, the essential oils from the *Mentha* genus can be extracted by hydrostillation and isolation of menthol is completed by fractional distillation.[8] At room temperature, menthol is a clear crystalline substance with a distinctive mint-like smell.

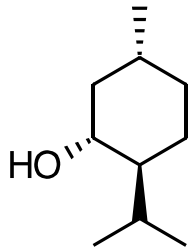

Figure. 3 Chemical structure of -(-)menthol

Both menthol and 1-8,cineole are within a class of terpenes labelled oxygenated monoterpenoids, signifying their individual chemical structures consist of a ten carbon backbone.[9] These cyclic organic compounds are well utilized in the flavour and fragrance industries as cooling compounds due to their associated cooling sensation.[7,10] Alongside their anti-inflammatory medicinal properties, the psychophysical cooling effect of both menthol and 1,8-cineole allow these compounds to interact with sensory receptors, producing the sensation of a increasing respiratory flow.[10]

The volatility of essential oils, including the parent oils of menthol and 1,8-cineole, causes these compounds to be challenging in analysis. Of the available methods in characterizing volatile organic substances, gas chromatography-mass spectrometry, GC-MS, is one of the most widely utilized due to its sensitivity and selectivity. There are two main components to GC-MS, the separation of compounds within a sample, and the identification of the individual compounds by the mass spectrometer. Many factors influence the separation of compounds, including the polarity, column temperature, carrier gas flow rate, and column temperature.[11] Interactions between the stationary, being the GC-MS column, and mobile phase, being the carrier gas, is one of the most important elements when considering compound separation. The stronger the interaction between a compound and the stationary phase, the longer the retention time of the compound. Due to the presence of oxygen-hydrogen bonds within the chemical structures of menthol and 1,8-cineole, both compounds are polar and will have short retention times when utilizing a non-polar column, such as the HP-5MS, compared to a polar column. Additionally, the usage of an appropriate solvent that is able to solubilize both compounds and not interfere

with peak identification is important and was considered in method development. The polarity of the analytes indicates a polar solvent is optimal.

Static headspace gas chromatography-mass spectrometry is a technique employed in the analysis of volatile and semi-volatile organic compounds. As demonstrated in Figure. 4, there are many components that compose a headspace sample. Within the headspace vial, there is the sample, or aqueous, phase containing the sample and solvent of choice, as well as the gas phase, otherwise known as headspace. When equilibrium is achieved between the headspace and sample phase, an aliquot is taken from the headspace to be utilized in the analysis.[12] This technique is useful for analyzing volatile organic compounds as included within the method is an incubation time, which heats the sample to release the volatile analytes within the sample phase to the headspace. As a result, the non-volatile analytes are not included in the analysis as they are left in the sample phase, effectively minimizing matrix effects, and allowing for a more efficient and instrument-friendly analysis. Static headspace GC-MS is able to analyze both solid and liquid samples and is a particularly appropriate method when analyzing viscous samples, such as a sample of Vicks VapoRub.

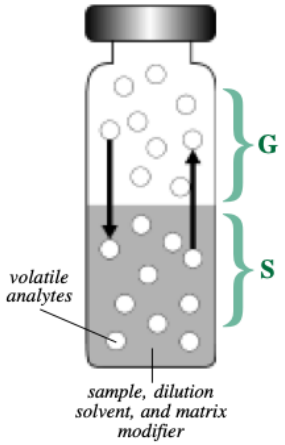

Figure. 4 Phases of headspace vail. G refers to the gas phase (headspace) and S refers to the sample phase.[12]

The partitioning coefficient of an analyte between the sample and gas phases in headspace GC-MS factors into the efficacy of an analysis. As presented in Figure. 5, the equation for determining partition coefficients includes a ratio between the concentration of compounds within each of the phases. For a comprehensive investigation of a sample, an equilibrium between the two phases must be achieved, however compounds can have a range of affinities towards the original sample phase. Further, since the instrument will use an aliquot of the

headspace, the objective is to have the compounds favor vaporizing to the headspace moreso than the unwanted compounds found within the sample matrix. Therefore, a low *K* value within the partition coefficient equation is optimal in the analysis of an analyte within a sample. Additionally, for the quantification of an analyte within a sample, method development should focus on maintaining a uniform the partition coefficient across all standards as well as samples. The GC-MS headspace technique, as well as the standard addition method, decreases matrix effects and allows for equal partitioning throughout all analyses.

$$K = \frac{C_S}{C_G}$$

Where:

- K is the partition coefficient of a given compound between sample (liquid) phase and the gas (headspace) phase
- C_S is the concentration of that compound in the sample (liquid) phase
- C_G is the concentration of that compound in the gas (headspace) phase

Figure. 5 Partition coefficient equation.[13]

The prevalence and wide utilization of Vicks VapoRub as an at-home medicinal remedy in North America encouraged this project in an effort towards quality control and assurance. As a result, this project aimed to simultaneously analyze 1,8-cineole and menthol in Vicks VapoRub utilizing static headspace GC-MS. The objective of Week 1 included developing an efficient method to identify the analytes within a sample of Vicks VapoRub, and in Week 2 the preparation of standards for an external calibration curve was the focus. Finally, Week 3 concentrated on the technique of standard addition for accurate quantification of the analytes.

Experimental

Table. 1 Instrument parameters

Instrument	Agilent 7890B GC System and 5977A MS Detector with PAL autosampler
Column Type	HP-5MS (5% phenyl/95% dimethylpolysiloxane, 30m length, 0.25 mm id, 0.25µm film thickness)
Column Dimensions	30.0m (nominal) x 0.25mm id; 0.25µm film thickness
Carrier Gas	Helium at a flow rate of 0.9 mL/min
Split Ratio	20:1
Split Flow	20 mL/min
Autosampler	Agitator: 250 rpm 500 µL injection
Incubation Time	Method 1 and 2: 5 min Method 3 and 4: 2 min
Injector Temperature	220 ℃

Table. 2 Temperature program for Method 1 with a total runtime of 26.1 minutes

Ramp (℃/min)	Value (℃)	Hold Time (min)
	40	3
12	180	5
40	240	5

Table. 3 Temperature program for Method 2 with a total runtime of 15.2 minutes

Ramp (℃/min)	Value (℃)	Hold Time (min)
	40	5
20	180	2
50	240	2

Table. 4 Temperature program for Method 3 with a total runtime of 15.2 minutes

Ramp (℃/min)	Value (℃)	Hold Time (min)
	40	3
20	180	2
50	240	2

Table. 5 Temperature program for Method 4 with a total runtime of 25.7 min

Ramp (°C/min)	Value (°C)	Hold Time (min)
	40	3
8	180	2
50	240	2

Table. 6 Solutions and reagents utilized

Sparged TOC Grade Water
Methanol
100 % Ethanol
(-)-Menthol
99% 1,8-Cineole
(1*R*)-(+)- Camphor
100 g (115 mL) jar of Vicks VapoRub Ointment, Lemon Scented

Table. 7 Amount of 1,8-cineole and menthol reported within Vicks VapoRub

Eucalyptus Oil	1.2 % W/W
Menthol	2.6 % W/W

Table. 8 Equipment utilized

20 GC-MS Static Headspace Autosampler Vials with Caps
100, 500 µL Glass Syringes
1 mL Repeater Pipette
2x 2 mL Volumetric Flask
8x 10 mL Volumetric Flask
Vortex Mixer
Assorted Beakers
Stir Bar
Scoopula
Spatula
Marker
Weigh Boat

Table. 9 Vicks VapoRub sample weights – Week 1

Sample 1	0.4374 g
Sample 2 in volumetric flask	0.030 g
Sample 2 in autosampler vial	0.5773 g
Sample 3 in volumetric flask	0.052 g
Sample 3 in autosampler vial	0.8333 g

Table. 10 Weights of menthol and 1,8-cineole in stock solution – Week 2

1,8-cineole	0.2958 g
Menthol	0.5148 g

Table. 11 Volume of stock solution and TOC grade water required for external standards and the concentrations of each 1,8-cineole and menthol within standards – Week 2

Standard	**Concentration of 1,8-Cineole (g/mL)**	**Concentration Menthol (g/mL)**	**Volume of Stock Solution (mL)**	**Volume of Sparged TOC Grade Water (mL)**
1	0.00000246	0.00000415	0.08	9.92
2	0.000299	0.000515	1.00	9.00
3	0.000887	0.00154	3.00	7.00
4	0.00177	0.00309	6.00	4.00
5	0.00237	0.00412	8.00	2.00
6	0.00296	0.00515	10.00	0.00

Table. 12 Spiked sample concentration of 1,8-cineole and menthol – Week 2

Concentration of 1,8-Cineole (g/mL)	**Concentration Menthol (g/mL)**
0.000150	0.000258

Table. 13 Data for standard addition series – Week 3

Standard	Spiked Volume of Stock Solution (mL)	Volume of Sparged TOC Grade Water	Extra Ethanol (mL)	Mass of Sample (g)
0	0.00	1.00	1.00	0.4729
1	0.10	1.00	0.90	0.5220
2	0.20	1.00	0.80	0.4880
3	0.30	1.00	0.70	0.4850
4	0.40	1.00	0.60	0.4845
5	0.5	1	0.5	0.5080
				Average: 0.4934 ± 0.0286

Table. 14 Concentration of added menthol and 1,8-cineole in standard addition series – Week 3

Standard	Concentration of 1,8-Cineole (g/mL)	Concentration of Menthol (g/mL)
0	0.00	0.00
1	1.48×10^{-4}	2.57×10^{-4}
2	2.96×10^{-4}	5.15×10^{-4}
3	4.44×10^{-4}	7.72×10^{-4}
4	5.92×10^{-4}	1.03×10^{-3}
5	7.40×10^{-4}	1.29×10^{-3}

Experimental Procedure – Week 1

1. Prepare GC-MS by loading method 1
2. Prepare Sample 1 of Vicks VapoRub for static headspace analysis
    a. Weigh a headspace autosampler vial
    b. Transfer small sample of Vicks VapoRub into autosampler vial
    c. Weigh the autosampler vial with sample
    d. Analyze sample using Method 1 on GC-MS
3. Prepare a sparged TOC grade water blank for static headspace analysis
    a. Weigh a headspace autosampler vial
    b. Transfer small sample of TOC grade water into autosampler vial
    c. Weigh the autosampler vial with water
    d. Analyze using Method 2 on GC-MS

4. Prepare Sample 2 of Vicks VapoRub with TOC grade water for static headspace analysis
    a. Weigh a 2 mL volumetric flask
    b. Transfer small sample of Vicks VapoRub into volumetric flask
    c. Weigh the volumetric flask with sample
    d. Fill to mark with sparged TOC grade water
    e. Mix with vortex mixer for 2 min
    f. Transfer to autosampler vial
    g. Analyze sample using Method 3 on GC-MS
5. Analyze Sample 1 utilizing Method 4 on GC-MS
6. Prepare Sample 3 of Vicks VapoRub with Methanol for static headspace analysis
    a. Weigh a 2 mL volumetric flask
    b. Transfer small sample of Vicks VapoRub into volumetric flask
    c. Weigh the volumetric flask with sample
    d. Fill to mark with sparged methanol
    e. Mix with vortex mixer for 2 min
    f. Transfer to autosampler vial
    g. Analyze sample using Method 3 on GC-MS

Experimental Procedure – Week 2

- Prepare Stock Solution of Menthol and 1,8-Cineole
    a. Weigh out approximately 5 g of menthol and 3 g of 1,8-cineole
    b. Add the pre-weighed analytes to a 10 mL volumetric flask
    c. Dilute to mark with 100 % ethanol

  Disclaimer: Prepared experiment to include camphor, however due to solubility issues did not include within stock solution
- Prepare external standards as per Table. 8
    a. Utilize 10 mL volumetric flasks and fill to mark with sparged TOC grade water
    b. Add 1 mL of each standard to autosampler vial
    c. Analyze each standard using Method 3 on GC-MS
- Prepare standard using standard addition
    a. Transfer 0.4261 g of Vicks VapoRub to autosampler vial

b. Add 1 mL of standard 1
c. Add 1 mL of sparged TOC grade water
d. Analyze standard using Method 3 on GC-MS

Experimental Procedure – Week 3

1. Prepared a standard addition series as per Table. 9
    a. Transfer approximately 0.5 g of Vicks VapoRub directly into autosampler vial
    b. Add, according to Table. 9, set amount of stock solution, TOC grade water and ethanol into the autosampler vial
    c. Mix with vortex mixer for 2 min
    d. Analyze each standard using Method 3 on GC-MS
2. Prepare a sparged TOC grade water blank for headspace analysis
    a. Weigh a headspace autosampler vial
    b. Transfer small sample of TOC grade water into autosampler vial
    c. Weigh the autosampler vial with water
    d. Analyze using Method 3 on GC-MS

Data and Results

Table. 15 GC-MS chromatogram retention time and peak area results from Sample 1 using Method 1 – Week 1

Compound	Retention Time (min)	Peak Area
1,8-Cineole	8.808	98,500,200
Menthol	10.786	23,784,695

Table. 16 GC-MS chromatogram retention time and peak area results from Sample 1 using Method 4 – Week 1

Compound	Retention Time (min)	Peak Area
1,8-Cineole	10.233	86,779,161
Menthol	13.104	14,435,616

Table. 17 GC-MS chromatogram retention time and peak area results from Sample 2 – Week 1

Compound	Retention Time (min)	Peak Area
1,8-Cineole	7.395	18,988,980
Menthol	8.640	5,500,902

Table. 18 GC-MS chromatogram retention time and peak area results from Sample 3 – Week 1

Compound	Retention Time (min)	Peak Area
1,8-Cineole	7.401	136,412

Table. 19 GC-MS chromatogram retention time and peak area results for 1,8-cineole in external standards – Week 2

Standard	Retention Time (min)	Peak Area
1	7.394	45,543,309
2	7.395	178,731,418
3	7.395	252,211,010
4	7.394	43,080,277
5	7.396	3,503,067
6	7.395	4,742,008

Table. 20 GC-MS chromatogram retention time and peak area results for menthol in external standards – Week 2

Standard	Retention Time (min)	Peak Area
1	8.639	1,0452,097
2	8.635	42,324,130
3	8.634	37,128,200
4	0	0
5	0	0
6	0	0

Table. 21 GC-MS chromatogram retention time and peak area results for 1,8-cineole and menthol in spiked sample – Week 2

Analyte	Retention Time (min)	Peak Area
1,8-Cineole	7.392	77,296,542
Menthol	8.639	5,427,944

Table. 22 GC-MS chromatogram retention time and peak area results for 1,8-cineole in standard addition series – Week 3

Standard	Retention Time (min)	Peak Area
0	7.394	39,667,798
1	7.392	45,414,204
2	7.392	45,941,722
3	7.393	46,520,643
4	7.393	45,468,427
5	7.393	50,038,035

Table. 23 GC-MS chromatogram retention time and peak area results for menthol in standard addition series – Week 3

Standard	Retention Time (min)	Peak Area
0	8.643	1,498,703
1	8.642	2,334,778
2	8.645	1,343,927
3	8.641	3,379,864
4	8.641	2,689,129
5	8.641	2,774,253

Table. 24 Results for equation of the line for standard addition series, calculated concentration of 1,8-Cineole in Vicks VapoRub and % W/W of 1,8-Cineole in Vicks VapoRub

Equation of the Line	$y = (1.02 \times 10^{10})x + 4.18 \times 10^{7}$
Concentration	4.09×10^{-3} g/mL
% W/W	1.66
Percent Difference of % W/W	38 %

Table. 25 Results for equation of the line for standard addition series, calculated concentration of menthol in Vicks VapoRub and % W/W of menthol in Vicks VapoRub

Equation of the Line	$y = (1.05 \times 10^{9})x + 1.66 \times 10^{6}$
Concentration of Menthol	1.58×10^{-3} g/mL
% W/W of Menthol	0.640
Percent Difference of % W/W	75 %

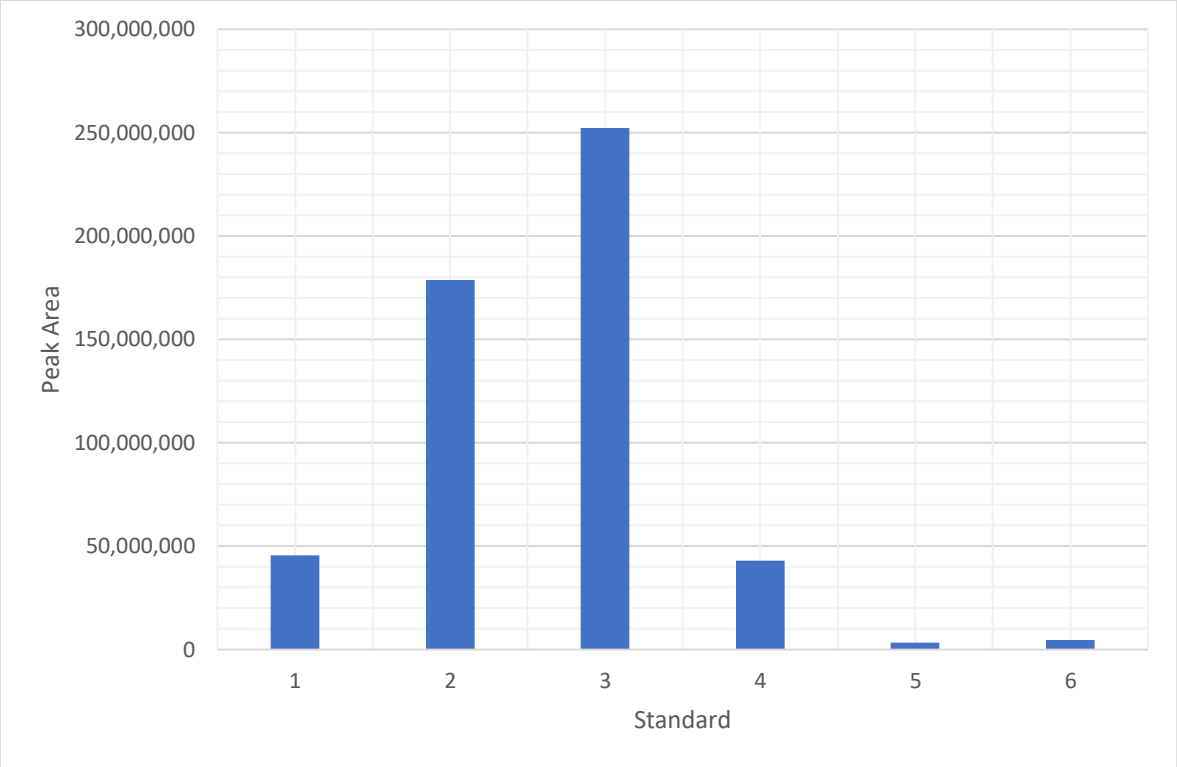

Figure. 6 GC-MS peak area results of 1,8-cineole within each standard from Week 2

