Identification and Quantization of Unknown Mixture Alkanes via Gas Chromatography

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Abstract

Two important functions of gas chromatography are identification and quantitation of volatile materials in a sample. The goal of this research was to do both, analyzing an unknown sample known to contain two or three alkanes from a set of the following: n-hexane, n-heptane, n-octane, n-nonane, and/or n-decane, and determining their percent composition. Using GOW-MAC 400 series gas chromatograph the unknown components were identified and quantified.

Retention times of standards were compared to the unknowns' chromatograms to identify the constituents. The normalized peak areas were used to quantify those constituents. Peak area normalization was the method utilized to determine the quantitative composition. Unknown sample #23, analyzed in this experiment, contained 70.1% octane, and 29. 9% nonane.

All standards and unknowns were deemed statistically valid, with relative standard deviations for the retention times below 2% and well below 20% for the peak areas.

Introduction

In laboratory work, a chemist is frequently called upon to test for or identify a substance.[1] Qualitative analyses are critical to many branches of chemistry, from discovering new substances to determining the outcome of a synthesis. Quantitative analysis forms the second leg of analytical chemistry, quantifying substances that have been identified.

Gas chromatography supports both qualitative and quantitative inquiry into samples of unknown composition, so long as they are volatile and thermally stable. The process involves the vaporization of the sample within the gas chromatograph and traversing the GC column carried along by the carrier gas. The compounds usually travel at differing rates depending on the substances boiling point and chemical affinities to the column material. The carrier gas, referred to as the mobile phase, is typically an inert gas like either helium, nitrogen, or hydrogen. The mobile phase travels, with the sample, through the column, packed with what is referred to as the stationary phase, to a detector. Detectors can be of many types. The type used in this experiment was a thermal conductance detector relying on the differing heat capacities of the sample materials. In practice a liquid sample is injected into the injection port of the gas chromatograph, where it is vaporized and is carried by the mobile phase gas along the stationary phase of the column. The different affinities of the sample unknowns to the stationary phase and mobile phase results in separation of the different compounds in the sample, being expressed as peaks as they pass the detector and are displayed on a computer screen, or in the past, a paper recording strip. [2]

The length of time it takes for a compound to traverse a column between injection and detection is called the retention time. The same compound should have very similar retention times if run on the same GC

under the same conditions. This allows the use of a standard in helping identify an unknown run, so long as they're run on the same machine and under the same conditions. A close match is a strong indicator that they are the same. Comparing derived values, such as adjusted retention time and retention factor, helps normalize results for comparison[3].

By measuring the area under the peak created by the sample passing the detector, the quantity of sample present can be determined. Again, comparing with a standard of known concentration is used in the quantitative determination of the compounds present[4]. The gas chromatograph used in this set of trials uses a thermal conductivity method of detection. It has a limitation of varied sensitivities to compounds with different heat capacity/thermal conductivity. To deal with this variability when deriving quantitation information is by use of area normalization. One method to account for this is the use of peak area normalization. This method utilizes the peak areas from known samples of given concentrations, generates correction or normalization factors, then normalizes unknown sample areas based on this to generate corrected areas and concentrations.

The purpose of this experiment is to identify the alkanes present in sample #23 and quantify the relative percent composition in the sample using the peak area and the peak area normalization method.

Experimental

Chemicals:

The following chemicals were used for preparation of the standards and unknowns for the experiments described in this paper.

Chemicals: n-hexane: 99+%, Acros Organics, Fair Lawn, NJ (CAS# 110-54-3), n-heptane: 99+%, Acros Organics, Fair Lawn, NJ (CAS# 142-82-5), n-octane: 99+%, Acros Organics, Fair Lawn, NJ (CAS# 111-65-9), n-nonane: 99+%, Acros Organics, Fair Lawn, NJ (CAS# 111-84-2), and n-decane: 99+%, Acros Organics, Fair Lawn, NJ (CAS# 124-18-5).

Equipment:

The equipment included Series 400 Gas Chromatograph with thermal conductivity detector made by GOW-MAC instrument Co., a U1231A True RMS Multimeter by Keysight, GFM Pro Gas Flowmeter by Thermo Scientific, a 10 μl glass syringe #701, Hamiliton Co. of Reno, NV, a Handheld Meter Logger Software by Keysight Technologies, version 2014-2015, and Microsoft Excel.

