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INTRODUCTION OF THE GAS CHROMATOGRAPH INTO THE INTRODUCTORY CHEMISTRY LABORATORY

A thesis presented to the Committee on Graduate Studies and the Department of Chemistry of Union College, Schenectady, New York, in partial fulfillment of the requirements for the degree of Master of Arts in Teaching.

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A general chemistry laboratory experiment, which incorporates relevant "real-world" samples, methods and techniques, has been developed. In this new experiment, students will employ a gas chromatograph (GC) to analyze volatile compounds and mixtures, including a sample that simulates a forensic analysis. This analysis is designed to pique students' natural curiosity.

Students will work cooperatively, which will produce a more effective learning environment. This experiment will reinforce the student's knowledge of intermolecular forces, and other chemistry concepts, and introduce the theory of chromatographic separations while engaging the student in the experiment.

This experiment reflects the excitement of chemistry and its use in solving "mysteries" while giving the general chemistry student hands-on training using state-of-the-art instrumentation. The end result will be a more meaningful laboratory experience for the general chemistry student, which will serve to cognitively challenge and interest the student in the field.

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CHAPTER ONE: INTRODUCTION AND THEORY

The inadequacies of the traditional chemistry laboratory programs have been the topic of an increasing number of articles in the past decade (1-4). The structure and format of the introductory chemistry laboratory must be adapted to meet the needs of the field of chemistry for two reasons: (a.) the need to get students interested in chemistry through the use of "real-life" experiments and methods which are actually used in industry and research and, (b.) the need to expose the students to modern techniques and state-of-the-art instrumentation that anticipates their future workplace.

The traditional introductory chemistry laboratory uses classical, "wet chemistry", techniques and simple apparatus to carry out cookbook-type experiments. These methods often give students imprecise and inaccurate results. Kildahl suggest that these kinds of introductory chemistry experiments fail the students in a couple of ways (5). Students are misled about the role of laboratory work in chemistry and the quality of the data that modern chemists are able to obtain. As a result, the general chemistry laboratory is often a negative experience for students. A student may lose interest and choose not to pursue a degree and career in chemistry because of the negative experience he or she had in the introductory chemistry laboratory. The negative experience may "turn-off" a student to the field of chemistry. Chemistry is a science of discovery. The introductory chemistry laboratory should reflect the element of discovery.

The need to expose students studying chemistry to modern techniques

and instrumentation is important to increase the students' marketability in the now very competitive job market. Employers want to hire workers that have strong background knowledge as well as practical experience. Unfortunately, many undergraduate chemistry majors have only a limited amount of exposure to the technology and techniques that are currently being used in industrial and research laboratories. It isn't usually until a student's junior or senior year of study in chemistry that he or she will be exposed to the techniques and instrumentation that are used in "real-world" laboratories. Exposing students from the beginning of their chemistry education to laboratory settings that anticipate their future workplace will increase the students' marketability after graduation.

The need for scientists to separate mixtures into their individual components is extremely important in chemistry laboratories around the world. The most widely used analytical technique to separate materials is called chromatography. Chromatography was invented by a Russian botanist, Mikhail Tswett, in the first part of the 20th century. Tswett found that when he passed extracts of green leaves through a glass column packed with finely divided calcium carbonate the solutions separated as colored bands on the column. Since Tswett's separations involved bands of colors, Tswett called this new technique chromatography (Greek *chroma* meaning color and *graphein* meaning to write).

Chromatography applications have grown tremendously in the past

fifty years. Chromatography now encompasses a wide range of separation methods that allow scientists to separate, isolate, and identify related components of complex mixtures. All methods of chromatography are based on the same principles and use two phases, a stationary phase and a mobile phase. The mobile (moving) phase carries the sample through the stationary (non-moving) phase. Partitioning of the components between the mobile and stationary phases serves to separate the components.

Martin and Synge, and James and Martin, used the separation principles of chromatography in the development of an instrument which uses an inert gas as the mobile phase (6). The method they developed is called gas chromatography (GC). Gas chromatography revolutionized separations in the chemistry laboratory by allowing chemists to separate mixtures of close-boiling volatile liquids.

Gas chromatography is a widely used technique for the separation of compounds in industrial and research laboratories. Students with practical working knowledge of the principles and techniques of gas chromatography are highly marketable in many different areas, such as pharmaceutical and environmental chemistry.