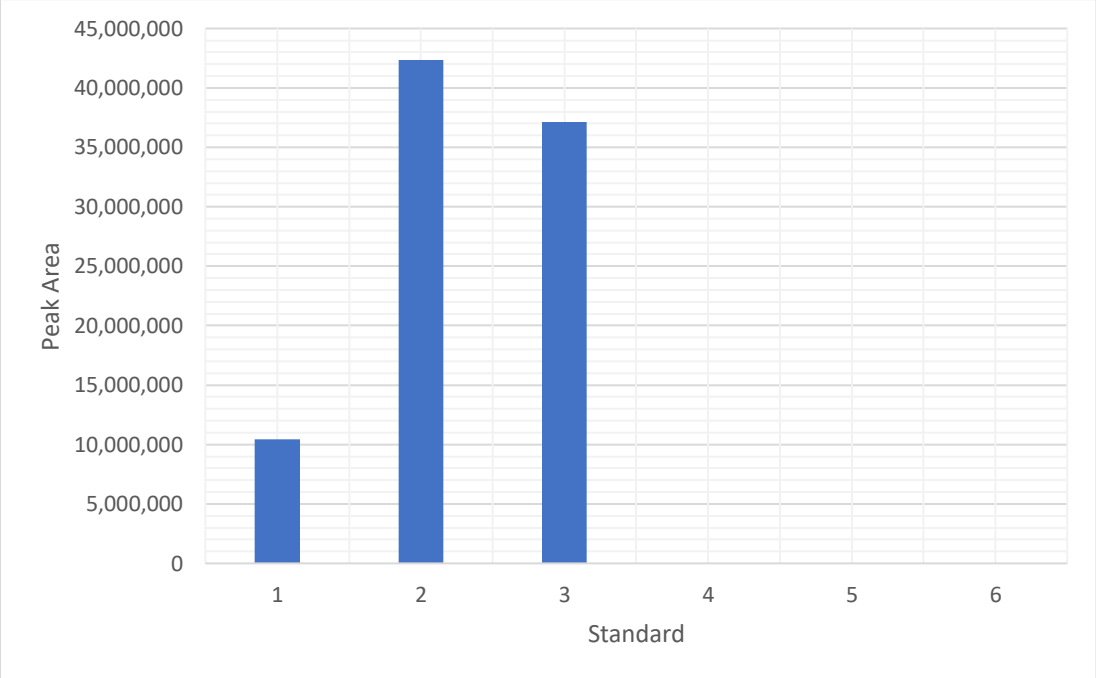

Figure. 7 GC-MS peak area results of menthol within each standard from Week 2.

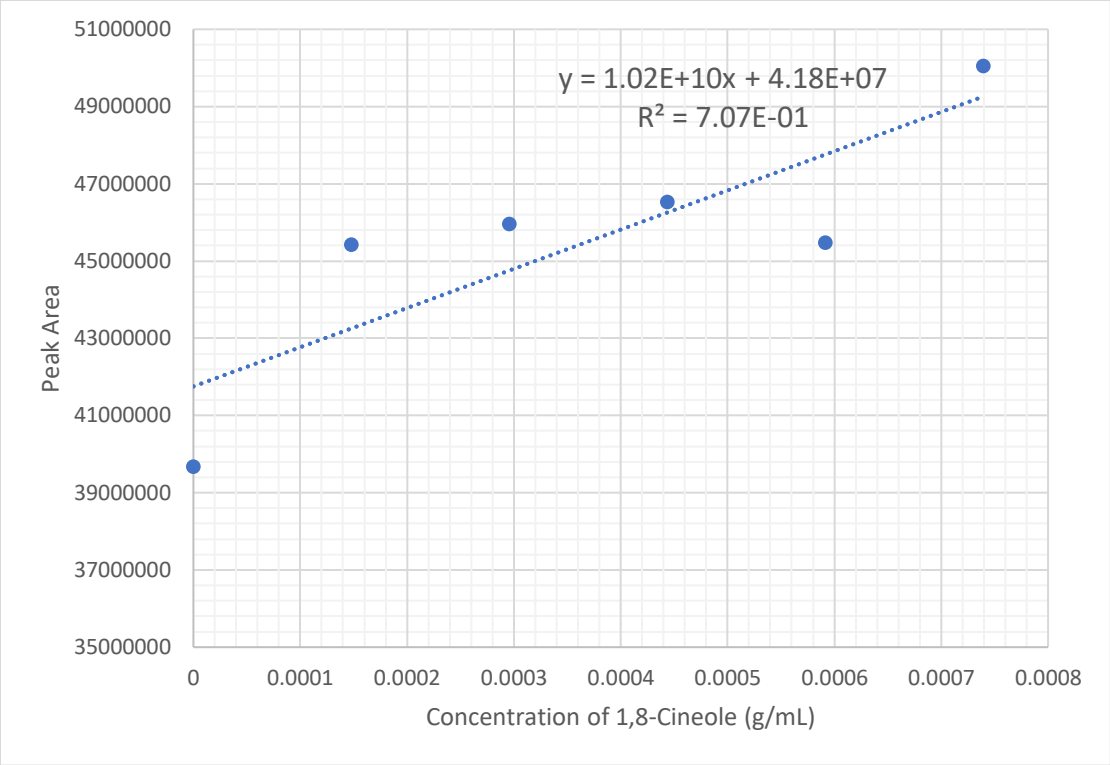

Figure. 8 GC-MS peak area results for 1,8-cineole in standard addition series

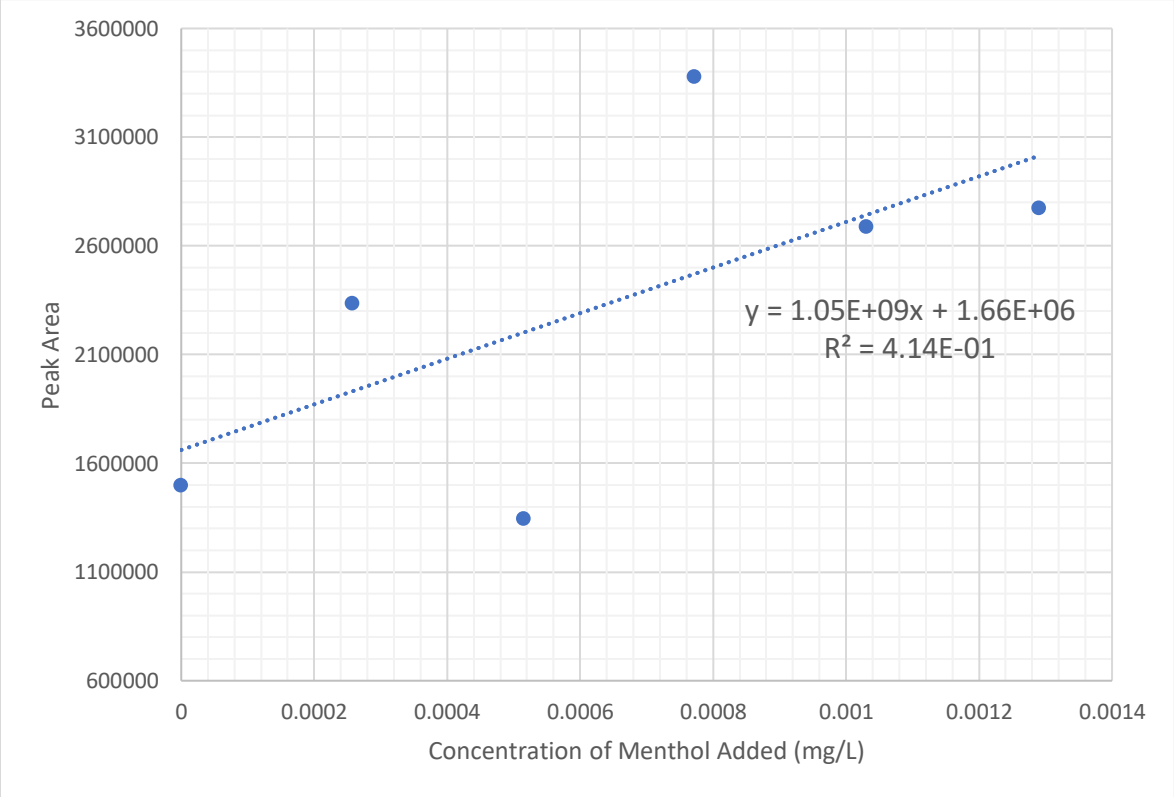

Figure. 9 GC-MS peak area results for menthol in standard addition series

Calculations

Week 2

Sample Calculation of Stock Solution Concentration for Menthol

$$\text{Concentration} = \frac{mass}{volume}$$
$$= \frac{0.5148\ g}{100.00\ mL}$$
$$= 0.005148\ \text{g/mL}$$

Sample Calculation of Standard 2 for Menthol

$$C_1V_1 = C_2V_2$$
$$(0.005148\ \text{g})(0.00100\ \text{mL}) = C_2\ (0.01000\ \text{mL})$$
$$C_2 = 0.000515\ \text{g/mL}$$

Week 3

Sample Calculation of Standard 1 for Menthol

$$C_1V_1 = C_2V_2$$
$$(0.005148\ \text{g})(0.100\ \text{mL}) = C_2\ (2.00\ \text{mL})$$
$$C_2 = 0.000257\ \text{g/mL}$$

Concentration of 1,8-Cineole in Vicks VapoRub Sample

$$y = (1.02 \times 10^{10})x + 4.18 \times 10^{7}$$

$$0 = (1.02 \times 10^{10})x + 4.18 \times 10^{7}$$

$$\left|\frac{-4.18 \times 10^{7}}{1.02 \times 10^{10}}\right| = x$$

$$x = 4.09 \times 10^{-3} \text{ g/mL}$$

Calculation for % w/w of 1,8-Cineole in Vicks VapoRub

Volume of ethanol + TOC grade water in each standard for standard addition series = 2.00 mL

Average mass of Vicks VapoRub used in standard addition series = 0.4934 g

$(4.09 \times 10^{-3} \text{ g/mL})(2.00 \text{ mL})$

$= 8.18 \times 10^{-3} \text{ g}$

$$\frac{8.18\ x\ 10^{-3} g}{0.4934\ \text{g}} \times 100\%$$

= 1.66 % w/w

Percent Difference for % w/w of 1,8-Cineole in Vicks VapoRub

$$\frac{|1.2 - 1.66|}{1.2} \times 100\ \%$$

= 38 %

Discussion

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This project focused on developing a method for the simultaneous analysis of menthol and 1,8-cineole in a commercial sample of Vicks VapoRub using static headspace gas chromatography-mass spectrometry, GC-MS. Method development spanned three weeks, with each session utilizing the results from the previous to further optimize the procedure.

Week 1

The first week concentrated on adapting a method previously published in the *ACS Omega* for analysis of a sample of Vicks VapoRub.[14] During this session, method development aimed to characterize menthol and 1,8-cineole within the sample and reduce analysis time. As a result, incubation time was minimized and the temperature program, outlined in Tables. 2, 3, 4 and 5, was adjusted. Utilizing the optimized Method 3, shown in Tables. 1 and 4, a measured amount of Vicks VapoRub, Sample. 1, as well as a sample of Vicks VapoRub within two different solvents, Samples 2 and 3, were all analyzed. The purpose for analyzing the sample within different solvents was to analyze the effects of the solvents on the partitioning of the analytes between the gas and sample matrix. Results from these trials are outlined in Tables. 15, 16, 17 and 18. A NIST database search of the chromatograms was able to identify both analytes within Sample 1 as well as when using sparged TOC grade water as a solvent, Sample 2. Interestingly, when using methanol as a solvent for analysis, 1,8-cineole was detected but menthol was not, as shown in Table. 18. Further, the peak area of 1,8-cineole is much less using methanol as a solvent than sparged TOC grade water or ethanol. Methanol's polarity and organic nature indicated it would be a suitable solvent for analysis of both analytes, however a possible issue with the analysis could be due to solubility. Despite both 1,8-cineole and menthol are reported to be soluble in methanol, it is evident that the analytes were unevenly distributed between the headspace and liquid phases.[15,16] The first session of this project successfully produced a suitable method for GC-MS analysis and the results indicated an appropriate solvent, being TOC grade water, for the following weeks.

Week 2

Utilizing the results of Week 1, the subsequent session aimed to prepare a range of standards containing the target analytes for an external calibration curve. It is worth mentioning

the original focus of the project was to identify and quantify three analytes within a sample of Vicks VapoRub, namely camphor, 1,8-cineole and menthol. However, due to solubility issues with the solvent determined most effective from the previous week, sparged TOC grade water, camphor was not included in the ensuing analyses. Additionally, more research indicated both menthol and 1,8-cineole were not readily soluble in sparged TOC grade water, although alcohols, such as 100 % ethanol, easily dissolved these analytes. As a result, the standards were prepared with a mixture of 100 % ethanol and sparged TOC grade water, as shown in Table. 11.

When reviewing the peak areas of both 1,8-cineole and menthol, indicated in Tables. 19 and 20 and Figures. 6 and 7, it is evident the analytes were not partitioning into the headspace in correspondence to their expected concentrations. Focusing on Figures. 6 and 7, these graphs indicate there is a cross-over point between standards 3 and 4 as the peak areas of both 1,8-cineole and menthol dramatically drop from standard 3 to 4. The substantial loss of signal for both analytes can be attributed to the ratio of ethanol to sparged TOC grade water. In standard 3 there was more water present within the matrix, being 7:3, whereas standard 4 had more ethanol present within the matrix, being 4:6. Following Henry's Law, the distribution of the analytes between the liquid and headspace phases depends on their solubilities.[17] As both 1,8-cineole and menthol have a higher affinity for ethanol than sparged TOC grade water, these compounds would rather prefer to remain in the liquid phase than vaporize to the headspace if there is more ethanol than TOC grade water present in the liquid phase. This could be the reason behind the low analyte signals of standards 4, 5, and 6, and since there is more TOC grade water than ethanol in standards 1, 2 and 3, these standards have larger analyte signals.

Further, when focusing on standards 1, 2, and 3, the analytes peak areas indicate that there is an optimal ratio of TOC grade water to ethanol. Figures. 6 and 7 demonstrate this increase between the standards illustrating that too much TOC grade water, as in standard 1, will produce a low analyte signal for both 1,8-cineole and menthol. Additionally, although 1,8-cineole demonstrates very low signals for standards 4, 5 and 6, the lack of menthol signal within these standards indicates that menthol is not partitioning into the headspace as readily as 1,8-cineole is and is therefore has a higher affinity for ethanol than 1,8-cineole.

Lastly, a spiked sample of Vicks VapoRub was prepared and analyzed during this experimental session. The result of this analysis is presented in Table. 21. Despite the comparable concentration of added analytes, shown in Tables. 12 and 14, the peak areas of both

analytes within the spiked sample are greater than that of standard 1 of Week 3, whose results are shown in Tables. 22 and 23. The differences between these standards lie in the volume of solvents included in analysis. The majority of the spiked sample from Week 2 consisted of TOC grade water, with only a minimal amount of ethanol whereas standard 1 from Week 3 had equal amounts of ethanol to TOC grade water. This supports the previously made claim concerning the effects of the ratio between TOC grade water and ethanol within the sample.

Although the results from Week 2 produced an unreliable external calibration curve, the analysis allows for a better understanding of the effects of solubility and partitioning during method development on the GC-MS.

Week 3

Constant standard addition was employed during this final session in an effort to develop a more reliable calibration curve for the quantification of 1,8-cineole and menthol within a sample of Vicks VapoRub. The results from this series of standards are outlined in Tables. 22 and 23 as well as Figures. 8 and 9. Despite the proportion of variance, indicated by the low R^2 values on Figures. 8 and 9, the positive slopes allowed the determination of concentration and % W/W of 1,8-cineole and menthol within a sample of Vicks VapoRub. Indicated in Tables. 24 and 25, the concentration and subsequent % W/W of 1,8-cinele was 4.09×10^{-3} g/mL and 1.66 respectively, whereas menthol was determined to be 1.58×10^{-3} g/mL and 0.640 respectively. When comparing these values to those on the manufacturer label of Vicks VapoRub, being 1.2 % W/W eucalyptus oil and 2.6 % W/W menthol as shown in Table. 7, there is a 38 % difference between the values for 1,8-cineole and 75 % difference between the values for menthol. There are many possible explanations for the difference between experimental and literature values such as the volatility of the analytes. Since both 1,8-cineole and menthol are monoterpenoids, they are characteristically volatile compounds and despite GC-MS dominance in analyzing volatile and semi-volatile analytes, there may have been some loss of analytes during sample preparation. Addressing this issue may take the form of transferring the sample and solvents into the GC-MS autosampler vial by injecting it through the septum. Injection through septum would minimize the exposure of analytes to the outside environment and thus decrease the loss of these volatile analytes. Additionally, menthol had a noticeably larger variation between literature and experimental which may be due to its increased solubility in ethanol than 1,8-cineole. This issue,

also present within the results of Week 2, can be minimized by experimenting with different solvents for the each of the analytes. Further, it is interesting to note that although the concentration of added menthol to each standard was greater than the concentration of added 1,8-cineole, the peak areas of menthol are consistently smaller than 1,8-cineole. This discrepancy can be associated with menthols apparent reluctance to vaporize into the headspace phase, further indicating the equilibrium between the contents of the autosampler vial was not achieved.

