Isolation and analysis of essential oils using gas chromatography assignment

Isolation and Analysis of Essential Oils using Gas Chromatography Lyndon Justin T. Guzman Institute of Chemistry, University of the Philippines, Diliman, Quezon City Date Performed: February 2; February 4, 2011 Date Submitted: February 18, 2011 Abstract The purpose of this experiment is to isolate the essential oil from eucalyptus leaves as a pure compound; moreover, the components of the essential oil, camphor and limonene, will be then separated using gas chromatography technique, identify the components by their retention times, and compute for the concentration and percentage content of each component by their peak areas and peak heights.

The volatile oil from eucalyptus leaves was isolated with the use of steam distillation setup, then using a separatory funnel to pipette out the extract from the distillate. A gas chromatography, with nitrogen gas as the carrier gas and a flame ionization detector, was used to separate and characterize the components of the essential oil. The retention times, peak areas, and peak heights were obtained for qualitative and quantitative analysis. A percentage of 0. 05% and 2. 85% were obtained as the content of camphor and limonene in the extracted oil, respectively.

It also goes to show that limonene has greater concentration than that of camphor in the essential oil extract. Indeed, steam distillation and gas chromatography techniques are essential methods for extracting essential oils and separating natural compounds from plants. I. Introduction Gas chromatography is used for separations of volatile or reasonably volatile organic liquids and solids. In this method of chromatography, the

components are partitioned between a liquid coating on the column (the stationary phase) and an inert gas (the mobile phase).

The stationary phase for gas chromatography is usually an organic polymer coated on the inside of a tube, such as long capillary, and the mobile phase is an inert gas, such as hydrogen, helium, or nitrogen. (Druelinger, 2000) Figure 1. Schematic diagram of a gas chromatographic system. http://www. cee. vt. edu/ewr/environmental/teach/smprimer/gc/gc. html A small volume (1-10 ? L) of a mixture of volatile substances (usually dissolved in a solvent) is injected by syringe onto a heated column through which an inert carrier gas is flowing.

The heat applied, as well as the gas flow, helps the molecules from the sample travel through the column. Smaller, more volatile molecules generally emerge first from the opposite end of the column and are detected. The detector is connected to a recorder/data system, which shows a deflection when a sample passes the detector in proportion to the amount of sample detected. Compounds are eluted through an exit port either in an intact form or as combustion products, depending on the type of detector used. (Druelinger, 2000)

The characteristic aromas of plants are due to the volatile oils, or also known as essential oils, which have been used since antiquity as a source of fragrances and flavorings. These oils occur in all living parts of the plant; they are often concentrated in twigs, leaves, flowers, and seeds. Essential oils are generally complex mixtures of hydrocarbons, alcohols, and carbonyl compounds mostly belonging to the broad group of plant products known as

terpenes. (Dalrymple and Moore, 1976) One of the many types of samples easily characterized by the technique of gas chromatography is the essential oil.

These essential oils are isolated from the plant tissue by steam distillation. Since organic compounds are generally miscible with one another, this phenomenon is usually observed only when one of the liquids is water with one or more immiscible organic liquids; in these cases, the distillation process is called steam distillation (Ault, 1983). The technique of steam distillation is based upon the principle that each component of immiscible liquid mixtures contributes to the total vapor pressure as if the other components were not there (Druelinger et. l. , 2000). As the temperature of such a mixture in an apparatus open to the atmosphere is raised, the vapor pressure of each substance increases until the total vapor pressure equals the pressure of the atmosphere. Since the total vapor pressure is the sum of the individual vapor pressures, the total vapor pressure must become equal to atmospheric pressure at a temperature below the boiling point of either pure substance (Ault, 1983). The mixture thus distills at a temperature below the boiling point of either pure component.