There are numerous experiments in the literature using gas chromatography in the college chemistry laboratory. However, most of these experiments are intended for the upper-level classes, such as organic chemistry and instrumental analysis (7-9). The amount of literature on the use of gas chromatography in the introductory chemistry laboratory is small

but is increasing (10-13).

Introductory chemistry students using gas chromatography in the laboratory will discover that liquid mixtures can be rapidly, easily, and quantitatively separated into their components. The students will observe that the separation of the components in a mixture is ultimately based on the molecular structure of the components. The students will discover that modern analysis can be carried out using very small sample volumes, and will realize the connection between chemicals used in the chemistry laboratory and in the household.

Gas chromatography is a surprisingly simple technique with great versatility which makes it an ideal candidate for incorporation into the introductory chemistry laboratory. Gas chromatography overcame the difficulties of using microfractionation columns for liquids that could not be separated by chemical methods. Microfractionation columns were used only if there was enough material, the boiling points were sufficiently different from each other, and azeotropic mixtures were avoided (14). The benefits of using gas chromatography in the introductory chemistry laboratory are the very small sample volume that is required for analysis and the tremendous resolution that can be obtained in a timely fashion. Rapid analysis and small sample volumes are also an important step towards reducing the students' exposure to potentially hazardous chemicals.

Gas chromatography has two major subdivisions which are based on the nature of the stationary phase. If the stationary phase is a solid, the

method is called gas-solid chromatography (GSC). If the stationary phase is a liquid, the method is called gas-liquid chromatography (GLS). The liquid phase is a thin film on solid particles or on the inner wall of the capillary column. GLS is employed in our work.

The gas chromatograph instrument is relatively simple. It consists of five basic parts: carrier gas supply, sampling device, chromatography column, detector, and recording device with data manipulation capabilities. See Figure 1-I for a schematic drawing of a typical gas chromatograph. Notice that the sample inlet, column, and detector are contained in a thermostat. The function of the thermostat is to regulate the temperature at which the analysis is to be run.

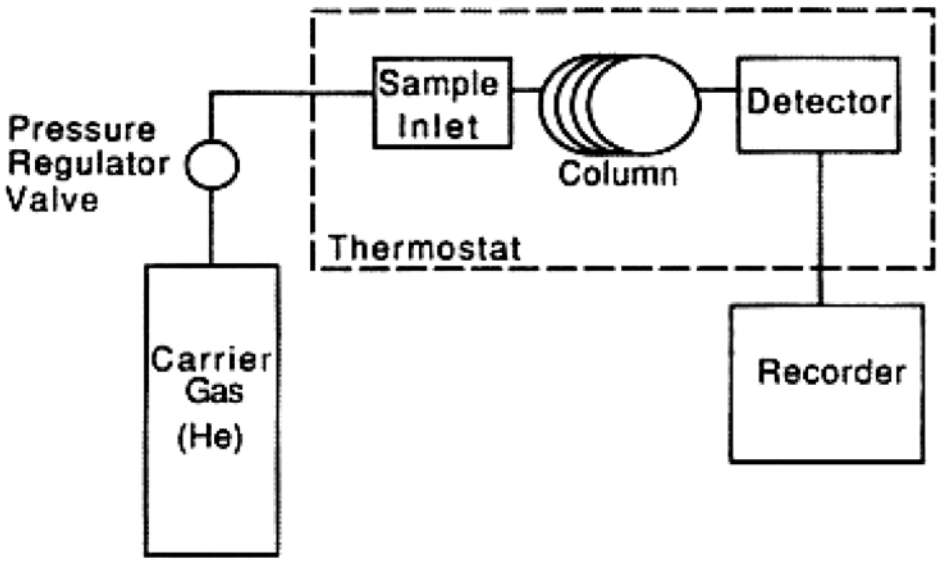

Figure 1-I Gas Chromatograph Schematic Drawing

A small amount of sample is injected through a rubber septum with a syringe into a heated injection port where it is immediately vaporized. An

inert gas, such as helium, used as the mobile phase (also called the carrier gas), sweeps the vaporized sample into a column which contains the stationary phase. The column consists of a tube packed with a support material, such as fused silica. The support material is coated with a nonvolatile liquid phase, such as methyl silicone, which is the stationary phase. Each component of the sample then distributes itself in a characteristic manner between the gaseous phase and the stationary liquid phase.