Future Work

This project produced foundational knowledge in method development for identifying and quantifying 1,8-cineole and menthol in commercial products through static headspace GC-MS. Continuing this analysis should focus on increasing the accuracy and reliability of the method. As mentioned previously, the volatility of these compounds during sample preparation may contribute to the loss of analyte signal. Future experiments should concentrate on minimizing the analyte loss, such as injecting the solvents and viscous sample through the septum of the autosampler vial. Additionally, the discrepancy between menthols expected and actual peak area is an element within the method that should be addressed. Utilizing different solvents in sample preparation, such as propylene glycol or chloroform, and analyzing the effects of the analysis would increase accuracy and reproducibility. Lastly, the partitioning of the samples and standards would be an interesting focus for future work. Utilizing headspace GC-MS, the goal is for the analytes to reach equilibrium between the liquid and headspace phases while minimizing the partition coefficient. An analysis that concentrates on identifying the partition coefficients of the analytes between ethanol and TOC grade water would indicate the efficacy of these solvents within this project. Further, to ensure equilibrium is achieved between the liquid and headspace phases, future work can determine the effects of altering the incubation time, temperature, or agitation rate. Additionally, since Vicks VapoRub is so viscous, it was difficult to ensure the sample was completely mixed within the solvents. As a result, increasing vortex mixing time or changing the method for mixing the sample within the solvent should allow the matrix to achieve equilibrium.

Conclusion

This project developed a method for the simultaneous analysis of 1,8-cineole and menthol in a sample of Vicks VapoRub through static headspace GC-MS. The % W/W of 1,8-cineole and menthol within the sample of Vicks VapoRub was determined to be 1.66 and 0.640 respectively. These values are 38 % and 75 % different from the manufacturers label of Vicks VapoRub for 1,8-cineole and menthol respectively.

References

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Appendix

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct 6\
Data File : SampleVapoRub1.D
Acq On : 06 Oct 2022 08:51
Operator :
Sample : SampleVapoRub1
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	7.043	0.30	D:\MassHunter\Library\NIST14.L			
			(+)-3-Carene	16050	000498-15-7	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16245	000508-32-7	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16251	000508-32-7	94
2	7.135	0.27	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16269	028634-89-1	93
			.alpha.-Phellandrene	16095	000099-83-2	91
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16278	002867-05-2	91
3	7.253	52.68	D:\MassHunter\Library\NIST14.L			
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	96
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
4	7.500	1.07	D:\MassHunter\Library\NIST14.L			
			Camphene	16029	000079-92-5	97
			Camphene	16039	000079-92-5	96
			Camphene	16040	000079-92-5	96
5	7.962	15.48	D:\MassHunter\Library\NIST14.L			
			.beta.-Pinene	16055	000127-91-3	94
			.beta.-Pinene	16060	000127-91-3	94
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	94
6	8.178	0.31	D:\MassHunter\Library\NIST14.L			
			.beta.-Myrcene	16066	000123-35-3	97
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16276	028634-89-1	80
			.beta.-Phellandrene	16088	000555-10-2	72
7	8.394	0.18	D:\MassHunter\Library\NIST14.L			
			.alpha.-Phellandrene	16096	000099-83-2	91
			.alpha.-Phellandrene	16095	000099-83-2	90
			.alpha.-Phellandrene	16093	000099-83-2	72
8	8.484	0.37	D:\MassHunter\Library\NIST14.L			
			3-Carene	16036	013466-78-9	95
			3-Carene	16035	013466-78-9	93
			3-Carene	16028	013466-78-9	93
9	8.584	0.16	D:\MassHunter\Library\NIST14.L			
			1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	16253	000099-86-5	96
			1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	16247	000099-86-5	96

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Figure. 10 Page 1 of library search report of Sample 1 using Method 1 – Week 1.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct6\
Data File : SampleVapoRub1.D
Acq On : 06 Oct 2022 08:51
Operator :
Sample : SampleVapoRub1
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			(+)-4-Carene	16052	029050-33-7	96
10	8.704	0.99	D:\MassHunter\Library\NIST14.L			
			o-Cymene	15144	000527-84-4	97
			p-Cymene	15143	000099-87-6	97
			o-Cymene	15146	000527-84-4	97
11	8.888	10.87	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	96
			Eucalyptol	27467	000470-82-6	86
12	9.213	0.34	D:\MassHunter\Library\NIST14.L			
			.gamma.-Terpinene	16078	000099-85-4	96
			.gamma.-Terpinene	16074	000099-85-4	94
			.gamma.-Terpinene	16077	000099-85-4	94
13	9.655	0.35	D:\MassHunter\Library\NIST14.L			
			Fenchone	25806	001195-79-5	93
			D-Fenchone	25813	004695-62-9	93
			Fenchone	25805	001195-79-5	90
14	9.894	0.78	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hexan-3-one, 4-methy 1-1-(1-methylethyl)-, [1S-(1.alpha .,4.beta.,5.alpha.)]-	26163	000471-15-8	94
			Bicyclo[3.1.0]hexan-3-one, 4-methy 1-1-(1-methylethyl)-	26116	001125-12-8	90
			Thujone	25794	000546-80-5	86
15	10.046	0.15	D:\MassHunter\Library\NIST14.L			
			Thujone	25784	000546-80-5	95
			Thujone	25794	000546-80-5	94
			Bicyclo[3.1.0]hexan-3-one, 4-methy 1-1-(1-methylethyl)-, [1S-(1.alpha .,4.beta.,5.alpha.)]-	26164	000471-15-8	94
16	10.444	13.05	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			Camphor	25791	000076-22-2	97
17	10.786	2.63	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-	29273	001490-04-6	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, (1.alpha.,2.beta.,5.alpha .)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, (1.alpha.,2.beta.,5.alpha .)-(.+/-.)-	29318	015356-70-4	91

Figure. 11 Page 2 of library search report of Sample 1 using Method 1 – Week 1.

Area Percent Report

Data Path : D:\Data\GC-EM 4400\Oct 6\
Data File : SampleVapoRub1.D
Acq On : 06 Oct 2022 08:51
Operator :
Sample : SampleVapoRub1
Misc :
ALS Vial : 2 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\GC-EM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: SampleVapoRub1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	7.043	1011	1029	1038	BV	149099	2726990	0.57%	0.301%
2	7.135	1038	1047	1058	PV	139282	2461355	0.52%	0.272%
3	7.253	1058	1069	1102	VV	24749782	477257861	100.00%	52.683%
4	7.500	1102	1116	1127	VV	498506	9723063	2.04%	1.073%
5	7.962	1170	1203	1236	BV	7823840	140256315	29.39%	15.482%
6	8.178	1236	1244	1263	VV	126043	2853384	0.60%	0.315%
7	8.394	1276	1285	1294	VV	88430	1652956	0.35%	0.182%
8	8.484	1294	1302	1313	VV	196894	3396074	0.71%	0.375%
9	8.584	1313	1320	1331	VV	79008	1412137	0.30%	0.156%
10	8.704	1331	1343	1348	PV	499608	8984777	1.88%	0.992%
11	8.808	1348	1363	1401	VB 4	4390492	98500200	20.64%	10.873%
12	9.213	1419	1439	1460	BB	152202	3073438	0.64%	0.339%
13	9.655	1505	1523	1540	BV	164033	3203487	0.67%	0.354%
14	9.894	1560	1568	1585	VV	395087	7078732	1.48%	0.781%
15	10.046	1585	1596	1619	VB 3	53450	1351509	0.28%	0.149%
16	10.444	1648	1672	1698	BV	6981591	118190897	24.76%	13.047%
17	10.786	1698	1736	1778	VB	1153066	23784695	4.98%	2.626%

Sum of corrected areas: 905907870

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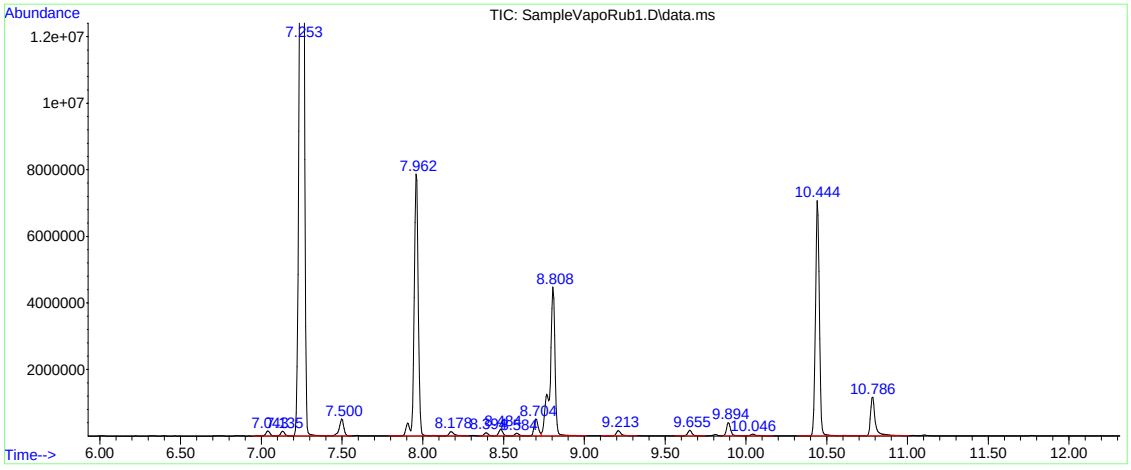


Figure. 12 GC-MS chromatogram of Sample 1 using Method 1 – Week 1.

Library Search Report

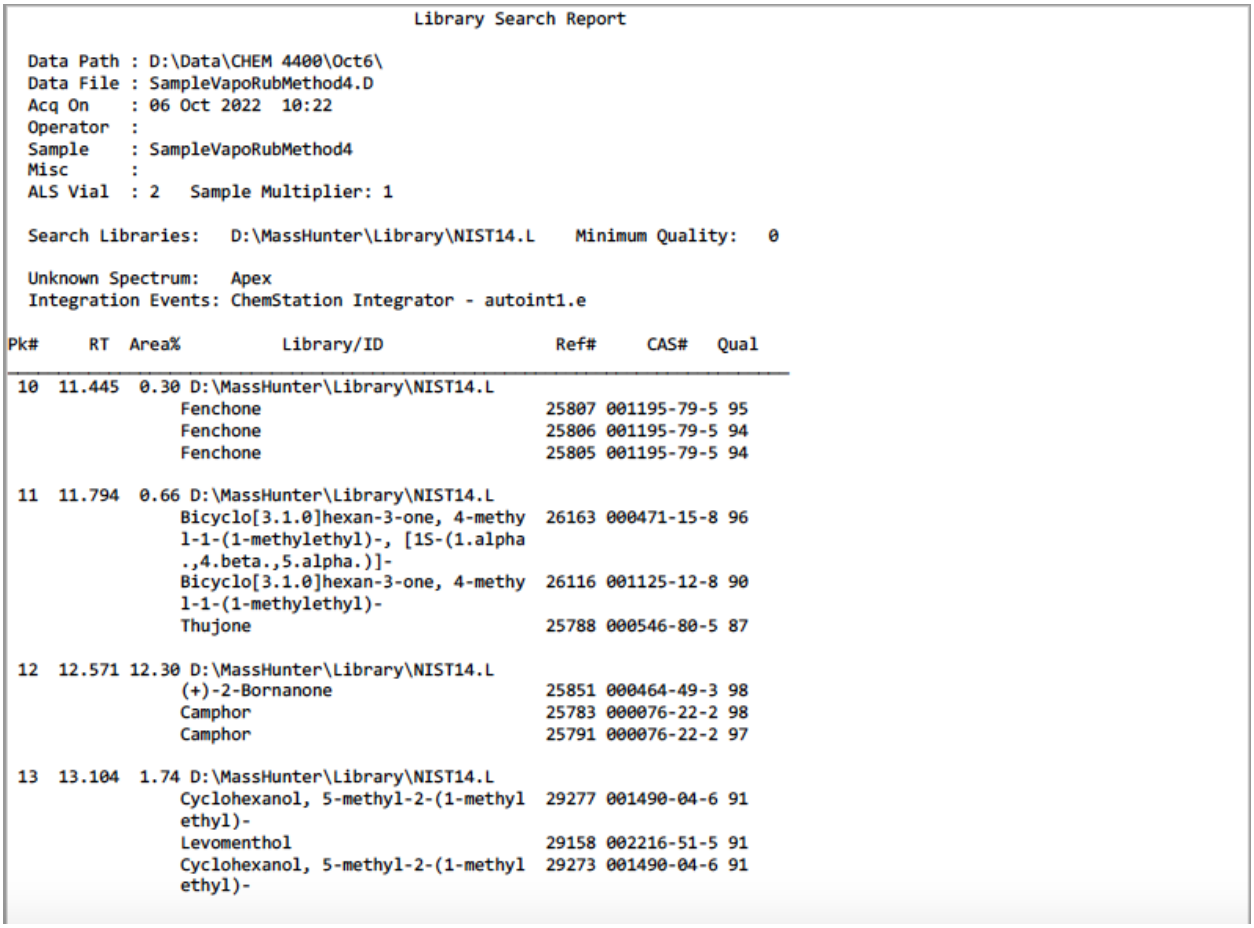
Data Path : D:\Data\CHEM 4400\Oct 6\
Data File : SampleVapoRubMethod4.D
Acq On : 06 Oct 2022 10:22
Operator :
Sample : SampleVapoRubMethod4
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	7.787	0.28	D:\MassHunter\Library\NIST14.L			
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16245	000508-32-7	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16251	000508-32-7	94
			(+)-3-Carene	16050	000498-15-7	94
2	7.924	0.25	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16271	002867-05-2	96
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16269	028634-89-1	93
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16278	002867-05-2	91
3	8.073	56.11	D:\MassHunter\Library\NIST14.L			
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	96
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
4	8.402	1.04	D:\MassHunter\Library\NIST14.L			
			Camphene	16029	000079-92-5	96
			Camphene	16039	000079-92-5	96
			Camphene	16040	000079-92-5	96
5	9.038	15.48	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	94
			.beta.-Pinene	16060	000127-91-3	94
			.beta.-Pinene	16055	000127-91-3	94
6	9.782	0.36	D:\MassHunter\Library\NIST14.L			
			3-Carene	16028	013466-78-9	95
			(+)-3-Carene	16050	000498-15-7	95
			3-Carene	16036	013466-78-9	95
7	10.102	0.73	D:\MassHunter\Library\NIST14.L			
			o-Cymene	15145	000527-84-4	97
			o-Cymene	15146	000527-84-4	97
			p-Cymene	15143	000099-87-6	97
8	10.233	10.49	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	95
			Eucalyptol	27467	000470-82-6	86
9	10.835	0.25	D:\MassHunter\Library\NIST14.L			
			.gamma.-Terpinene	16077	000099-85-4	96
			.gamma.-Terpinene	16078	000099-85-4	95
			.gamma.-Terpinene	16074	000099-85-4	94

Figure. 13 Page 1 of library search report of Sample 1 using Method 4 – Week 1.