This can be explained using a combination of Dalton’s and Raoult’s Law: Patm = XAP?? A + XBP?? B where Patm is the atmospheric pressure, XA and XB are the mole fractions of compounds A and B, and P?? A and P?? B are the vapor pressures of pure liquids A and B. Their individual contributions are dependent on their respective mole fractions, and both liquids contribute to the vapor pressure of the system (Institute of Chemistry, UPD, 2010). In this

experiment, a major constituent of volatile oils from eucalyptus leaves will be isolated as a pure compound with high purity via steam distillation.

These essential oils are camphor and limonene and by using the gas chromatography technique, the different components of the eucalyptus essential oil will be separated. This experiment also aims to manipulate the gas chromatography system and change conditions in order to effectively and efficiently separate the components, and therefore achieve a good resolution. The components will be identified by determination of their retention times relative to those of a homologous series of n-alkanes by co-injection with authentic (standard) samples. II. Methodology Extraction of Essential Oils by Steam Distillation

A steam distillation setup was prepared as shown in Figure 2 below (Note 1). The sample (Note 2) was cut into small pieces and an amount enough to fill three-fourths of a 1-L round bottom flask was collected. 400-mL distilled water was weighed and added into the flask. The mixture was steam distilled rapidly until you have about 100 mL of the distillate. Figure 2. Steam distillation setup. http://www. pharmainfo. net/reviews/fractional-distillation-binary-solvent-mixture The distillate was placed in a separatory funnel and 2. 0 g NaCl was added. The funnel was left to stand until separation of layers occurred.

All the extract was then pipetted out (Note 3). The mixture was dried by adding enough anhydrous sodium sulfate to the mixture until the sodium sulfate swirled freely. If the entire drying agent clumped, another spatula-full anhydrous sodium sulfate was added. The mixture was then swirled. The

mixture was dry if there were no visible signs of water and the drying agent flowed freely in the container. The essential oil and aqueous distillate were stored in separate tightly-sealed, properly-labeled containers (vials) in the freezer for future use in the FT-IR analysis experiment or GC experiment or special project (Note 4).

Gas Chromatographic Analysis of Essential Oils Solution Preparation 1. Reference Solution. Separate stock solutions of 500 ppm of camphor and limonene in acetone were prepared. 50-300 ppm working standard solutions were also prepared (50, 100, 150, 200, 250 ppm) (Note 5). 2. Essential Oil Extract. 0. 5 mL of the pure extract (from steam distillation) was measured and diluted with acetone in a 10-mL volumetric flask. Instrumentation Gas chromatography was performed using a Shimadzu GC-14B using Equity 1 (30 m x 0. 25 ? m ID, 0. 25 mm film thickness) capillary column with N2 as a carrier gas.

The following were the operating conditions: N2 flow rate| 1. 0 mL/min| Column temperature| initial 50?? C (at 4 mins)| Ramp| 20?? C/min to 210?? C| Injector temperature| 200?? C| Detector temperature| 250?? C| Before the start of the experiment, the GC must have warmed up. Refer to the GC condensed procedure. Gas Chromatographic Analysis 1. 0 ? L of the standard camphor solution was injected and its chromatogram was generated. The procedure was repeated using limonene standard solution. The recorded retention times and peak areas of these substances were noted. 1. 0 ? L of the test solution (essential oil extract) was injected.

Using the retention times determined from the chromatogram with the standard solution, the components of the standard solution was located on the chromatogram obtained with the test solution (Note 6). After all solutions have been injected and data computer-processed, the GC was left to run at the highest column temperature used in the experiment with the N2 gas flowing at a slower rate than the experimental flow rate for 15-20 minutes. The injector temperature was set to room temperature and slowly lowered the column temperature down to room temperature with the N2 gas still flowing in the system.