Experimental Conditions:

The detector temperature was set and read 202 °C. The injector temperature was set and read 202 °C. The column temperature was set and read 133 °C. The helium flow rate was set to 59.9 mL/min and the detector current was set to 100 mA. The column/stationary phase used was packed with poly dimethyl siloxane and the sample size used was 5 µL.

Procedures:

The unknown for this experiment was designated unknown #23. The standards used contain 20% each of each of the 5 n-alkanes, hexane, heptane, octane, nonane, and decane. Six runs were performed, three runs for the 5-alkane standard mixture and three runs for unknown #23 sample. The 5-alkane standard mixture contained 20% each of n-hexane, n-heptane, n-octane, n-nonane, and n-decane. Unknown #23 contained 2 or 3 alkanes of those in the 5-alkane standard sample, but in an unknown concentration.

Each run used 5 µL of sample. Prior to each injection, the syringe was washed with acetone three or four times, then air-dried by repeatedly drawing air into the syringe. The gas chromatograph was a Series 400 Gas Chromatograph number 5, with thermal conductivity detector made by GOW-MAC instrument Co. The non-polar stationary phase used was polydimethylsiloxane in a packed column. Helium was used as the mobile phase for this experiment. Prior to turning on the detector, the helium flow was set and read 59.9 mL/min. The detector temperature was set and read 202°C, the injector and column temperatures were set and read 202°C and 133°C, respectively. The detector current was set to 100 mA, and the RMS multimeter voltage zeroed.

For each trial a 10 µL #701 glass syringe was used to inject 5 µL of sample into port B. The multimeter was zeroed prior to injecting samples and the recording software was immediately started. The run continued until peaks had completed their transit and been recorded. The software was refreshed after each run.

The above procedure was executed for three trials of 5-alkane standard mixture and three trials of unknown #23. Each of the standard run took about 9 minutes to complete and each of the unknown sample runs took approximately 7 minutes to complete.

Results and Discussion

Standards Mixture Trials

Table one contains the data from the three 5-alkane standard mixture runs. Retention times (s), average area (mV.s) under the peak, averages (s and mV.s), standard deviations (s and mV.s), coefficient of variations (%), A1% (mV.s), and area correction factors (CF) are in the table below.

Table 1 5-alkane standard runs, statistics, and computed correction factors

	hexane	**heptane**	**octane**	**nonane**	**decane**
Trial 1 - t_R (s)	63	101	166	281	481
Trial 2 - t_R (s)	63	104	170	286	480
Trial 3 - t_R (s)	64	102	168	283	486
Avg t_R (s)	63.3	102.3	168.0	283.3	482.3
StDev t_R (s)	0.577	1.528	2.000	2.517	3.215
RSD t_R (%)	0.91	1.49	1.19	0.888	0.666
Trial 1 - A (mV.s)	105.2	105.7	114.0	119.4	123.5
Trial 2 - A (mV.s)	106.3	105.1	110.1	112.4	115.3
Trial 3 - A (mV.s)	106.4	105.4	113.2	116.0	119.7
Avg A (mV.s)	105.97	105.40	112.43	115.93	119.50
StDev A (mV.s)	0.666	0.300	2.06	3.50	4.10
RSD A (%)	0.63	0.28	1.83	3.02	3.43
A1% (mV.s)	5.30	5.27	5.62	5.80	5.98
CF	1.00	0.99	1.06	1.09	1.13

Experimental Conditions: the detector temperature was set and read 202 °C. The injector temperature was set and read 202 °C. The column temperature was set and read 133 °C. The helium flow rate was set to 59.9 mL/min and the detector current was set to 100 mA. The column/stationary phase used was polydimethylsiloxane in a packed column and the sample size used was 5 µL.

The alkane retention times (t_R) had an acceptable coefficient of variations, shown in Table 1 and all being below 2.0%. All the area (A) measurements were acceptable and below the 20% cutoff.

The average retentions times for hexane was 63.3 seconds, for heptane was 102.3 seconds, for octane was 168.0 seconds, for nonane was 283.3 seconds, and for decane was 482.3 seconds. They eluted in this order due to the progressively higher vapor pressure each has. Looking at n-alkanes, each additional carbon increases both the molecular weight and the vapor pressure/boiling point of that alkane. This is why the alkanes tested here elute in the order seen.