Library Search Report

Data Path : D:\Data\CHEM 4400\Oct6\
Data File : SampleVapoRubMethod4.D
Acq On : 06 Oct 2022 10:22
Operator :
Sample : SampleVapoRubMethod4
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
10	11.445	0.30	D:\MassHunter\Library\NIST14.L			
			Fenchone	25807	001195-79-5	95
			Fenchone	25806	001195-79-5	94
			Fenchone	25805	001195-79-5	94
11	11.794	0.66	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hexan-3-one, 4-methy l-1-(1-methylethyl)-, [1S-(1.alpha .,4.beta.,5.alpha.)]-	26163	000471-15-8	96
			Bicyclo[3.1.0]hexan-3-one, 4-methy l-1-(1-methylethyl)-	26116	001125-12-8	90
			Thujone	25788	000546-80-5	87
12	12.571	12.30	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			Camphor	25791	000076-22-2	97
13	13.104	1.74	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-	29277	001490-04-6	91
			Levomenthol	29158	002216-51-5	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-	29273	001490-04-6	91

Figure. 14 Page 2 of library search report of Sample 1 using Method 4 – Week 1.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 6\
Data File : SampleVapoRubMethod4.D
Acq On : 06 Oct 2022 10:22
Operator :
Sample : SampleVapoRubMethod4
Misc :
ALS Vial : 2 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: SampleVapoRubMethod4.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	7.787	1160	1170	1183	BV	110644	2321374	0.50%	0.280%
2	7.924	1183	1196	1210	PV	99351	2105393	0.45%	0.254%
3	8.073	1210	1224	1268	PB	20381613	464353014	100.00%	56.109%
4	8.402	1269	1286	1305	BV	366134	8600652	1.85%	1.039%
5	9.038	1385	1406	1447	BB	5964696	128119360	27.59%	15.481%
6	9.782	1537	1547	1565	VV	139466	2993757	0.64%	0.362%
7	10.102	1596	1607	1615	BV	252245	6052384	1.30%	0.731%
8	10.233	1615	1632	1679	VB 2	3225183	86779161	18.69%	10.486%
9	10.835	1719	1745	1778	BB 2	65300	2077260	0.45%	0.251%
10	11.445	1848	1861	1886	BB	100772	2448496	0.53%	0.296%
11	11.794	1917	1926	1952	BB 2	230037	5475463	1.18%	0.662%
12	12.571	2059	2073	2113	BB	4883881	101825549	21.93%	12.304%
13	13.104	2162	2174	2222	BB	499997	14435616	3.11%	1.744%

Sum of corrected areas: 827587479

Carmen Chlo...rm Lab_F22.M Thu Oct 06 11:09:47 2022

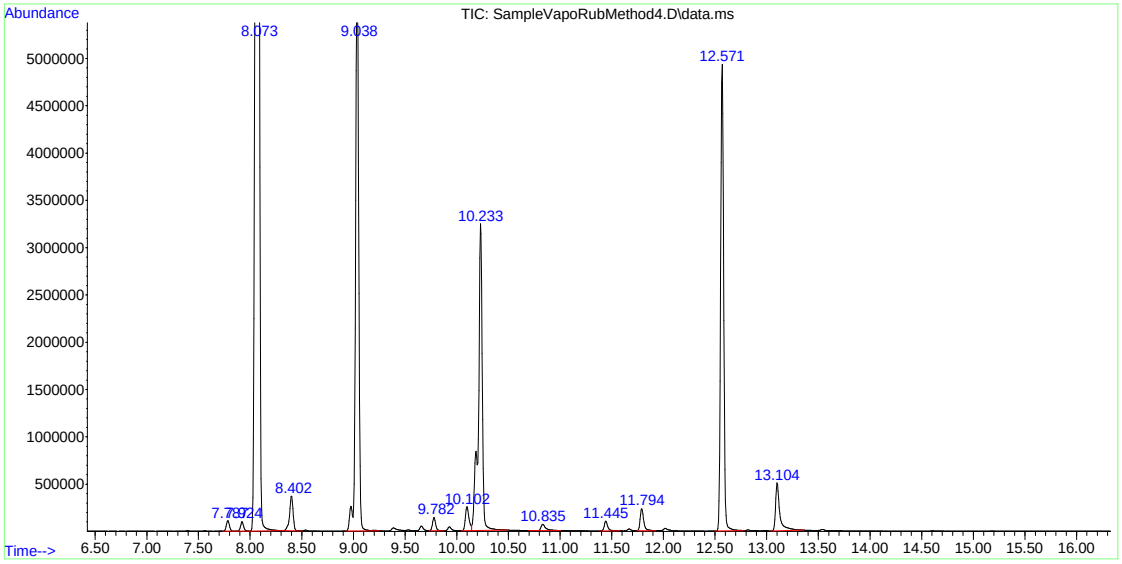


Figure. 15 GC-MS chromatogram of Sample 1 using Method 4 – Week 1.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct 6\
Data File : VapoRubH2OSample1.D
Acq On : 06 Oct 2022 09:59
Operator :
Sample : VapoRubH2OSample1
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.369	2.82	D:\MassHunter\Library\NIST14.L			
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	95
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	94
2	6.850	1.50	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16282	018172-67-3	94
			.beta.-Pinene	16055	000127-91-3	94
			.beta.-Pinene	16060	000127-91-3	91
3	7.395	34.93	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	95
			Eucalyptol	27467	000470-82-6	86
4	7.939	0.98	D:\MassHunter\Library\NIST14.L			
			Fenchone	25803	001195-79-5	90
			Fenchone	25804	001195-79-5	83
			Fenchone	25805	001195-79-5	83
5	8.087	3.57	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-	26163	000471-15-8	94
			Thujone	25788	000546-80-5	87
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-	26164	000471-15-8	87
6	8.182	0.80	D:\MassHunter\Library\NIST14.L			
			Thujone	25784	000546-80-5	96
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-	26164	000471-15-8	95
			Thujone	25787	000546-80-5	76
7	8.438	45.27	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			Camphor	25791	000076-22-2	97
8	8.640	10.12	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29318	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-	29273	001490-04-6	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29317	015356-70-4	91

Carmen Chlo...rm Lab_F22.M Thu Oct 06 11:08:39 2022 1

Figure. 16 Library search report of Sample 2 – Week 1.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 6\
Data File : VapoRubH2OSample1.D
Acq On : 06 Oct 2022 09:59
Operator :
Sample : VapoRubH2OSample1
Misc :
ALS Vial : 4 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: VapoRubH2OSample1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.369	891	902	924	BB	100777	1531999	6.23%	2.818%
2	6.850	977	993	1013	BB	51921	817073	3.32%	1.503%
3	7.395	1079	1096	1135	BB	1296400	18988980	77.16%	34.932%
4	7.939	1190	1199	1209	BV	35177	533218	2.17%	0.981%
5	8.087	1209	1226	1239	VV	104810	1943024	7.90%	3.574%
6	8.182	1239	1244	1270	VB 3	15447	434006	1.76%	0.798%
7	8.438	1282	1293	1323	BV	1685475	24609999	100.00%	45.273%
8	8.640	1323	1331	1366	PV	233249	5500902	22.35%	10.120%

Sum of corrected areas: 54359201

Carmen Chlo...rm Lab_F22.M Thu Oct 06 11:07:59 2022

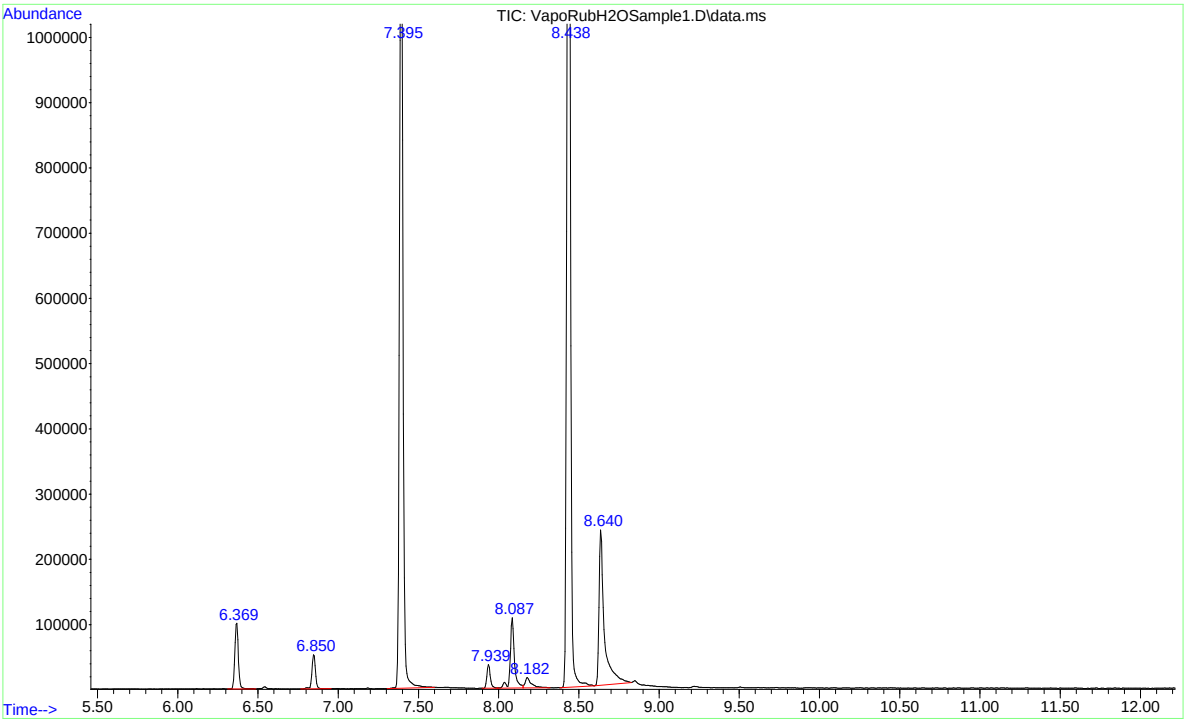


Figure. 17 GC-MS chromatogram of Sample 2 – Week 1.

Library Search Report

Data Path : D:\Data\CH-EM 4400\Oct 6\
Data File : SampleVapoRubMethanol 1.D
Acq On : 06 Oct 2022 10:54
Operator :
Sample : SampleVapoRubMethanol 1
Misc :
ALS Vial : 5 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	6.368	80.15	D:\MassHunter\Library\NIST14.L			
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	97
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	95
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	94
2	6.542	1.37	D:\MassHunter\Library\NIST14.L			
			1,3,6-Heptatriene, 2,5,5-trimethyl	16167	029548-02-5	58
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	53
			Bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl-	16209	000464-17-5	53
3	6.849	14.02	D:\MassHunter\Library\NIST14.L			
			.beta.-Pinene	16055	000127-91-3	94
			.beta.-Pinene	16058	000127-91-3	91
			.beta.-Pinene	16060	000127-91-3	91
4	7.401	1.31	D:\MassHunter\Library\NIST14.L			
			1H-4-Azacyclopropa[cd]indene, octahydro-4-methyl-	17236	016967-50-3	25
			Histidine, 1,N-dimethyl-4-nitro-	91772	1000129-55-1	25
			Benzyl alcohol, p-hydroxy-.alpha.-[(methylamino)methyl]-	37757	000094-07-5	23
5	8.444	1.06	D:\MassHunter\Library\NIST14.L			
			Camphor	25791	000076-22-2	95
			Camphor	25783	000076-22-2	95
			(+)-2-Bornanone	25850	000464-49-3	95
6	13.915	0.82	D:\MassHunter\Library\NIST14.L			
			1,2-Propanediamine	800	000078-90-0	7
			3-Propoxyamphetamine	58649	1000124-04-4	7
			1,2-Propanediamine	796	000078-90-0	5
7	14.222	1.27	D:\MassHunter\Library\NIST14.L			
			Benzeneethanamine, N-methyl-	15957	000589-08-2	9
			Amphetamine	15918	000300-62-9	9
			Benzeneethanamine, N-methyl-	15953	000589-08-2	9

Figure. 18 Library search report of Sample 3 – Week 1.

Area Percent Report

Data Path : D:\Data\CH-EM 4400\Oct 6\
Data File : SampleVapoRubMethanol 1.D
Acq On : 06 Oct 2022 10:54
Operator :
Sample : SampleVapoRubMethanol 1
Misc :
ALS Vial : 5 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CH-EM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: SampleVapoRubMethanol 1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.368	893	902	928	PV	548990	8341510	100.00%	80.149%
2	6.542	928	935	951	VB 3	7170	142950	1.71%	1.374%
3	6.849	964	993	1024	BB	92795	1458868	17.49%	14.017%
4	7.401	1075	1097	1117	BB 5	6248	136412	1.64%	1.311%
5	8.444	1270	1294	1307	BB 3	6887	109896	1.32%	1.056%
6	13.915	2287	2327	2335	VV 3	1075	85364	1.02%	0.820%
7	14.222	2335	2385	2401	VV 3	1235	132552	1.59%	1.274%

Sum of corrected areas: 10407552

Carmen Chlo...rm Lab_F22.M Thu Oct 06 11:11:54 2022

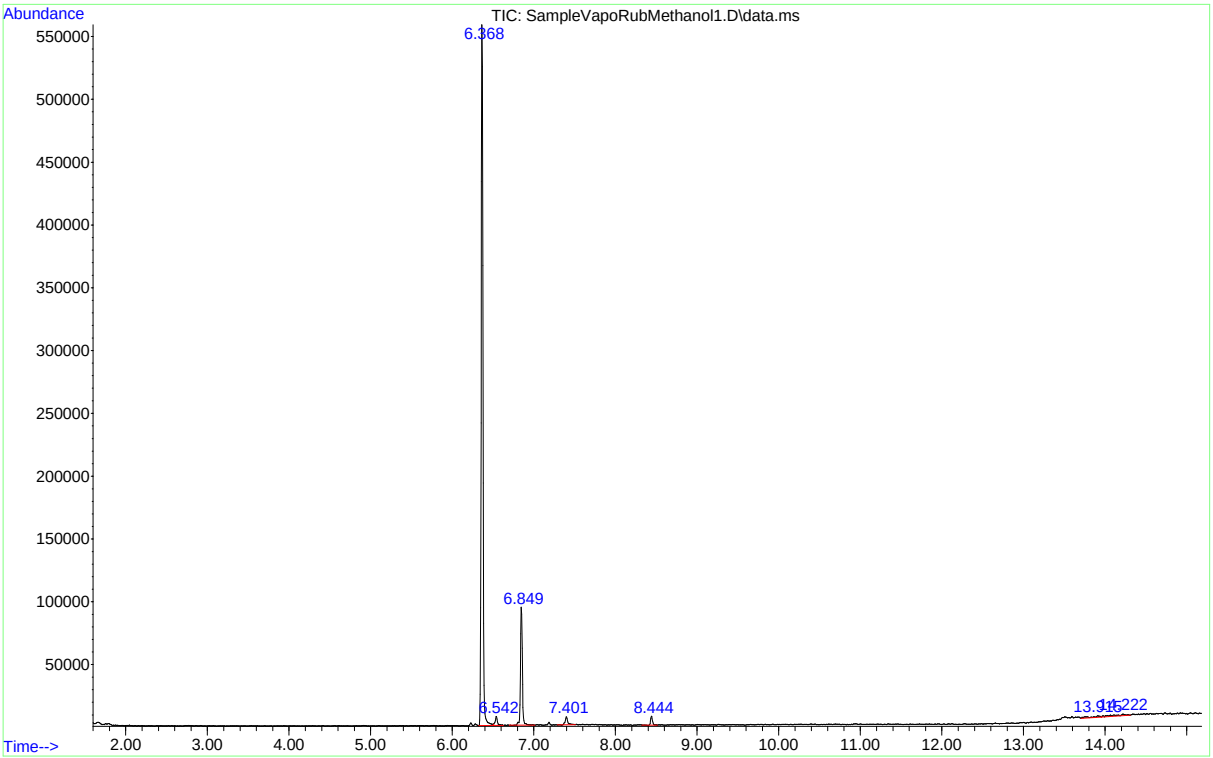


Figure. 19 GC-MS chromatogram of Sample 3 – Week 1.

Library Search Report

Data Path : D:\Data\CH-EM4400\Oct13\
Data File : Std0.D
Acq On : 13 Oct 2022 11:34
Operator :
Sample : Standard0
Misc :
ALS Vial : 8 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.394	81.33	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	95
			Eucalyptol	27467	000470-82-6	86
2	8.639	18.67	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-	29273	001490-04-6	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.beta.,5.beta.)]-	29314	023283-97-8	90

Figure. 20 Library search report of standard 1 – Week 2.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 13\
Data File : Std0.D
Acq On : 13 Oct 2022 11:34
Operator :
Sample : Standard0
Misc :
ALS Vial : 8 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: Std0.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. %max.	% of total
1	7.394	1069	1096	1139	BB	3220467	45543309	100.00%	81.334%
2	8.639	1306	1331	1417	BB	371157	10452097	22.95%	18.666%

Sum of corrected areas: 55995406

Carmen Chlo...rm Lab_F22.M Thu Oct 13 12:04:13 2022

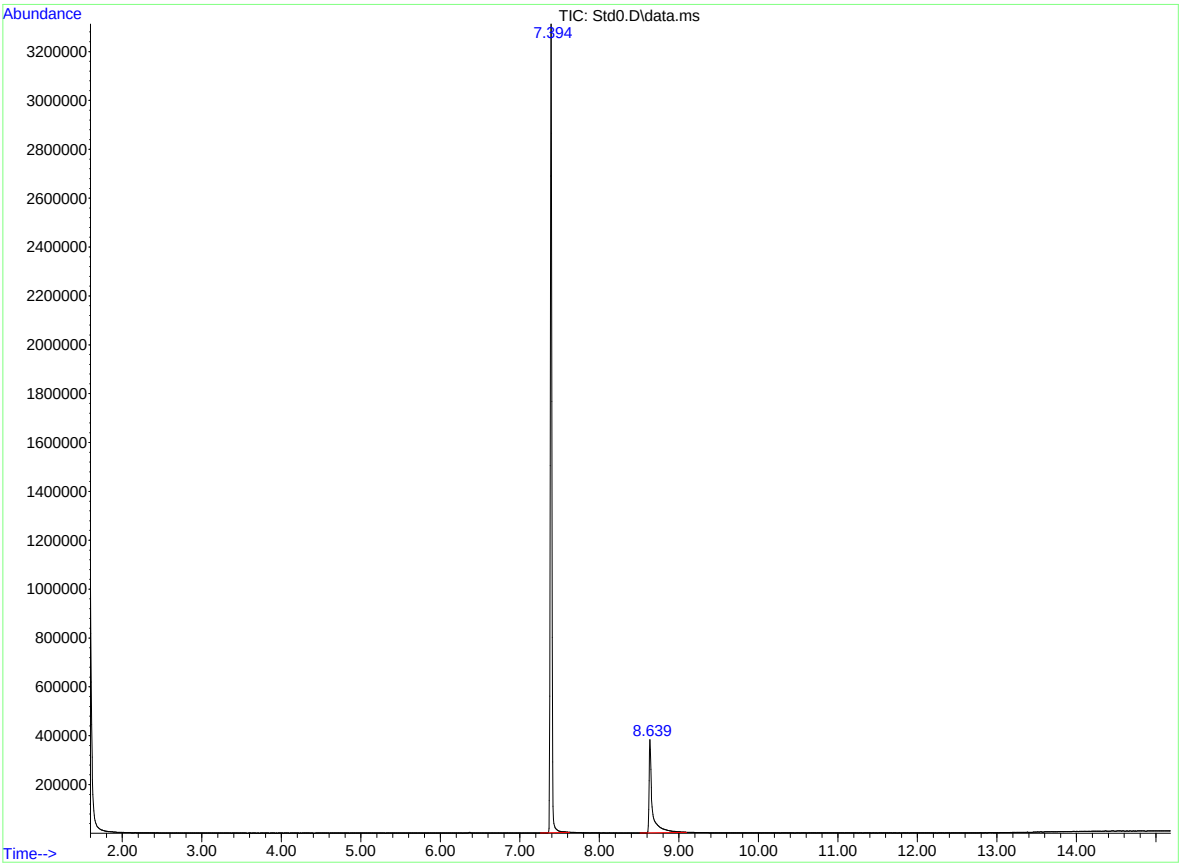


Figure. 21 GC-MS chromatogram of standard 1 – Week 2.