The rate of migration for the components is determined by the time the sample spends in the gaseous mobile phase compared to the time the sample spends in the liquid stationary phase. An individual component will undergo many thousands of transfers between the stationary and mobile phase as it passes through the column during analysis. If the analysis conditions are well chosen, the components of the mixture are all separated and each passes in the effluent stream from the column through a detector at a different time.

Chromatography column dimensions vary in length and diameter. Capillary column lengths can be as short as ten meters or as long forty meters. The inside diameter of a capillary column is usually about 0.20 to 0.40 millimeters. The column is coiled to allow it to fit inside the thermostat, which is often called the oven. The degree of separation of the components is determined ultimately by their molecular structures.

The characteristic manner in which a sample distributes itself between the mobile phase and the stationary phase in the column is highly

temperature-dependant. The temperature of the column is adjusted to obtain the proper vapor pressures for the components to be separated. Vapor pressure is a measure of the tendency for the molecules of a given substance to escape from the liquid phase and enter the gas phase at a given temperature. A component's vapor pressure is determined by the degree of intermolecular bonding. The vapor pressure will be low if the intermolecular forces are strong between a component and the stationary phase. Conversely, the vapor pressure will be high if the intermolecular forces between a component and the stationary phase are weak. An individual component of a substance can migrate through a column only during residence in the moving gaseous phase; hence, a component's vapor pressure will determine the rate that it will migrate through the column. For example, a component with a high vapor pressure (and weak intermolecular forces) will migrate through a column faster then a component with a low vapor pressure (and strong intermolecular forces). Increasing the analysis temperature will increase the rates of migration through the column for all of the components of a mixture because all of the components of a mixture will have higher vapor pressures at higher temperatures. Conversely, lowering the analysis temperature will decrease the rates of migration through the column for all the components of a mixture.

Temperature programs are used during gas chromatography analysis to facilitate the separation of both high- and low-vapor-pressure components in a mixture. In temperature programs, the temperature of the column is

increased gradually (at a pre-set rate) during analysis. Components that have high vapor pressures are separated at lower temperatures, while components with low vapor pressures are moved at faster rates through the column at higher temperatures. Temperature programing shortens the time needed to separate components with low vapor pressures.

The continuous detection of the effluent gas stream results in a chromatogram as depicted (not to scale) in Figure 1-II. The shape of the peaks typically corresponds to that of the normal Gaussian distribution curve unless there is a distortion due to instrumental or operational factors. The average amount of time required from sample injection for a component to pass or elute from the column is called the retention time, t_R. Retention times are a measure of the amount of time that each component is retained by the stationary phase.

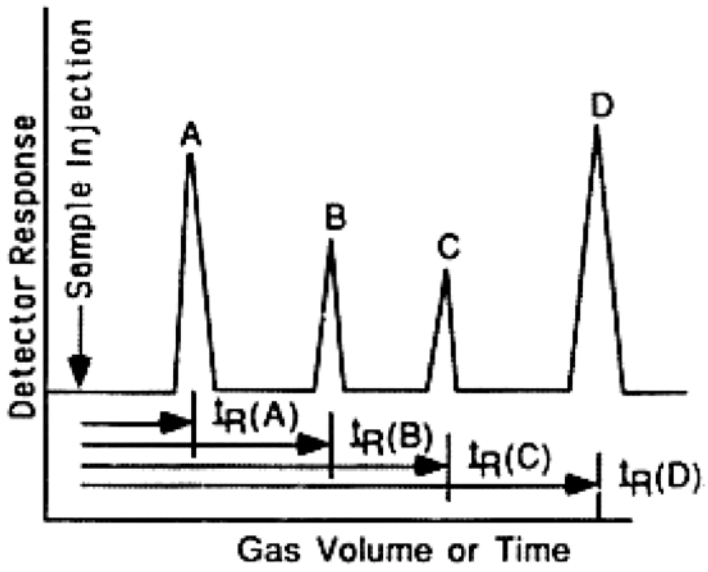

Figure 1-II Elution Chromatogram

The chromatogram that is produced from an analysis provides the chemist with both quantitative and qualitative results. Retention times are used to identify the components in the sample by comparison to the retention times of known standards run under the same analysis conditions. The area under each peak is proportional to concentration and can be used to determine relative amounts of each component in a mixture.