Excepting standard statistical computations, the formulas used for the above calculations can be found in the Appendix.

Figure 1, below, contains an overlay of the three 5-alkane standards runs. The early portion of the graph contains a small spike in run 1. This is presumed to be residual acetone, not properly cleared from the syringe.

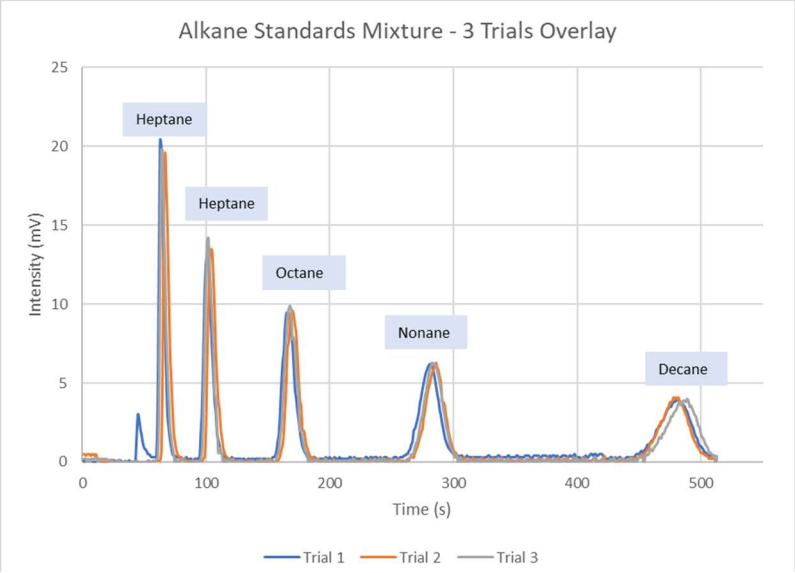

Figure 1 An overlay chromatogram of three trials of the 5-alkane mixture

Unknown Trials

Table two contains the data from the three unknown # 23 mixture runs. Retention times (s), average area (mV.s) under the peak, averages (s and mV.s), standard deviations (s and mV.s), coefficient of variations (%), corrected area (Acor in mV.s), area correction factors (CF) used, and % component composition are in the table below.

Table 2 The retention times, peak areas, standard deviations, RSDs, correction factors, corrected areas, and percent composition from the three unknown #23 trials.

	peak 1	**peak 2**
Trial 1 - t_R (s)	169	284
Trial 2 - t_R (s)	169	289
Trial 3 - t_R (s)	172	292
Avg t_R (s)	170.0	288.3
StDev t_R (s)	1.73	4.04
RSD t_R (%)	1.02	1.40
Trial 1 - A (mV.s)	377.2	166
Trial 2 - A (mV.s)	383.5	173.6
Trial 3 - A (mV.s)	381.6	162.4
Avg A (mV.s)	380.77	167.33
StDev A (mV.s)	3.23	5.72
RSD A (%)	0.85	3.42
Identity	octane	nonane
CF	1.06	1.09
Area corr (mV.s)	358.9	152.9
% component	70.1	29.9

Experimental Conditions: the conditions are the same for the unknown trials as used for the standards trials.

Figure 2, below, contains an overlay of the three unknown #23 runs. The early portion of the graph starts at 75 s, but before any informative data appears. This was done to increase the differentiation between the three runs.