Library Search Report

Data Path : D:\Data\GCMS-QP2020\2022\Oct 13\
Data File : Std1.D
Acq On : 13 Oct 2022 09:45
Operator :
Sample : Standard1
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	7.395	80.85	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	95
			Eucalyptol	27467	000470-82-6	86
2	8.635	19.15	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-	29273	001490-04-6	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29318	015356-70-4	91

Figure. 22 Library search report of standard 2 – Week 2.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 13\
Data File : Std1.D
Acq On : 13 Oct 2022 09:45
Operator :
Sample : Standard1
Misc :
ALS Vial : 2 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: Std1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	7.395	1075	1096	1119	BB	12523287	178731418	100.00%	80.854%
2	8.635	1321	1330	1356	BB	2606469	42324130	23.68%	19.146%

Sum of corrected areas: 221055548

Carmen Chlo...rm Lab_F22.M Thu Oct 13 10:05:17 2022

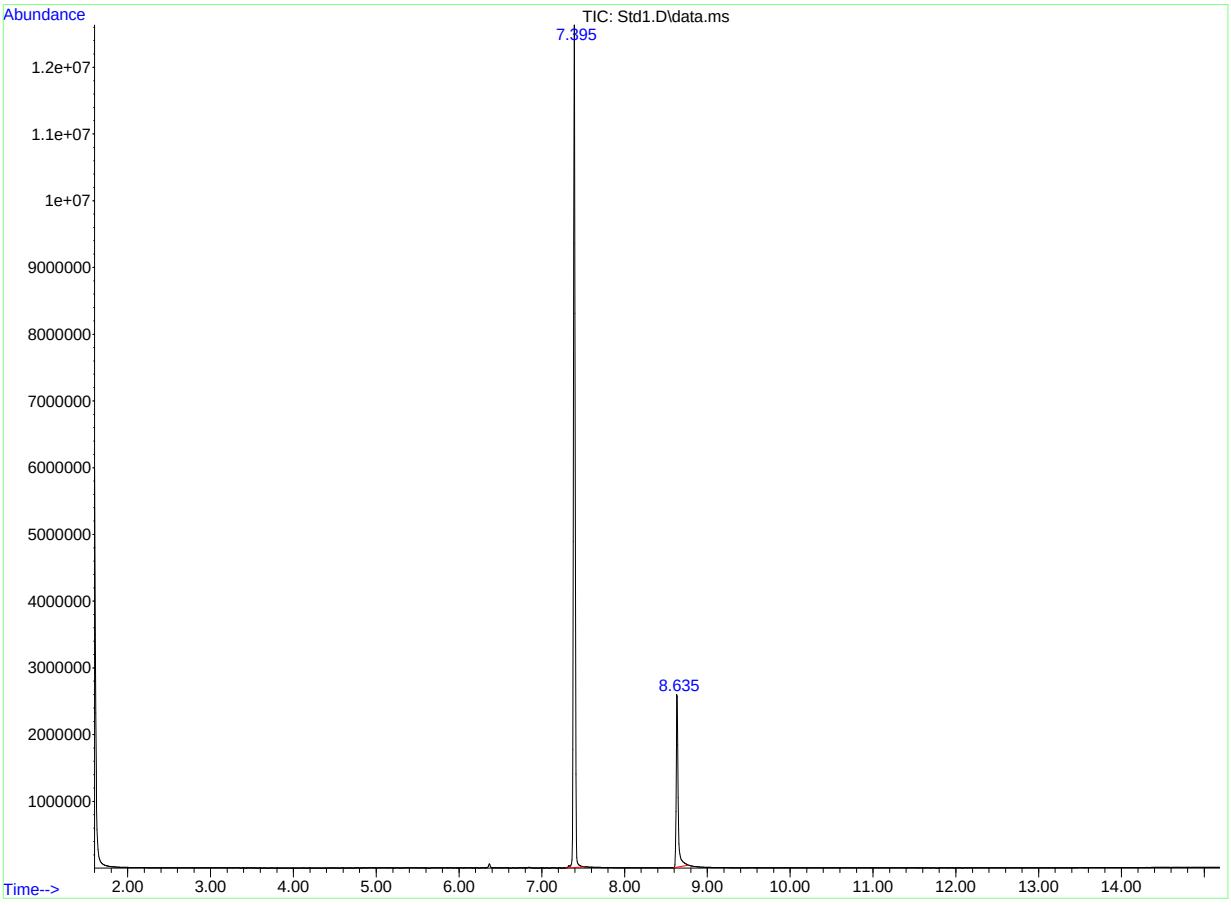


Figure. 23 GC-MS chromatogram of standard 2 – Week 2.

Library Search Report

Data Path : D:\Data\GC-EM 4400\Oct 13\
Data File : Std2.D
Acq On : 13 Oct 2022 10:28
Operator :
Sample : Standard2
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.368	0.73	D:\MassHunter\Library\NIST14.L			
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	97
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
2	6.850	0.13	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	94
			.beta.-Pinene	16060	000127-91-3	94
			Cyclohexene, 4-methylene-1-(1-methylethyl)-	16216	000099-84-3	91
3	7.395	86.42	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	95
			Eucalyptol	27464	000470-82-6	89
4	8.634	12.72	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29318	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-	29273	001490-04-6	91

Figure. 24 Library search report of standard 3 – Week 2.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 13\
Data File : Std2.D
Acq On : 13 Oct 2022 10:28
Operator :
Sample : Standard2
Misc :
ALS Vial : 4 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: Std2.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.368	879	902	923	BB	139837	2136106	0.85%	0.732%
2	6.850	972	993	1016	BB	23348	382538	0.15%	0.131%
3	7.395	1063	1096	1215	BB	16831351	252211010	100.00%	86.416%
4	8.634	1302	1330	1435	BB	1910532	37128200	14.72%	12.721%

Sum of corrected areas: 291857854

Carmen Chlo...rm Lab_F22.M Thu Oct 13 10:47:23 2022

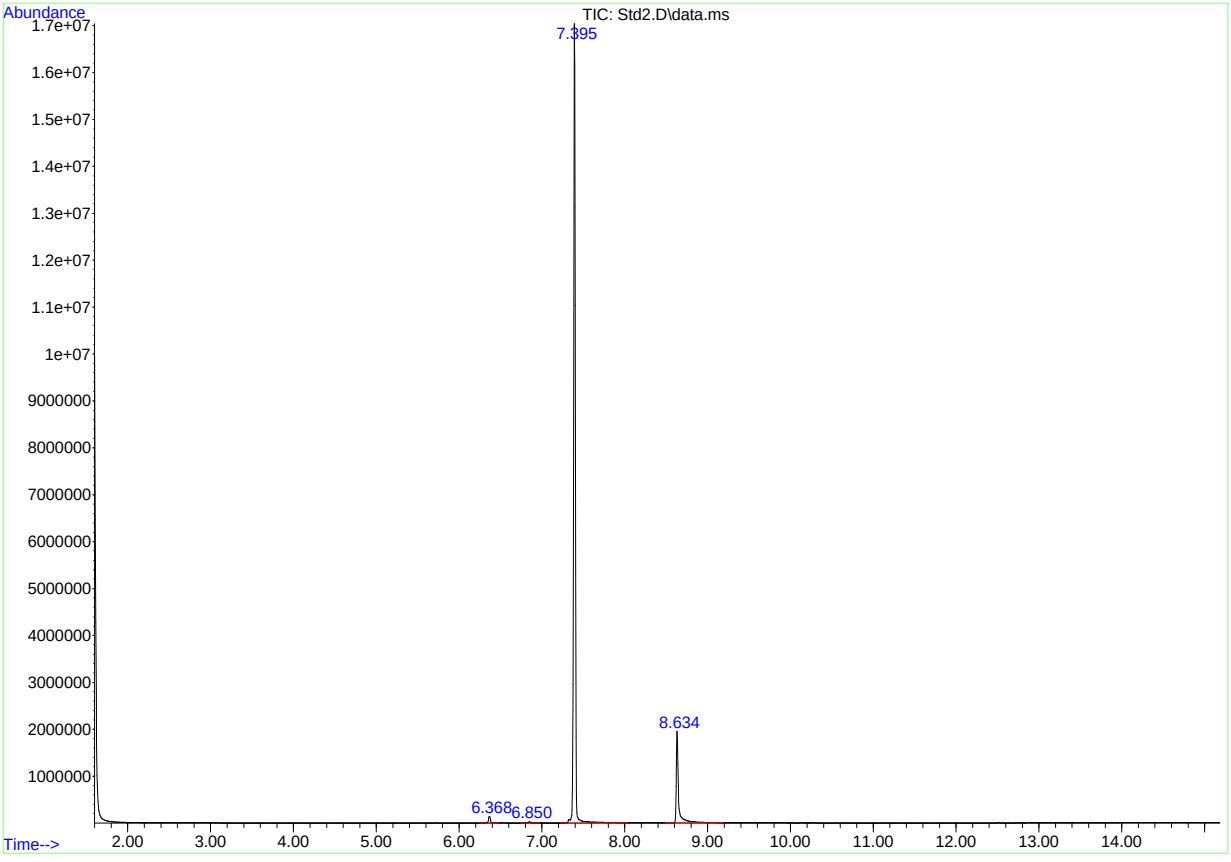


Figure. 25 GC-MS chromatogram of standard 3 – Week 2.

Library Search Report

Data Path : D:\Data\GCMS-QP2020\Oct 13\
Data File : Std3.D
Acq On : 13 Oct 2022 10:50
Operator :
Sample : Standard3
Misc :
ALS Vial : 5 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.369	2.71	D:\MassHunter\Library\NIST14.L			
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	95
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	95
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	94
2	7.394	97.29	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	96
			Eucalyptol	27467	000470-82-6	86

Figure. 26 Library search report of standard 4 – Week 2.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 13\
Data File : Std3.D
Acq On : 13 Oct 2022 10:50
Operator :
Sample : Standard3
Misc :
ALS Vial : 5 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: Std3.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.369	879	902	925	BB	77667	1200932	2.79%	2.712%
2	7.394	1064	1096	1140	BB	2983642	43080277	100.00%	97.288%

Sum of corrected areas: 44281208

Carmen Chlo...rm Lab_F22.M Thu Oct 13 11:10:33 2022

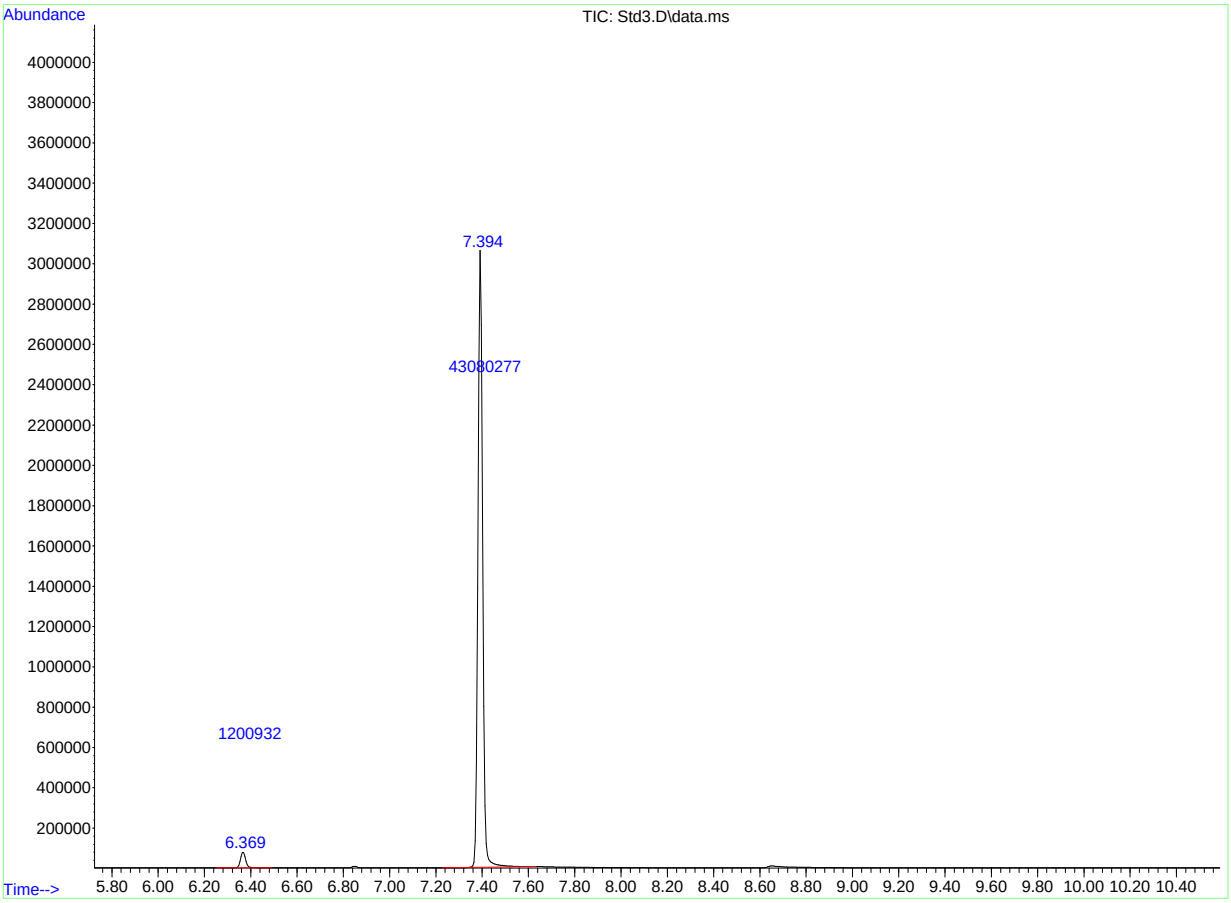


Figure. 27 GC-MS chromatogram of standard 4 – Week 2.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct 13\
Data File : Std4.D
Acq On : 13 Oct 2022 11:12
Operator :
Sample : Standard4
Misc :
ALS Vial : 6 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.396	100.00	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	96
			Eucalyptol	27464	000470-82-6	93

Figure. 28 Library search report of standard 5 – Week 2.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 13\
Data File : Std4.D
Acq On : 13 Oct 2022 11:12
Operator :
Sample : Standard4
Misc :
ALS Vial : 6 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: Std4.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	7.396	1074	1096	1122	BB	230624	3503067	100.00%	100.000%

Sum of corrected areas: 3503067

Carmen Chlo...rm Lab_F22.M Thu Oct 13 12:03:04 2022

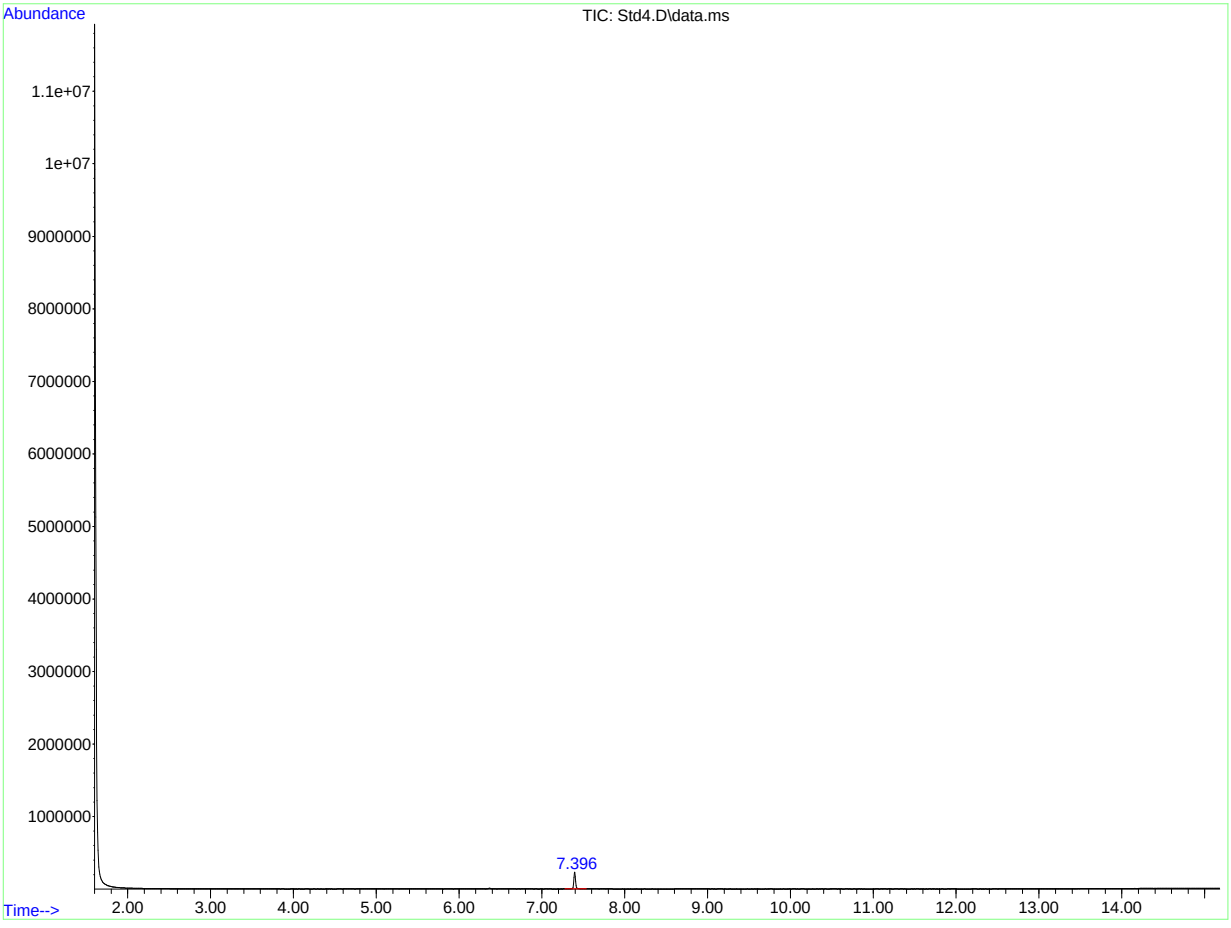


Figure. 29 GC-MS chromatogram of standard 5 – Week 2.