Once everything reached room temperature, the gas flow was left to stand for another 10-15 minutes, after which the GC can be turned off. Notes 1. Boiling chips were added to the steam generator and sample flasks. 2. Each group must use different plant samples. 3. There should be two layers after the addition of NaCl solution. One was mostly water. The other was mostly extracted oil. To find out which is which, a small amount of water was added to the flask, whichever layer dissolved the water drop was the aqueous layer. The layer of essential oil was then carefully pipetted out. . If you have to get more of the organic layer out of the water, you can perform a back-extraction (solvent extraction) experiment. 5. All standard and sample solutions were stored in a well-filled, airtight container, protected from light and a temperature not exceeding 25?? C. 6. The assay was not valid unless the number of theoretical plates calculated for the peak due to limonene at 110?? C was at least 30000; the resolution between the peaks corresponding to limonene and cineole was at least 1. 5. Waste Disposal All solid wastes were disposed in the trash can.

Waste acetone was poured into properly labeled waste container exclusively for acetone. Do not pour waste acetone in the sink! III. Results and Discussion The extraction of the essential oils, camphor and limonene, from the eucalyptus leaves sample was carried out using the steam distillation technique. camphor Figure 3. Structural formulas for camphor and limonene. The boiling point of the oily, aqueous distillate will never exceed the boiling point of water. This is because both water and the oily component each contribute to the total vapor pressure as if the other component was not present.

The mixture boils when the combined vapor pressures of water and oil equal the atmospheric pressure. The oil has a small, but significant vapor pressure at 100?? C, so that the boiling point of the mixture will be just below the boiling point of water. (Druelinger, 2000) The mass of the eucalyptus leaves that were extracted was 112. 98 g. 100 mL of the distillate was produced from the steam distillation. Only a small amount of oil was extracted within the distillate by a separatory funnel. The components of the oil sample were then separated and characterized using the gas chromatography technique with a flame ionization detector.

Nitrogen gas served as the carrier gas or the mobile phase that moved the sample throughout the column. The chromatograms, plots of detector response versus time, of the standards and the sample were taken. Retention times were noted for qualitative analysis. Peak areas and peak heights were also recorded for and quantitative analysis of the essential oils. Table 1. Retention times of camphor and limonene standard and sample solutions. Solution| Retention Time (min)| pure standard camphor| 9. 021|

pure standard limonene| 7. 908| extracted camphor sample| 9. 347| extracted limonene sample| 7. 89| From the given data above for the retention time, the retention time of the camphor and limonene from the standard solutions, 9. 021 min and 7. 908 min, respectively, were close to the retention time of the camphor and limonene with that of the sample solution, 9. 347 min and 7. 889 min. It was deduced that the camphor and limonene from the sample solution were present compounds in the extracted oil from eucalyptus leaves. Below is a table shown for the resulted peak areas and peak heights from the chromatograms of camphor and limonene standard solutions. Table 2.

Peak areas and peak heights of camphor and limonene standard solutions. Solution| Peak Area| Peak Height| pure standard camphor| 46848| 17109| 50 ppm| 4427| 1113| 150 ppm| 12904| 4627| 200 ppm| 20417| 6065| 250 ppm| 15683| 5282| pure standard limonene| 56156| 20681| 50 ppm| 4419| 1236| 150 ppm| 15058| 4946| 200 ppm| 20464| 6452| 250 ppm| 20875| 5913| Figure 4. Camphor standard solutions vs. Peak area. Figure 5. Camphor standard solutions vs. Peak height. Table 3. Determination of the concentration of camphor component in the sample. Camphor Sample| Value| Concentration (ppm)| Peak Area| 5820| 67. 8| Peak Height| 1544| 61. 57| From the plotted calibration curve on the peak height and peak area for the camphor component, a regression equation is formulated in each curve with linearities almost equal to 1. From the acquired data on peak area and peak height of the camphor sample, the concentration of the camphor is 67. 98 ppm when the peak area is 5820 and 61. 57 ppm when the peak height is 1544. Figure 6. Limonene standard solutions vs. Peak area. Figure 7.

Limonene standard solutions vs. Peak height. Table 4. Determination of the concentration of limonene component in the sample.