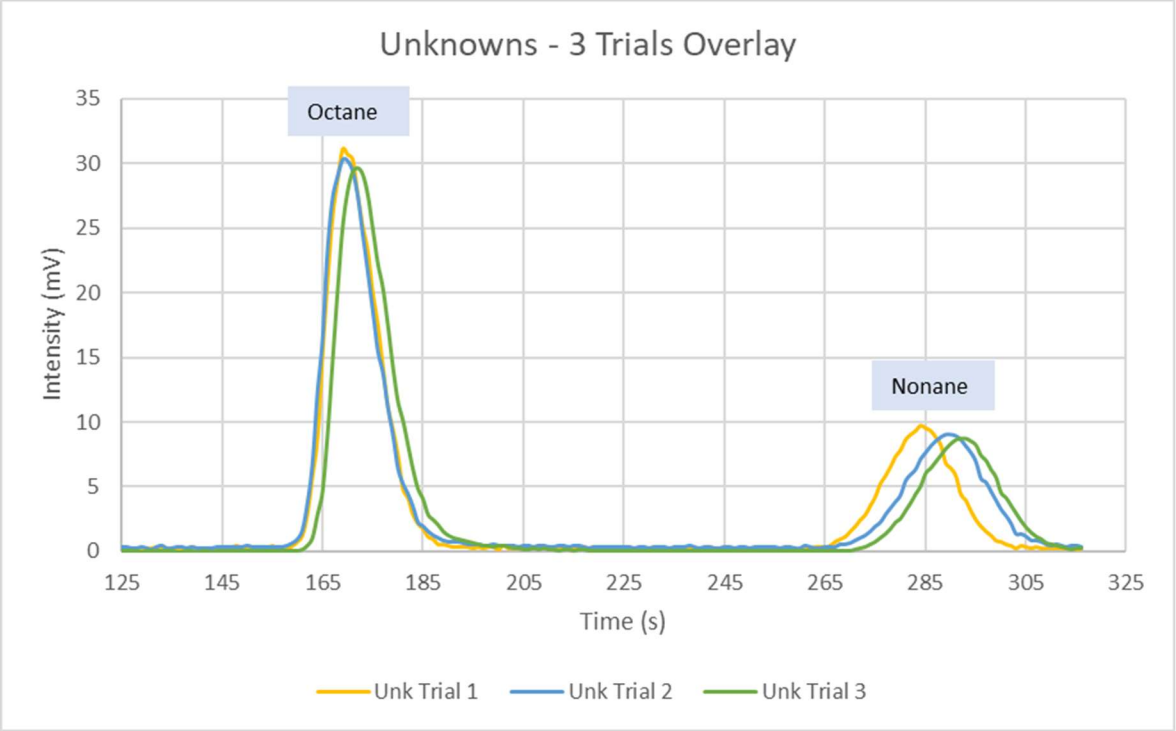

Figure 2 An overlay chromatogram of three unknown #23 trials.

Identification of these alkanes are by retention time correlation. The identified component retention times showed a good correlation with those of the standards. The octane t_R was within 1.2%, and the nonane t_R was within 1.7%.

For the unknown number 23 trials, peak one, the average retention time was 170.0 seconds, with a standard deviation of 1.73 seconds and a coefficient of variation (RSD) of 1.02%. The average peak area was 380.77 millivolt seconds, a standard deviation of 3.23 millivolt seconds, and an RSD of 0.85%.

For the second peak the average retention time was 288.3 seconds, with a standard deviation of 4.04 seconds, and a coefficient of variation (RSD) of 1.40%. The average peak area was 167.33 millivolt seconds, a standard deviation of 5.72 millivolt seconds, and an RSD of 3.42%.

For peak two, the average retention times were 288.3 seconds, with a standard deviation of 1.73 and a coefficient of variation (RSD) of 1.02%

All results for the retention times and areas for Unknown #23 were within the acceptable range for coefficient of variation, being under 2.0 and 20% respectively.

Peak one correlated with the retention time of octane, a calculated area correction factor of 1.06, a corrected area of 358.9 millivolt seconds, and a percent composition of 70.1%.

Peak two correlated with the retention time of nonane, a calculated area correction factor of 1.09, a corrected area of 152.9 millivolt seconds, and a percent composition of 29.9%. The computation method used for computing percent composition is the peak area normalization method.

Computations

The gas chromatograph employed for this test uses a thermal conductivity detector (TCD). It has the advantage of being useful for most compounds, but a drawback is they have a different responses to different materials, because materials have different thermal conductivities. The following computations normalizes the measurements from a TDC to allow for comparison between different compounds.

Computation of the correction (normalization) factor involved the two following equations. This example will be based on a standard mixture with 20% of each of five alkanes, C6 through C10:

A1% = avg Area (A) / (percent of component in standard mixture)

Example $A_{1\%}$ of heptane = 105 4 mV.s / 20 = 5.27 mV.s

CF_x (correction factor for component x) = $A_{1\%s}$ / $A_{1\%x}$

[where component s is a chosen reference. Hexane was the chosen reference.