Library Search Report

Data Path : D:¥Data¥CH-EM 4400¥Oct 13¥
Data File : Std5.D
Acq On : 13 Oct 2022 10:06
Operator :
Sample : Standard5
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: D:¥MassHunter¥Library¥NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	7.395	100.00	D:¥MassHunter¥Library¥NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	96
			Eucalyptol	27464	000470-82-6	93

Figure. 30 Library search report of standard 6 – Week 2.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 13\
Data File : Std5.D
Acq On : 13 Oct 2022 10:06
Operator :
Sample : Standard5
Misc :
ALS Vial : 3 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: Std5.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	7.395	1071	1096	1124	BB	315205	4742008	100.00%	100.000%

Sum of corrected areas: 4742008

Carmen Chlo...rm Lab_F22.M Thu Oct 13 10:23:16 2022

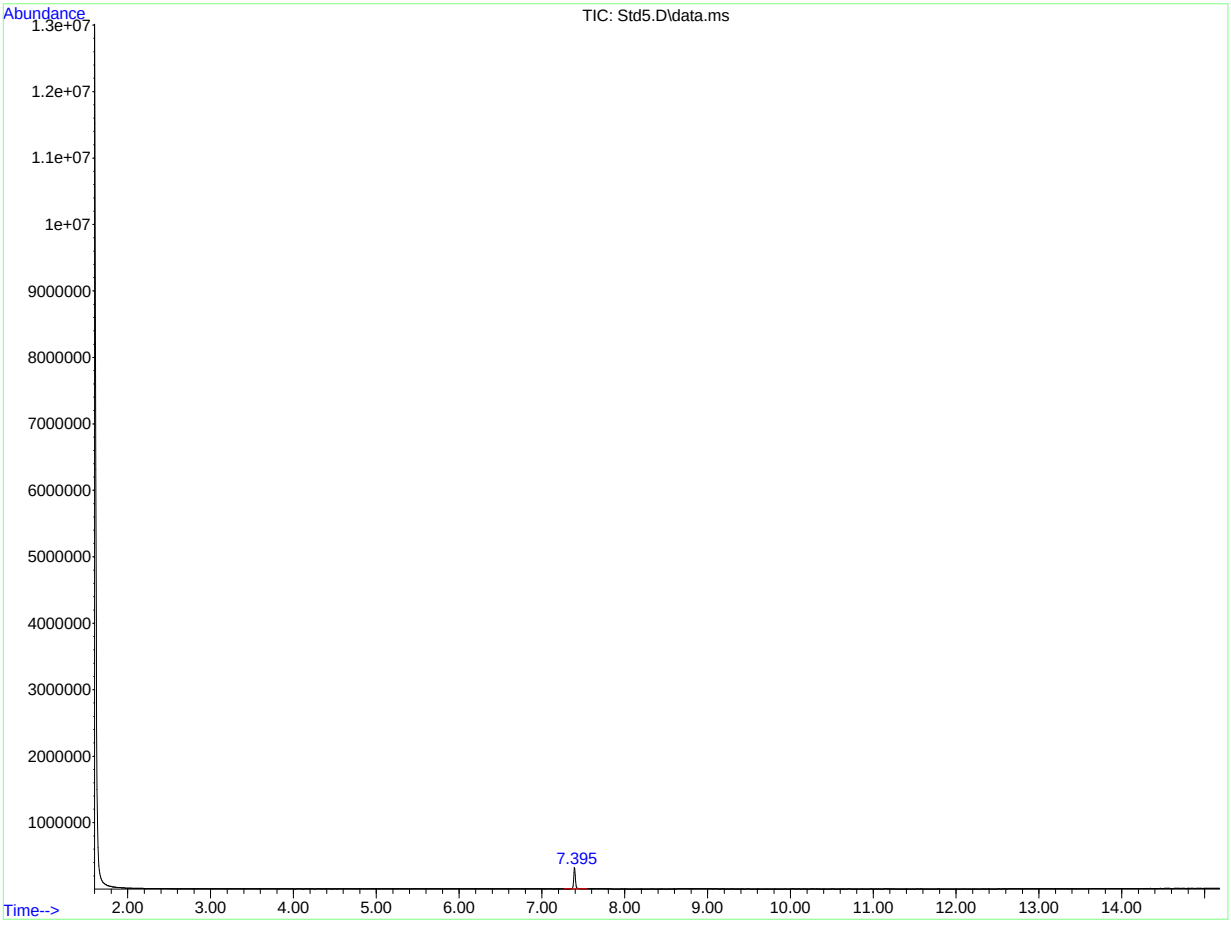


Figure. 31 GC-MS chromatogram of standard 6 – Week 2.

Library Search Report

Data Path : D:\Data\GC-EM 4400\Oct 13\
Data File : SpikedSampled1.D
Acq On : 13 Oct 2022 11:56
Operator :
Sample : SpikedSample1
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.369	60.30	D:\MassHunter\Library\NIST14.L			
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	96
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
2	6.540	1.37	D:\MassHunter\Library\NIST14.L			
			Camphene	16029	000079-92-5	96
			Camphene	16039	000079-92-5	96
			Camphene	16040	000079-92-5	96
3	6.847	18.23	D:\MassHunter\Library\NIST14.L			
			.beta.-Pinene	16055	000127-91-3	94
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	94
			.beta.-Pinene	16060	000127-91-3	94
4	7.392	11.25	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	96
			Eucalyptol	27467	000470-82-6	90
5	8.436	8.06	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			Camphor	25791	000076-22-2	97
6	8.639	0.79	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-	29273	001490-04-6	90
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,5.beta.)]-	29316	002216-52-6	90

Figure. 32 Library search report of spiked sample – Week 2.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 13\
Data File : SpikedSampled1.D
Acq On : 13 Oct 2022 11:56
Operator :
Sample : SpikedSample1
Misc :
ALS Vial : 2 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\Carmen Chloroform Lab_F22.M
Title :

Signal : TIC: SpikedSampled1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.369	893	902	925	VV	25922569	414378507	100.00%	60.296%
2	6.540	925	934	958	VB	537961	9420087	2.27%	1.371%
3	6.847	973	992	1011	BV	8284550	125302190	30.24%	18.233%
4	7.392	1074	1095	1122	VB	5 3774114	77296542	18.65%	11.247%
5	8.436	1239	1292	1315	BB	3914754	55419897	13.37%	8.064%
6	8.639	1322	1331	1354	BB	250020	5427944	1.31%	0.790%

Sum of corrected areas: 687245166

Carmen Chlo...rm Lab_F22.M Thu Oct 13 12:13:38 2022

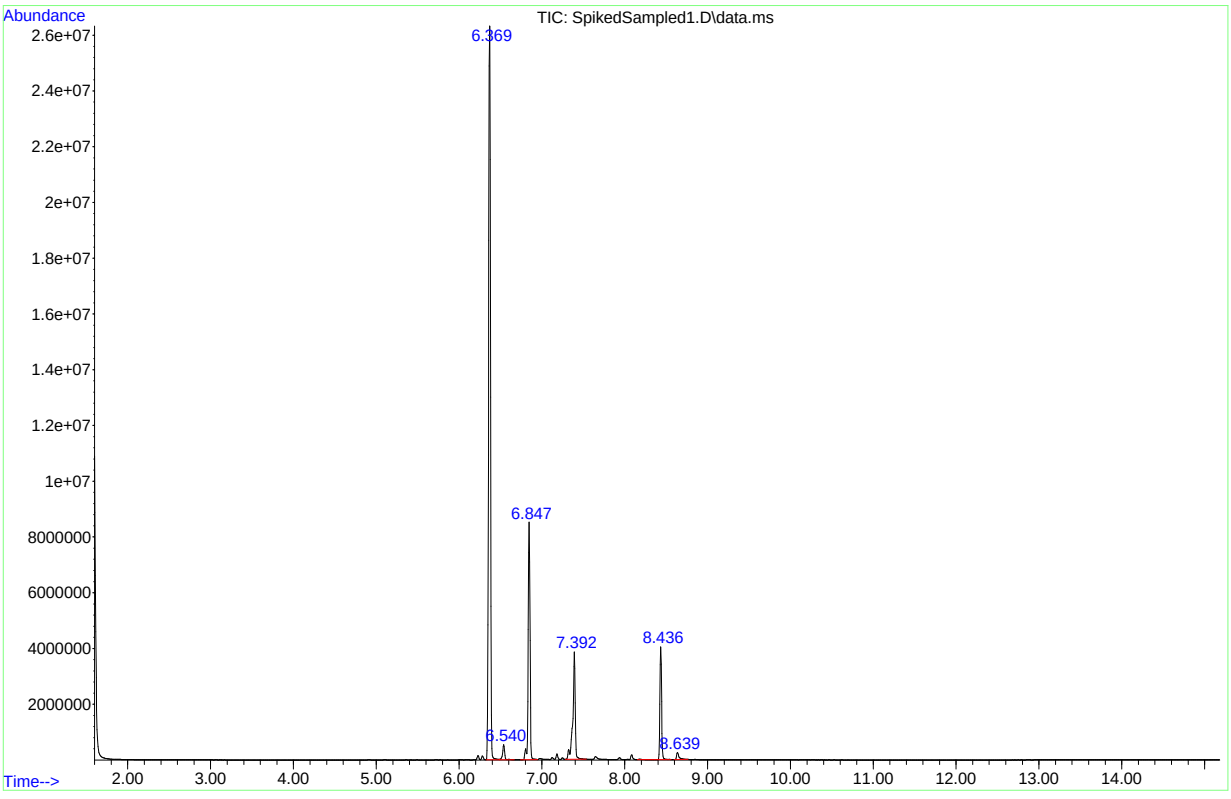


Figure. 33 GC-MS chromatogram of spiked sample – Week 2.

Library Search Report

Data Path : D:¥Data¥CHEM 4400¥Oct 20¥
Data File : Standard0.D
Acq On : 20 Oct 2022 09:24
Operator :
Sample : Standard 0
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:¥MassHunter¥Library¥NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	6.230	0.33	D:¥MassHunter¥Library¥NIST14.L			
			(+)-3-Carene	16050	000498-15-7	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16245	000508-32-7	94
			3-Carene	16036	013466-78-9	91
2	6.285	0.26	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16271	002867-05-2	97
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16269	028634-89-1	93
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16278	002867-05-2	91
3	6.369	65.00	D:¥MassHunter¥Library¥NIST14.L			
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	97
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
4	6.541	1.28	D:¥MassHunter¥Library¥NIST14.L			
			Camphene	16029	000079-92-5	96
			Camphene	16039	000079-92-5	96
			Camphene	16040	000079-92-5	96
5	6.806	0.63	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16270	003387-41-5	97
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16273	003387-41-5	91
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16276	028634-89-1	91
6	6.848	17.63	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	94
			.beta.-Pinene	16055	000127-91-3	94
			.beta.-Pinene	16060	000127-91-3	94
7	7.185	0.40	D:¥MassHunter¥Library¥NIST14.L			
			.gamma.-Terpinene	16074	000099-85-4	95
			3-Carene	16037	013466-78-9	95
			3-Carene	16036	013466-78-9	95
8	7.394	9.33	D:¥MassHunter¥Library¥NIST14.L			
			Eucalyptol	27458	000470-82-6	97
			Eucalyptol	27466	000470-82-6	95
			Eucalyptol	27467	000470-82-6	90
9	8.088	0.34	D:¥MassHunter¥Library¥NIST14.L			
			Thujone	25794	000546-80-5	83
			Bicyclo[3.1.0]hexan-3-one, 4-methy	26119	001125-12-8	72

EDVapoRub3.M Thu Oct 20 09:43:28 2022 Page: 1

Figure. 34 Page 1 of library search report of standard 0 – Week 3.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct20\
Data File : Standard0.D
Acq On : 20 Oct 2022 09:24
Operator :
Sample : Standard 0
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			l-1-(1-methylethyl)-			
			Thujone	25787	000546-80-5	64
10	8.438	4.44	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			Camphor	25791	000076-22-2	97
11	8.643	0.35	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, (1.alpha.,2.beta.,5.alpha .)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-	29273	001490-04-6	90
			Cyclohexane, 1-methyl-4-(1-methyle thenyl)-, cis-	17416	001879-07-8	83

Figure. 35 Page 2 of library search report of standard 0 – Week 3.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard0.D
Acq On : 20 Oct 2022 09:24
Operator :
Sample : Standard 0
Misc :
ALS Vial : 2 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\EDVapoRub3.M
Title :

Signal : TIC: Standard0.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.230	843	876	881	BV	92448	1400168	0.51%	0.329%
2	6.285	881	886	893	VV	75882	1121087	0.41%	0.264%
3	6.369	893	902	925	PV	17819711	276312973	100.00%	65.005%
4	6.541	925	935	942	VV	332146	5422700	1.96%	1.276%
5	6.806	973	985	987	BV	216880	2681779	0.97%	0.631%
6	6.848	987	992	1013	VV	5174626	74926395	27.12%	17.627%
7	7.185	1051	1056	1063	VV	117185	1706420	0.62%	0.401%
8	7.394	1076	1096	1135	VB 5	1858292	39667798	14.36%	9.332%
9	8.088	1213	1227	1240	BV	86154	1441891	0.52%	0.339%
10	8.438	1285	1293	1313	BB	1314992	18886550	6.84%	4.443%
11	8.643	1317	1331	1356	BB	61495	1498703	0.54%	0.353%

Sum of corrected areas: 425066462

EDVapoRub3.M Thu Oct 20 09:43:55 2022

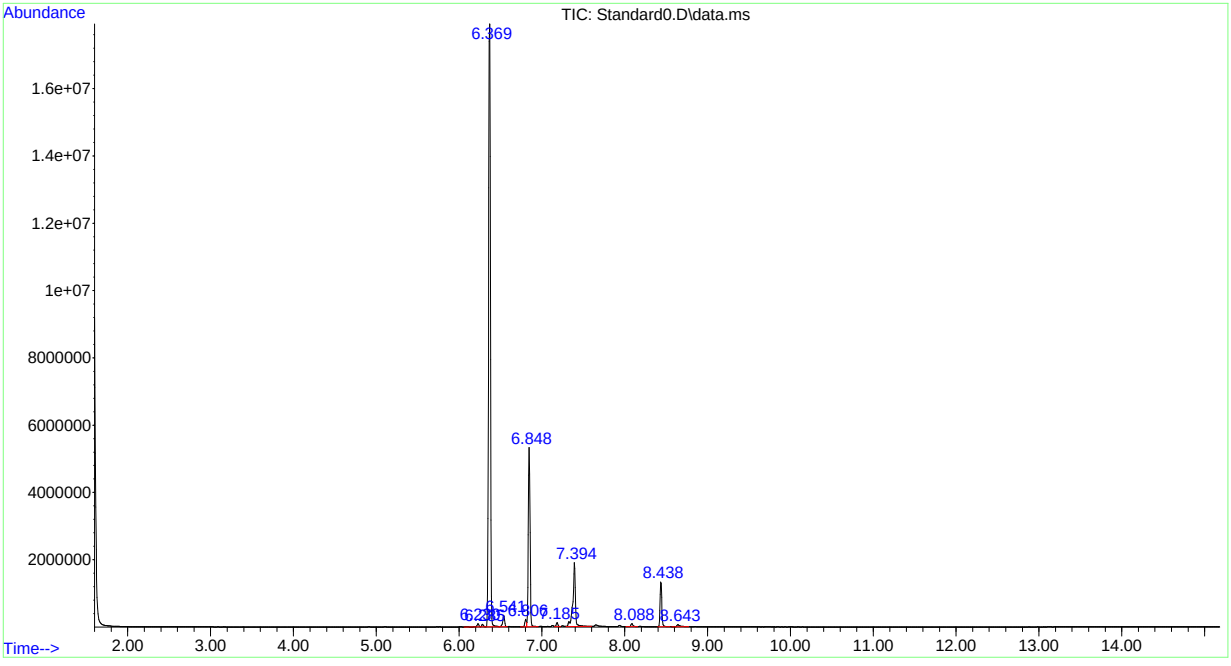


Figure. 36 GC-MS chromatogram of standard 0 – Week 3.