Limonene Sample| Value| Concentration (ppm)| Peak Area| 306384| 2875. 39| Peak Height| 102881| 2943. 95| From the plotted calibration curve on the peak height and peak area for the limonene component, a regression equation is formulated in each curve with linearities also almost equal to 1. From the obtained data on peak and peak height of the limonene sample, the concentration of the limonene is 2875. 39 ppm when the peak area is 306384 and 2943. 95 ppm when the peak height is 102881. To determine the percentage content of the components of the essential oil, the area normalization method is applied.

Determining the areas beneath all of the peaks of a chromatogram enables to assign percentages to each of the components of a sample. Table 5. Determination of the percentage content of camphor and limonene sample. Component| Area| Total Area| % Content| camphor| 5820| 10766407| 0. 05%| limonene| 306384| | 2. 85%| Using the formula for area normalization, the computed percentage contents for camphor and limonene are 0. 05% and 2. 85%, respectively. This suggests that there is a greater amount of limonene in the oil extracted from the eucalyptus leaves than that of camphor.

The very low percentage implies that extracting and separating natural organic compounds from essential oils give you a very low yield that’s why you need to have huge amounts of starting material to extract from to get a relatively high percentage of its components. IV. Conclusion In this

experiment, the essential oil from eucalyptus leaves was isolated as a pure compound by steam distillation. The components of the eucalyptus essential oil, camphor and limonene, were separated using the gas chromatography technique having a flame ionization detector.

The components were also identified through determination of their retention times and were confirmed that camphor and limonene are present, having a retention time of 9. 347 and 7. 889, respectively. Calibration curves on peak areas and peak heights on camphor and limonene were formed. Concentrations of the components were calculated and gave 67. 98 ppm and 61. 57 ppm for camphor, and 2875. 39 ppm and 2943. 95 ppm for limonene. The percentage contents of the components were also determined. The essential oil extracted constituted 0. 5% camphor and 2. 85% limonene. Steam distillation is a useful method for isolating high-boiling liquids, such as oils, from other non-volatile organic compounds, such as waxes, complex fats, proteins, and sugars (Druelinger, 2000). Natural oils can be isolated readily by steam distillation. Individual compounds can be separated from the essential oil by gas chromatography wherein the components of a vaporized sample are separated as a consequence of being partitioned between a mobile gaseous phase and a liquid stationary phase held in a column.

Gas chromatography is the most widely used technique for qualitative and quantitative analysis for analysis times are short, very small amounts of sample are required and an ideal tool for the microscale and miniscale organic laboratories. If you want to obtain large percentage of compounds from the extracted essential oil, you need to have huge amounts of

eucalyptus leaves and it will take you a long time to steam distill. The standards should be injected under the same set of conditions for if not, this will lead to erroneous comparisons of data.

You can manipulate some parameters like type of column, carrier gas flow rate, injector temperature, and column temperature to compare some effects on the quantitative breakdown of the experiment. V. References Skoog, D. A. , West, D. M. , Holler, F. J. and S. R. Crouch. 2004. Fundamentals of Analytical Chemistry, 8th edition. Thomson Learning Asia, Singapore. Institiute of Chemistry. Intermediate Chemistry Laboratory II Manual. 2010. University of the Philippines, Diliman, Philippines. Druelinger, M. L. , B. A. Gaddis and A. M. Schoffstall. 2000.

Microscale and Miniscale Organic Chemistry Laboratory Experiments. The McGraw-Hill Companies, Inc. , USA. Dalrymple, D. L. and J. A. Moore. 1976. Experimental Methods in Organic Chemistry, 2nd edition. W. B. Saunders Company, USA. Ault, A. 1983. Techniques and Experiments for Organic Chemistry, 4th edition. Allyn and Bacon, Inc. , USA. VI. Appendix Calculations Concentration of camphor component in the sample peak area = 5820 regression equation: y = 103. 48x ??? 1214. 9 5820 = 103. 48x ??? 1214. 9 x = 67. 98 ppm peak height = 1544 regression equation: y = 33. 317x ??? 507. 9 1544 = 33. 317x ??? 507. 29 x = 61. 57 ppm Concentration of limonene component in the sample peak area = 306384 regression equation: y = 106. 88x ??? 937. 57 306384 = 106. 88x ??? 937. 57 x = 2875. 39 ppm peak height = 102881 y = 35. 106x ??? 469. 43 102881 = 35. 106x ??? 469. 43 x = 2943. 95 ppm Percentage content of camphor sample