The x represents a different standard value]

Example $CF_{heptane}$ = 5.27 $mV.s_{hexane}$ / 5.30 $mV.s_{heptane}$ = 0.99

To compute a corrected area for an unknown, the measured peak area of the unknown is divided by its specific correction factor. This normalizes it.

Example Unknown Heptane Corrected Peak Area = UnknownHeptanePeakArea / $CF_{heptane}$

$CorrArea_{HeptaneUnk}$ = 2.33 mV.s / 0.99 = 2.35 mV.s

To compute percent composition, all that remains is to sum all the corrected areas, divide that into whatever specific corrected area component being computed, multiply by 100 and that's the percent composition.

Example: using 8.91 mV.s as the total corrected peak area, the percent composition is computed.

$$PercentComposition_{Heptane} = 2.35 / 8.91 * 100 = 26.4\%$$

The compounds identified and quantified were 70.1% octane, and 29.29% nonane.

The method of quantization used, as described above, was the peak area normalization method[5].

Error analysis

All retention times and area measurements, from both standards and unknowns, had coefficients of variation under 2.0% and 20.0 %, respectively. This was the criteria for acceptable measurements.

Conclusions

The goal of this research was to analyze an unknown sample containing two or three unknown alkanes, using gas chromatography, to determine the identities of its constituents and the relative composition of those compounds.

By running a five-alkane standard mixture containing 20% of C6 through C10 n-alkane, retention times were established for all alkanes the unknown sample #23 could contain. Based on the known concentrations in the standard, calculations using the known and unknown peak areas allowed the percent composition of the unknown components to be determined.

Three trials were run on both the standards mixture and the unknown mixture. This allowed identifications to be performed and produce peak area information. Using this data, determination of the component percent composition was completed with statistically acceptable results.

The unknown peaks correlated to octane and nonane, being within 1.2% and 1.7% of the standards retention times. The concentrations of the identified alkanes were calculated at 70.1% octane, and 29.9% nonane.

With the unknowns, the relative standard deviations for the retention times were 1.02% and 1.40%, corresponding to octane and nonane, which was comfortably under the 2.0% requirement. The relative standard deviations for the area under the peaks, for octane and nonane, were 0.85% and 3.42%, respectively, far under the 20.0% requirement for acceptability. The relative standard deviations for the standards mixture were also easily under the respective requirements for the retention times and peak areas.

The quantization was done via the peak area normalization method using the measured peak area data from the standards mixture runs.

References

[1] C. H. Sorum, An Introduction to semimicro Qualitative Analysis, pp 1, Prentice-Hall Inc., 1960

[2] Mark F. Vitha, Chromatography, Principles and Instrumentation, pp 3, 53, 61, Wiley, 2017

[3] C. F. Poole, S.K. Poole. Chromatography Today, pp.5-8. Elsevier Science Publishers, 1991

[4] L. R. Snyder, J.J.Kirkland, J.L.Glajch, Practical HPLC Method Development, pp. 654, 1997

[5] "Normalizing Gas-Chromatography-Mass Spectrometry Data: method choice after biological influence", Michael J. Noonan, Helga V. Tinnesand, and Christina D. Buesching, BioEssays Vol 40, issue 6, April 30, 2018, **https://doi.org/10.1002/bies.201700210**

Appendix

Run Details

GC Serial Number	MY59170086
GC Model Number	U1231A
Date	09/13/2023
Time	from 8.57 AM to 10:18 AM

Formulas

A1% = avg Area (A) / (percent of component in mixture)

Example A1% of heptane = 105 4 mV.s / 20 = 5.27 mV.s

CF_x (correction factor for component x) = A1%s / A1%x

[where component s is a chosen reference]

Example $CF_{heptane}$ = 5.27 mV.s / 5.30 mV.s = 0.99

$Acor_x$ (normalized area) = A_x / CF_x

Example A_{octane} = 380.77mV.s / 1.06 = 358.9 mV.s

$\%Component_x$ = $Acor_x$ / sum(all Acor) * 100

Example $\%_{octane}$ = 358.9 mV.s / (358.9+152.9) mV.s = 70.1%

Significant Figures

Significant figures used were the significant figures of the raw data plus one, for all calculation results.

Raw Data

Available on request.