Library Search Report

Data Path : D:¥Data¥CHEM 4400¥Oct 20¥
Data File : Standard1.D
Acq On : 20 Oct 2022 09:45
Operator :
Sample : Standard 1
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: D:¥MassHunter¥Library¥NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.230	0.33	D:¥MassHunter¥Library¥NIST14.L			
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16245	000508-32-7	94
			(+)-3-Carene	16050	000498-15-7	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16251	000508-32-7	91
2	6.285	0.26	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16271	002867-05-2	97
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16269	028634-89-1	93
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16277	002867-05-2	91
3	6.369	63.97	D:¥MassHunter¥Library¥NIST14.L			
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	97
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
4	6.540	1.32	D:¥MassHunter¥Library¥NIST14.L			
			Camphene	16029	000079-92-5	97
			Camphene	16039	000079-92-5	96
			Camphene	16040	000079-92-5	95
5	6.847	18.48	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	95
			.beta.-Pinene	16055	000127-91-3	94
			.beta.-Pinene	16060	000127-91-3	94
6	7.184	0.41	D:¥MassHunter¥Library¥NIST14.L			
			(+)-3-Carene	16050	000498-15-7	97
			3-Carene	16037	013466-78-9	95
			.gamma.-Terpinene	16077	000099-85-4	94
7	7.392	9.29	D:¥MassHunter¥Library¥NIST14.L			
			Eucalyptol	27458	000470-82-6	96
			Eucalyptol	27466	000470-82-6	95
			Eucalyptol	27464	000470-82-6	93
8	8.087	0.30	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,.4.beta.,5.alpha.)]-	26163	000471-15-8	96
			Thujone	25788	000546-80-5	91
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	26116	001125-12-8	87
9	8.437	5.16	D:¥MassHunter¥Library¥NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98

EDVapoRub3.M Thu Oct 20 10:03:08 2022 Page: 1

Figure. 37 Page 1 of library search report of standard 1 – Week 3.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct20\
Data File : Standard1.D
Acq On : 20 Oct 2022 09:45
Operator :
Sample : Standard 1
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Camphor	25791	000076-22-2	97
			(+)-2-Bornanone	25850	000464-49-3	97
10	8.642	0.48	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, (1.alpha.,2.beta.,5.alpha .)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, [1S-(1.alpha.,2.alpha.,5. beta.)]-	29316	002216-52-6	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-	29273	001490-04-6	87

Figure. 38 Page 2 of library search report of standard 1 – Week 3.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard1.D
Acq On : 20 Oct 2022 09:45
Operator :
Sample : Standard 1
Misc :
ALS Vial : 3 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\EDVapoRub3.M
Title :

Signal : TIC: Standard1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.230	843	876	881	BV	109602	1618084	0.52%	0.331%
2	6.285	881	886	893	VV	87250	1290498	0.41%	0.264%
3	6.369	893	902	925	PV	19763482	312874159	100.00%	63.975%
4	6.540	925	934	942	VV	393429	6432444	2.06%	1.315%
5	6.847	967	992	1013	BV	5992562	90391066	28.89%	18.483%
6	7.184	1050	1056	1063	VV	133215	1989414	0.64%	0.407%
7	7.392	1077	1095	1137	VB 5	2066239	45414204	14.52%	9.286%
8	8.087	1221	1226	1239	PV 2	88303	1464511	0.47%	0.299%
9	8.437	1278	1293	1316	BB	1757229	25250263	8.07%	5.163%
10	8.642	1316	1331	1361	BB	90232	2334778	0.75%	0.477%

Sum of corrected areas: 489059420

EDVapoRub3.M Thu Oct 20 10:03:23 2022

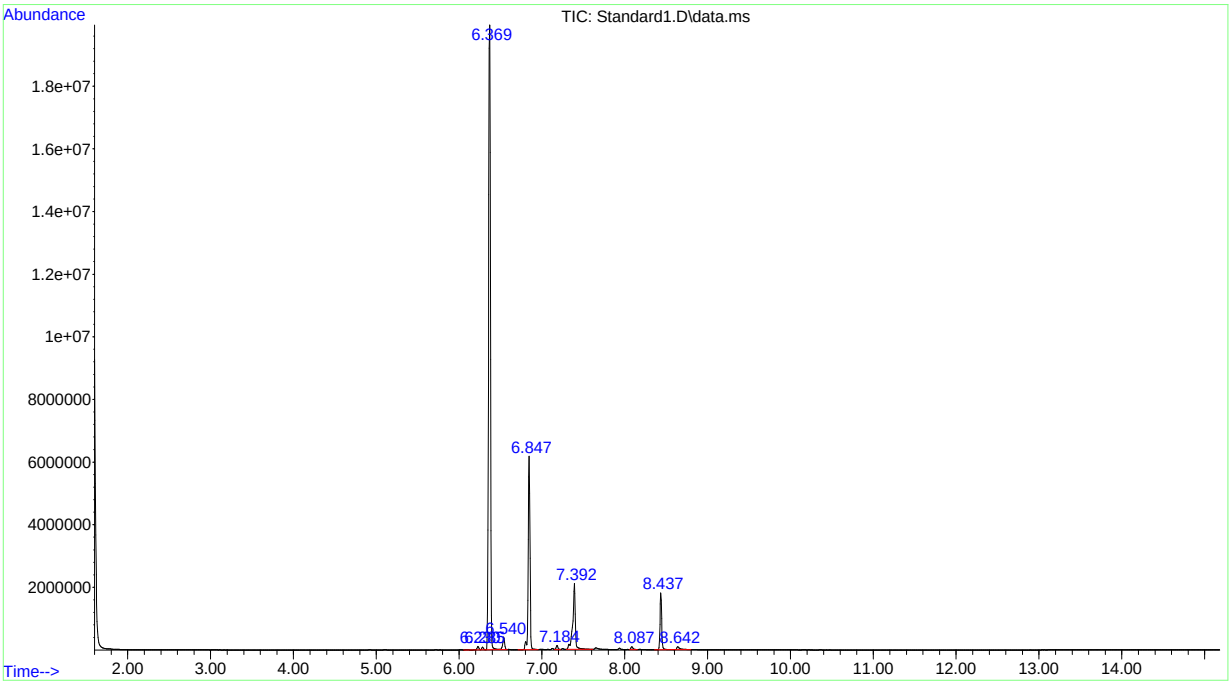


Figure. 39 GC-MS chromatogram of standard 1– Week 3.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard2.D
Acq On : 20 Oct 2022 10:07
Operator :
Sample : Standard 2
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.230	0.38	D:\MassHunter\Library\NIST14.L			
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16245	000508-32-7	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16251	000508-32-7	94
2	6.285	0.30	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16277	002867-05-2	91
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16271	002867-05-2	91
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16278	002867-05-2	91
3	6.370	65.57	D:\MassHunter\Library\NIST14.L			
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	96
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
4	6.540	1.42	D:\MassHunter\Library\NIST14.L			
			Camphene	16029	000079-92-5	97
			Camphene	16039	000079-92-5	96
			Camphene	16040	000079-92-5	96
5	6.805	0.73	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16270	003387-41-5	97
			.beta.-Phellandrene	16088	000555-10-2	91
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16273	003387-41-5	91
6	6.847	18.82	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	94
			.beta.-Pinene	16055	000127-91-3	94
			.beta.-Pinene	16060	000127-91-3	94
7	7.127	0.14	D:\MassHunter\Library\NIST14.L			
			.alpha.-Phellandrene	16096	000099-83-2	91
			.alpha.-Phellandrene	16093	000099-83-2	91
			.alpha.-Phellandrene	16095	000099-83-2	91
8	7.183	0.45	D:\MassHunter\Library\NIST14.L			
			.gamma.-Terpinene	16074	000099-85-4	95
			3-Carene	16036	013466-78-9	95
			.gamma.-Terpinene	16078	000099-85-4	95
9	7.328	0.56	D:\MassHunter\Library\NIST14.L			
			o-Cymene	15145	000527-84-4	97

EDVapoRub3.M Thu Oct 20 10:27:12 2022 Page: 1

Figure. 40 Page 1 of library search report of standard 2 – Week 3.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct20\
Data File : Standard2.D
Acq On : 20 Oct 2022 10:07
Operator :
Sample : Standard 2
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			o-Cymene	15146	000527-84-4	97
			p-Cymene	15143	000099-87-6	97
10	7.392	8.25	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27466	000470-82-6	96
			Eucalyptol	27458	000470-82-6	96
			Eucalyptol	27467	000470-82-6	94
11	7.653	0.30	D:\MassHunter\Library\NIST14.L			
			.gamma.-Terpinene	16077	000099-85-4	96
			.gamma.-Terpinene	16078	000099-85-4	95
			.gamma.-Terpinene	16076	000099-85-4	90
12	8.088	0.19	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-	26163	000471-15-8	96
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	26116	001125-12-8	90
			Thujone	25788	000546-80-5	81
13	8.437	2.65	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			Camphor	25791	000076-22-2	97
14	8.645	0.24	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,5.beta.)]-	29316	002216-52-6	90
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-	29273	001490-04-6	80
			L-Menthyl chloroformate	81125	014602-86-9	76

Figure. 41 Page 2 of library search report of standard 2 – Week 3.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard2.D
Acq On : 20 Oct 2022 10:07
Operator :
Sample : Standard 2
Misc :
ALS Vial : 4 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\EDVapoRub3.M
Title :

Signal : TIC: Standard2.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.230	861	876	881	BV	139487	2098843	0.57%	0.377%
2	6.285	881	886	893	VV	110802	1665447	0.46%	0.299%
3	6.370	893	902	925	VV	22794633	365225356	100.00%	65.570%
4	6.540	925	934	942	VV	484998	7928861	2.17%	1.423%
5	6.805	968	984	986	BV	328349	4049621	1.11%	0.727%
6	6.847	986	992	1012	VV	7187116	104818493	28.70%	18.818%
7	7.127	1039	1045	1050	VV	46711	792166	0.22%	0.142%
8	7.183	1050	1056	1063	VV	164767	2521300	0.69%	0.453%
9	7.328	1076	1083	1085	VV	218955	3093076	0.85%	0.555%
10	7.392	1085	1095	1136	VB 4	2078185	45941722	12.58%	8.248%
11	7.653	1136	1145	1177	BB	58270	1675522	0.46%	0.301%
12	8.088	1221	1227	1240	PV	64724	1086066	0.30%	0.195%
13	8.437	1278	1293	1319	BB	998721	14762784	4.04%	2.650%
14	8.645	1319	1332	1361	BB	44127	1343927	0.37%	0.241%

Sum of corrected areas: 557003183

EDVapoRub3.M Thu Oct 20 10:26:58 2022

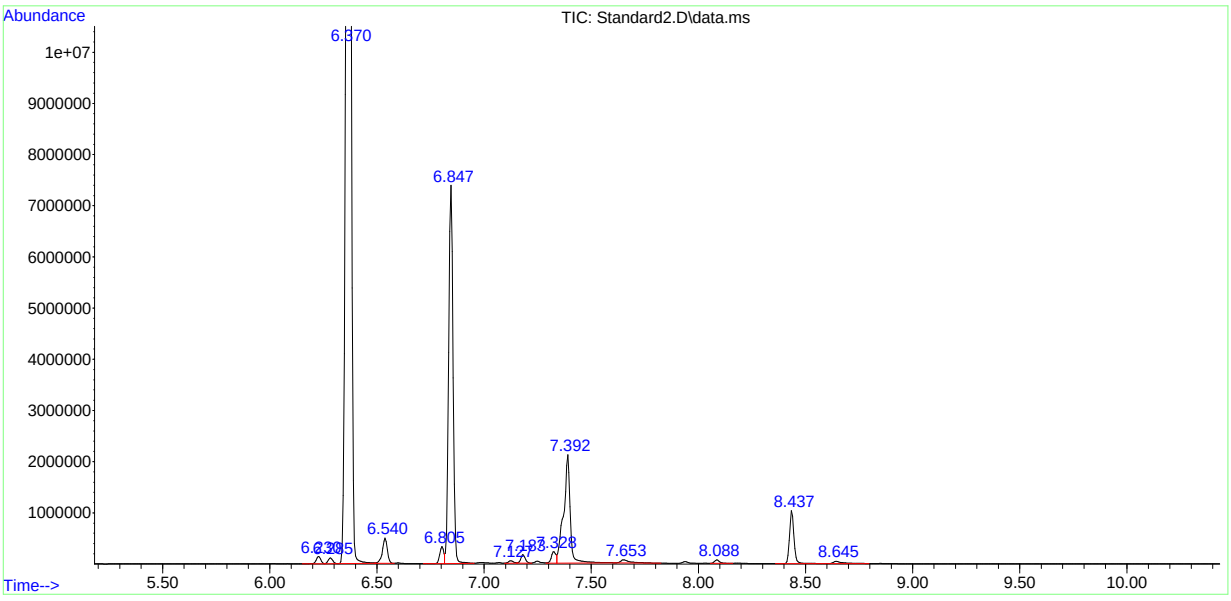


Figure. 42 GC-MS chromatogram of standard 2– Week 3.

Library Search Report

Data Path : D:¥Data¥CHEM 4400¥Oct 20¥
Data File : Standard3.D
Acq On : 20 Oct 2022 10:29
Operator :
Sample : Standard 3
Misc :
ALS Vial : 5 Sample Multiplier: 1

Search Libraries: D:¥MassHunter¥Library¥NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.230	0.35	D:¥MassHunter¥Library¥NIST14.L			
			(+)-3-Carene	16050	000498-15-7	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16245	000508-32-7	94
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16249	000508-32-7	91
2	6.285	0.28	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16271	002867-05-2	97
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16269	028634-89-1	93
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16278	002867-05-2	91
3	6.369	64.14	D:¥MassHunter¥Library¥NIST14.L			
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	97
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
4	6.540	1.34	D:¥MassHunter¥Library¥NIST14.L			
			Camphene	16029	000079-92-5	97
			Camphene	16040	000079-92-5	95
			Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	16285	005794-04-7	94
5	6.805	0.67	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16270	003387-41-5	97
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16273	003387-41-5	91
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16276	028634-89-1	91
6	6.847	17.70	D:¥MassHunter¥Library¥NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	94
			.beta.-Pinene	16055	000127-91-3	94
			.beta.-Pinene	16060	000127-91-3	94
7	7.184	0.41	D:¥MassHunter¥Library¥NIST14.L			
			(+)-3-Carene	16050	000498-15-7	96
			3-Carene	16036	013466-78-9	95
			3-Carene	16037	013466-78-9	95
8	7.328	0.50	D:¥MassHunter¥Library¥NIST14.L			
			p-Cymene	15143	000099-87-6	97
			o-Cymene	15144	000527-84-4	97
			o-Cymene	15146	000527-84-4	97
9	7.393	8.80	D:¥MassHunter¥Library¥NIST14.L			

Figure. 43 Page 1 of library search report of standard 3 – Week 3.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct20\
Data File : Standard3.D
Acq On : 20 Oct 2022 10:29
Operator :
Sample : Standard 3
Misc :
ALS Vial : 5 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Eucalyptol	27466	000470-82-6	96
			Eucalyptol	27458	000470-82-6	96
			Eucalyptol	27467	000470-82-6	90
10	7.654	0.35	D:\MassHunter\Library\NIST14.L			
			.gamma.-Terpinene	16077	000099-85-4	96
			.gamma.-Terpinene	16078	000099-85-4	96
			.gamma.-Terpinene	16076	000099-85-4	94
11	8.087	0.26	D:\MassHunter\Library\NIST14.L			
			Thujone	25794	000546-80-5	83
			Bicyclo[3.1.0]hexan-3-one, 4-methy l-1-(1-methylethyl)-, [1S-(1.alpha .,4.beta.,5.alpha.)]-	26164	000471-15-8	83
			Bicyclo[3.1.0]hexan-3-one, 4-methy l-1-(1-methylethyl)-	26119	001125-12-8	72
12	8.437	4.56	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			Camphor	25791	000076-22-2	97
13	8.641	0.64	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-	29273	001490-04-6	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, (1.alpha.,2.beta.,5.alpha .)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, [1S-(1.alpha.,2.alpha.,5. beta.)]-	29316	002216-52-6	90

Figure. 44 Page 2 of library search report of standard 3 – Week 3.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard3.D
Acq On : 20 Oct 2022 10:29
Operator :
Sample : Standard 3
Misc :
ALS Vial : 5 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\EDVapoRub3.M
Title :

Signal : TIC: Standard3.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.230	862	876	881	BV	122365	1835769	0.54%	0.347%
2	6.285	881	886	893	VV	98940	1466906	0.43%	0.277%
3	6.369	893	902	925	VV	21171109	339092347	100.00%	64.142%
4	6.540	925	934	942	VV	431925	7058666	2.08%	1.335%
5	6.805	968	984	986	BV	291873	3561417	1.05%	0.674%
6	6.847	986	992	1013	VV	6459113	93583640	27.60%	17.702%
7	7.184	1050	1056	1063	VV	145960	2170640	0.64%	0.411%
8	7.328	1076	1083	1085	VV	188466	2626206	0.77%	0.497%
9	7.393	1085	1095	1137	VV 4	2202866	46520643	13.72%	8.800%
10	7.654	1137	1145	1175	VB	56894	1868614	0.55%	0.353%
11	8.087	1221	1227	1240	PV	85492	1396542	0.41%	0.264%
12	8.437	1278	1293	1322	BB	1667859	24097957	7.11%	4.558%
13	8.641	1322	1331	1366	BV	120993	3379864	1.00%	0.639%