%content = (area / total area) x 100 %content = (5820/10766407) x 100

%content = 0. 05% Percentage content of limonene sample %content = (area / total area) x 100 %content = (306384/10766407) x 100 %content = 2. 85% Answers to Questions 1.

The gas chromatography technique is used for separations of volatile or reasonably volatile organic liquids and solids. 2. Thermal conductivity detectors (TCD), flame ionization detectors (FID), and electron capture detectors (ECD) are commonly used type of detectors. The thermal conductivity detector, which was one of the earliest detectors for gas chromatography, senses a difference in thermal conductivity of gases eluting from a GC column. The thermal conductivities of helium and hydrogen are roughly 6 to 10 times greater than those of most organic compounds.

Thus, even small amounts of organic species cause relatively large decreases in the thermal conductivity of the column effluent, which results in a marked rise in the temperature of the detector. (Skoog et. al. , 2004) Flame ionization detectors, the most widely used and applicable detector for GC, consist of a flame fueled by hydrogen gas. Functional groups, such as carbonyl, alcohol, halogen, and amine, yield fewer ions or none at all in a flame. The detector is insensitive towards non-combustible gases such as H2O, CO2, SO2, and NO2.

These properties make the FID a most useful detector for the analysis of most organic samples, including those that are contaminated with water and the oxides of nitrogen and sulfur. (Skoog et. al. , 2004) The electron capture detector has become one of the most widely used detectors for environmental samples because this detector selectively responds to

halogen-containing organic compounds, such as pesticides and polychlorinated biphenyls. (Skoog et. al. , 2004) 3. An elution with a single solvent or a solvent mixture of constant composition is isocratic.

For samples with a broad boiling range, it is often desirable to employ temperature programming, whereby the column temperature is either increased continuously or in steps as the separation proceeds. 4. polydimethyl siloxane ??? general-purpose nonpolar phase, hydrocarbons, polynuclear aromatics, steroids, PCBs polyethylene glycol ??? free acids, alcohols, ethers, essential oils, glycols 5% phenyl-polydimethyl siloxane ??? fatty acid methyl esters, alkaloids, drugs, halogenated compounds 50% cyanopropyl-polydimethyl siloxane ??? polyunsaturated fatty acids, rosin acids, free acids, alcohols . Table 6. Internal Standard method for GC. % analyte| Peak height analyte| Peak height internal std| Peak height ratio (analyte/internal std)| 0. 05| 18. 8| 50. 0| 0. 38| 0. 10| 48. 1| 64. 1| 0. 75| 0. 15| 63. 4| 55. 1| 1. 15| 0. 20| 63. 2| 42. 7| 1. 48| 0. 25| 93. 6| 53. 8| 1. 74| unknown| 58. 9| 49. 4| 1. 19| Figure 8. Peak Height Ratio vs. Percent Analyte. regression equation: y = 6. 9x + 0. 065 slope = 6. 9 y-intercept = 0. 065 concentration of unknown: . 19 = 6. 9x + 0. 065 x = 0. 16304 standard deviation = 0. 007939 Chromatograms Figure 9. 50 ppm standard solution chromatogram. Figure 10. 150 ppm standard solution chromatogram. Figure 11. 200 ppm standard solution chromatogram. Figure 12. 250 ppm standard solution chromatogram. Figure 13. Pure standard camphor solution chromatogram. Figure 14. Pure standard limonene solution chromatogram. Figure 15. Essential oil extract chromatogram.