Sum of corrected areas: 528659212

EDVapoRub3.M Thu Oct 20 10:45:52 2022

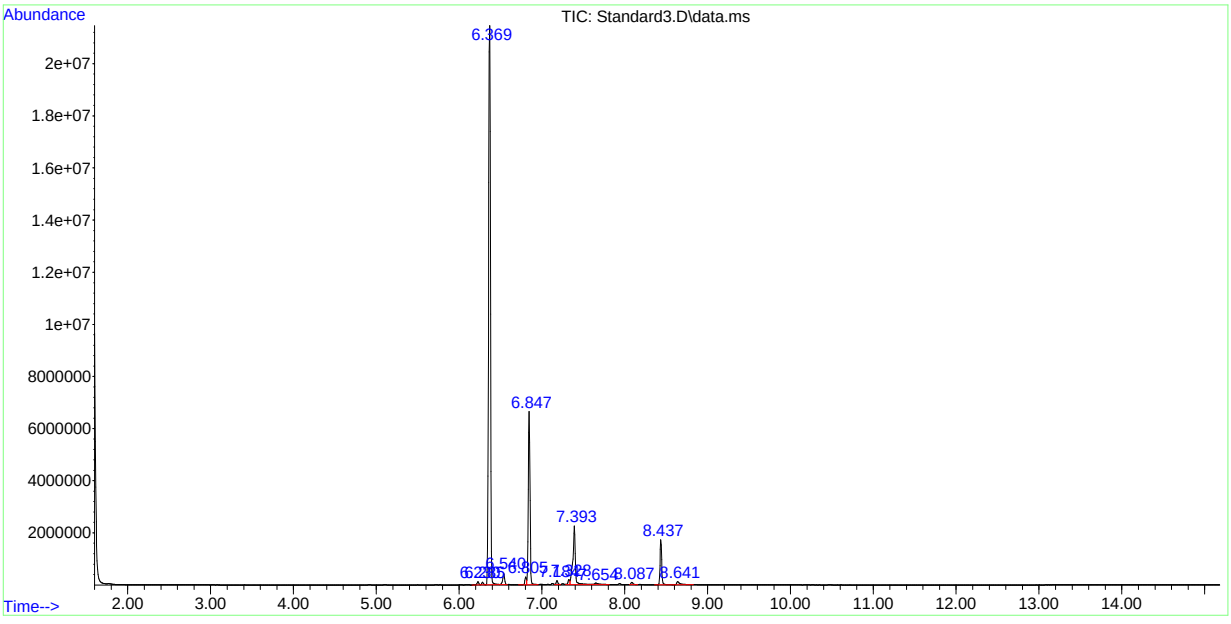


Figure. 45 GC-MS chromatogram of standard 3– Week 3.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard5.D
Acq On : 20 Oct 2002 1::5
Operator :
Sample : Standard 4
Misc :
ALS Vial : 6 Sample Multiplier: 1

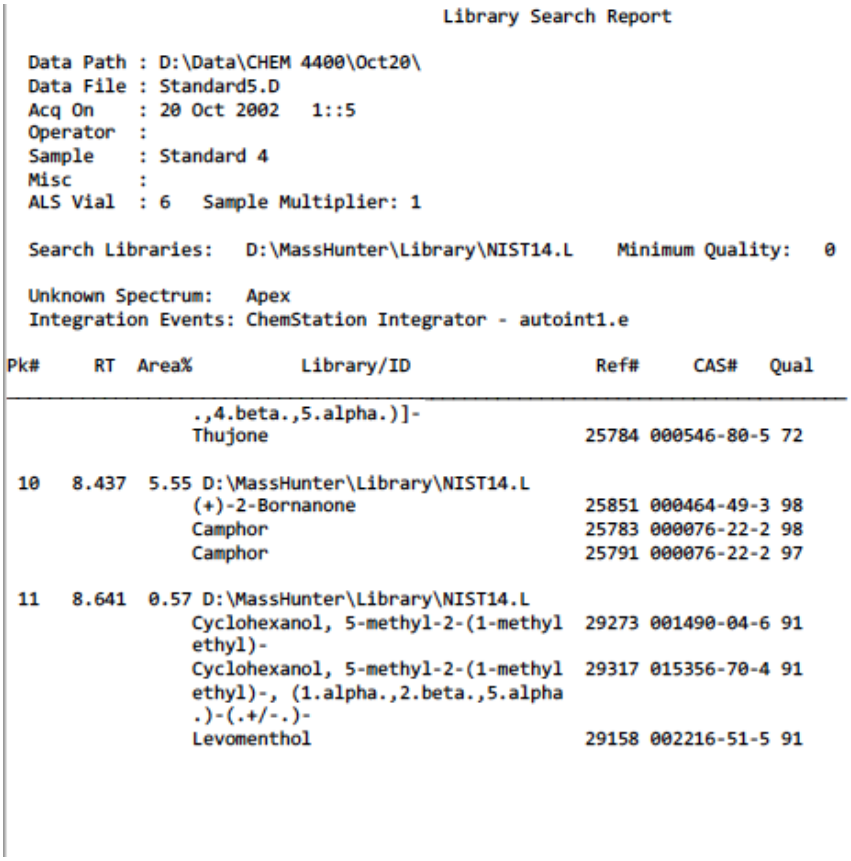
Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.230	0.33	D:\MassHunter\Library\NIST14.L			
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16245	000508-32-7	94
			(+)-3-Carene	16050	000498-15-7	94
			3-Carene	16035	013466-78-9	91
2	6.285	0.25	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16271	002867-05-2	96
			Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	16269	028634-89-1	93
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16277	002867-05-2	91
3	6.369	63.42	D:\MassHunter\Library\NIST14.L			
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	97
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
4	6.540	1.27	D:\MassHunter\Library\NIST14.L			
			Camphene	16029	000079-92-5	97
			Camphene	16039	000079-92-5	96
			Camphene	16040	000079-92-5	95
5	6.847	17.87	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	95
			.beta.-Pinene	16060	000127-91-3	94
			.beta.-Pinene	16055	000127-91-3	94
6	7.184	0.38	D:\MassHunter\Library\NIST14.L			
			(+)-3-Carene	16050	000498-15-7	95
			3-Carene	16037	013466-78-9	95
			3-Carene	16035	013466-78-9	95
7	7.393	9.67	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	96
			Eucalyptol	27466	000470-82-6	95
			Eucalyptol	27464	000470-82-6	93
8	7.654	0.33	D:\MassHunter\Library\NIST14.L			
			.gamma.-Terpinene	16077	000099-85-4	96
			.gamma.-Terpinene	16078	000099-85-4	95
			.gamma.-Terpinene	16076	000099-85-4	90
9	8.087	0.35	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-	26164	000471-15-8	80
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha	26163	000471-15-8	74

EDVapoRub3.M Thu Oct 20 11:10:05 2022 Page: 1

Figure. 46 Page 1 of library search report of standard 4 – Week 3.



Library Search Report

Data Path : D:\Data\CHEM 4400\Oct20\
Data File : Standard5.D
Acq On : 20 Oct 2002 1::5
Operator :
Sample : Standard 4
Misc :
ALS Vial : 6 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			.,4.beta.,5.alpha.)]-			
			Thujone	25784	000546-80-5	72
10	8.437	5.55	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			Camphor	25791	000076-22-2	97
11	8.641	0.57	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-	29273	001490-04-6	91
			Cyclohexanol, 5-methyl-2-(1-methyl ethyl)-, (1.alpha.,2.beta.,5.alpha .)-(.+/-.)-	29317	015356-70-4	91
			Levomenthol	29158	002216-51-5	91

Figure. 47 Page 2 of library search report of standard 4 – Week 3.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard5.D
Acq On : 20 Oct 2002 1::5
Operator :
Sample : Standard 4
Misc :
ALS Vial : 6 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\EDVapoRub3.M
Title :

Signal : TIC: Standard4.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.230	859	876	881	BV	103516	1547538	0.52%	0.329%
2	6.285	881	886	893	VV	80823	1192811	0.40%	0.254%
3	6.369	893	902	925	PV	19219115	298137944	100.00%	63.425%
4	6.540	925	934	942	VV	364327	5972596	2.00%	1.271%
5	6.847	969	992	1013	BV	5600586	83994413	28.17%	17.869%
6	7.184	1050	1056	1064	VV	119904	1801743	0.60%	0.383%
7	7.393	1077	1095	1138	VV	2193358	45468427	15.25%	9.673%
8	7.654	1138	1145	1173	VB	45943	1566274	0.53%	0.333%
9	8.087	1212	1227	1239	PV	95992	1626252	0.55%	0.346%
10	8.437	1277	1293	1317	BB	1802703	26070156	8.74%	5.546%
11	8.641	1318	1331	1366	BB	97907	2689129	0.90%	0.572%

Sum of corrected areas: 470067283

EDVapoRub3.M Thu Oct 20 11:09:26 2022

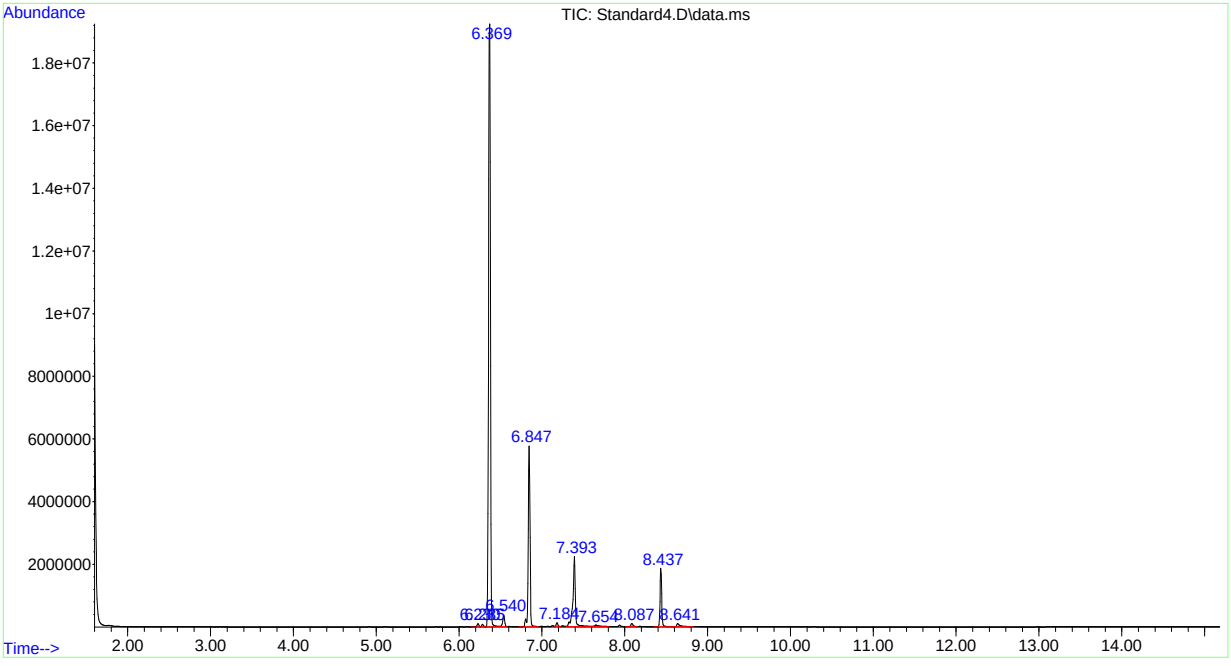


Figure. 48 GC-MS chromatogram of standard 4– Week 3.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard5.D
Acq On : 20 Oct 2022 11:13
Operator :
Sample : Standard 5
Misc :
ALS Vial : 7 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref #	CAS#	Qual
1	6.230	0.36	D:\MassHunter\Library\NIST14.L			
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16251	000508-32-7	96
			Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	16245	000508-32-7	94
			(+)-3-Carene	16050	000498-15-7	94
2	6.285	0.28	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16271	002867-05-2	97
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16277	002867-05-2	91
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16278	002867-05-2	91
3	6.370	63.48	D:\MassHunter\Library\NIST14.L			
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	97
			(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16223	007785-26-4	97
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16226	007785-70-8	95
4	6.540	1.35	D:\MassHunter\Library\NIST14.L			
			Camphene	16029	000079-92-5	97
			Camphene	16039	000079-92-5	96
			Camphene	16040	000079-92-5	95
5	6.805	0.69	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16270	003387-41-5	95
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16275	003387-41-5	91
			Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	16274	003387-41-5	91
6	6.847	17.92	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	16288	018172-67-3	95
			.beta.-Pinene	16060	000127-91-3	94
			.beta.-Pinene	16055	000127-91-3	94
7	7.128	0.15	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	16271	002867-05-2	91
			.alpha.-Phellandrene	16095	000099-83-2	91
			.alpha.-Phellandrene	16096	000099-83-2	90
8	7.184	0.45	D:\MassHunter\Library\NIST14.L			
			(+)-3-Carene	16050	000498-15-7	95
			3-Carene	16037	013466-78-9	95
			3-Carene	16036	013466-78-9	95
9	7.328	0.53	D:\MassHunter\Library\NIST14.L			

EDVapoRub3.M Thu Oct 20 11:29:04 2022 Page: 1

Figure. 49 Page 1 of library search report of standard 5 – Week 3.

Library Search Report

Data Path : D:\Data\CHEM 4400\Oct20\
Data File : Standard5.D
Acq On : 20 Oct 2022 11:13
Operator :
Sample : Standard 5
Misc :
ALS Vial : 7 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			o-Cymene	15146	000527-84-4	97
			p-Cymene	15143	000099-87-6	97
			o-Cymene	15145	000527-84-4	95
10	7.393	8.89	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27466	000470-82-6	96
			Eucalyptol	27458	000470-82-6	96
			Eucalyptol	27467	000470-82-6	90
11	7.653	0.38	D:\MassHunter\Library\NIST14.L			
			.gamma.-Terpinene	16078	000099-85-4	96
			.gamma.-Terpinene	16077	000099-85-4	96
			(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	16224	007785-70-8	95
12	8.087	0.24	D:\MassHunter\Library\NIST14.L			
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-	26163	000471-15-8	96
			Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-	26116	001125-12-8	90
			Thujone	25794	000546-80-5	83
13	8.437	4.77	D:\MassHunter\Library\NIST14.L			
			(+)-2-Bornanone	25851	000464-49-3	98
			Camphor	25783	000076-22-2	98
			(+)-2-Bornanone	25850	000464-49-3	97
14	8.641	0.49	D:\MassHunter\Library\NIST14.L			
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-	29273	001490-04-6	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29317	015356-70-4	91
			Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-	29318	015356-70-4	83

Figure. 50 Page 2 of library search report of standard 5 – Week 3.

Area Percent Report

Data Path : D:\Data\CHEM 4400\Oct 20\
Data File : Standard5.D
Acq On : 20 Oct 2022 11:13
Operator :
Sample : Standard 5
Misc :
ALS Vial : 7 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

Method : D:\Methods\CHEM 4400\F22\EDVapoRub3.M
Title :

Signal : TIC: Standard5.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.230	862	876	881	BV	135678	2042910	0.57%	0.363%
2	6.285	881	886	893	VV	107463	1597894	0.45%	0.284%
3	6.370	893	902	925	PV	21993637	357255798	100.00%	63.484%
4	6.540	925	934	942	VV	467278	7574414	2.12%	1.346%
5	6.805	968	984	986	BV	312526	3857694	1.08%	0.686%
6	6.847	986	992	1013	VV	6921582	100820717	28.22%	17.916%
7	7.128	1040	1045	1050	VV	47027	871650	0.24%	0.155%
8	7.184	1050	1056	1063	VV	159605	2539560	0.71%	0.451%
9	7.328	1076	1083	1085	VV	208804	3003780	0.84%	0.534%
10	7.393	1085	1095	1138	VV 4	2329819	50038035	14.01%	8.892%
11	7.653	1138	1145	1175	VB	64072	2159257	0.60%	0.384%
12	8.087	1221	1226	1238	VV 2	83518	1376864	0.39%	0.245%
13	8.437	1275	1293	1319	BB	1871601	26837510	7.51%	4.769%
14	8.641	1319	1331	1365	BB	104091	2774253	0.78%	0.493%

Sum of corrected areas: 562750337

EDVapoRub3.M Thu Oct 20 11:28:46 2022

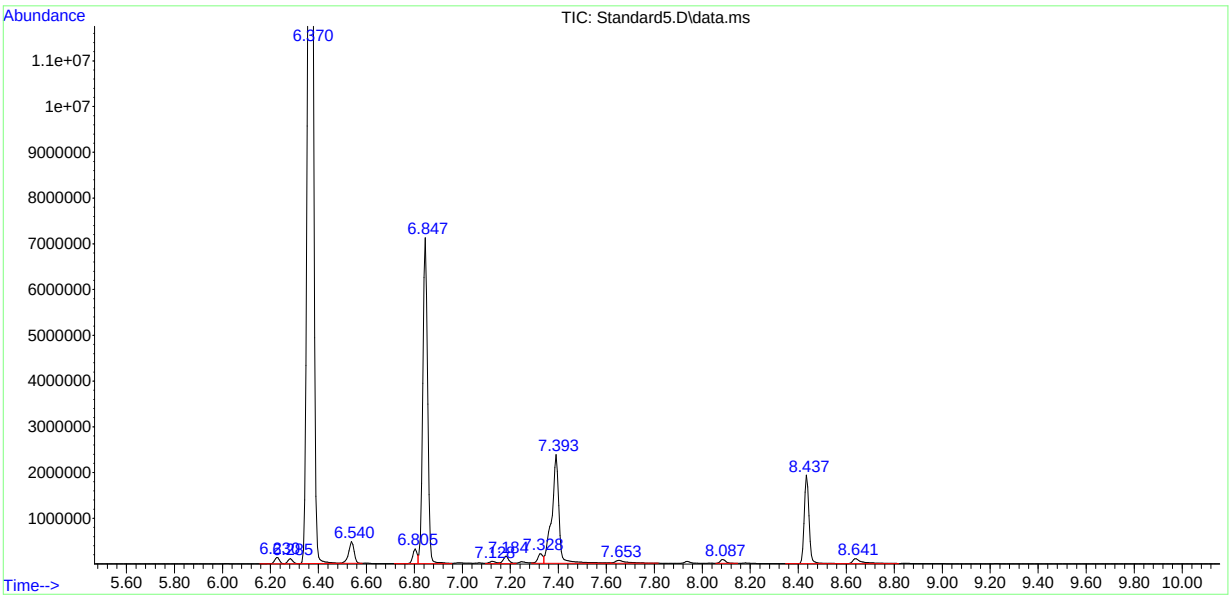


Figure. 51 GC-MS chromatogram of standard 5– Week 3.