In the instrument used for this work, the individual components in the effluent stream are detected by a flame ionization detector (FID). A schematic drawing of the flame ionization detector is shown in Figure 1-III. See "Principles of Instrumental Analysis" by Skoog for more detailed description of the principles of the FID (6). The flame ionization detector is based on the formation of ions that occurs when the sample passes through an air-hydrogen flame. These ions, which lower the resistance of the flame, are attracted to and captured in the collector. The lowered resistance is accompanied by current in a circuit that applies a large voltage across the flame. The change in current is converted into a signal which is transferred to a computer and a printer. The flame ionization detector will give a signal for any substance that is ionized in the flame. Therefore, the flame ionization detector is insensitive towards noncombustible gases such as H_2O, CO_2, SO_2, and NO_2. The main drawback of using the flame ionization detector is that the sample is destroyed during the analysis.

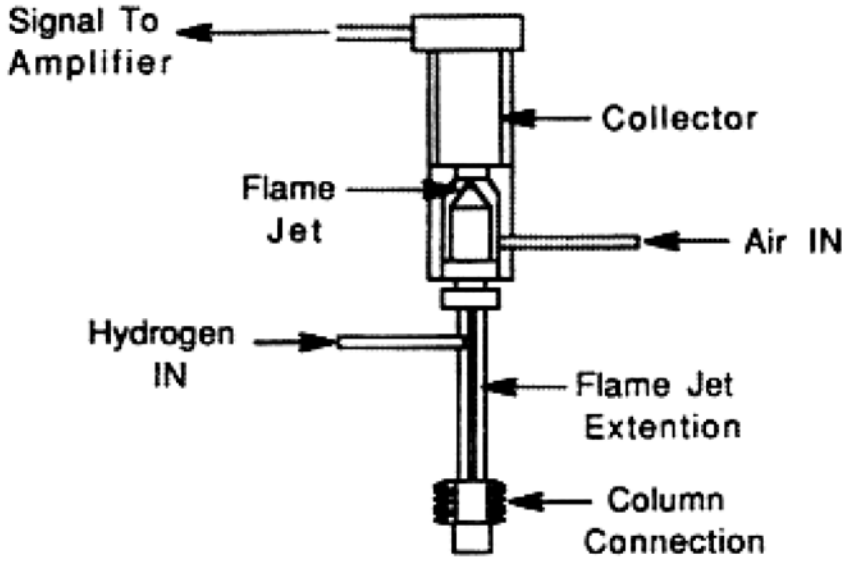

Figure 1-III Flame Ionization Detector Schematic Drawing

Szepesy summed up the advantages of gas chromatography as follows (16):

1. Gas chromatographs are relatively cheap compared with other instruments of similar performance (e.g. spectrophotometers) that are used in industrial and research laboratories. The maintenance cost of the gas chromatograph instrument is low.

2. Gas chromatographs are easy to operate, their operation requires no special previous qualifications, and they can be used in a number of widely varying analytical tasks.

3. Very long columns with extremely high separation power can be used since the moving gas phase has such a low viscosity. Consequentially, gas chromatographs can be used for the analysis of

permanent gases, high-boiling liquids, and volatile solids.

4. The rate of mass transfer between the mobile phase and stationary phases is high, resulting in high diffusion rates for the components being separated from a mixture. Therefore, gas chromatography analysis can be performed rapidly. The time required for a single analysis is generally about 8-20 minutes. With the use of special apparatus, such as mass-spectrometers, the analysis of multi-component systems can be performed in seconds.

5. Relatively simple and highly sensitive detectors can be used to detect the separated components in the mobile phase as they are eluted from the column. Consequentially very small samples can be used; typically 1-3 mL of gas and 1-10 μL of liquid will be enough for an analysis.

6. The signal from the detector can be recorded and stored instantly in a computer for evaluation and manipulation. Data reports can be produced in a matter of minutes.

7. With the proper choice of stationary phase in chromatography columns it is possible to separate components with identical boiling points or similar structures (e.g. optical isomers). This is the reason for the considerable utility of gas chromatography in pharmaceutical laboratories.

8. Gas chromatography has opened new ways to study chemical processes and reactions and is also used for the determination of the chemical structure of compounds.

Gas chromatography has tremendous potential for the introductory chemistry laboratory. The instrumental method is very versatile for the

analysis of many different types of samples. Gas chromatography is a simple technique that can be learned quickly by the introductory chemistry student.

All of the above advantages of gas chromatography make it an ideal candidate for introduction into the general chemistry laboratory. After completing the experiment, the general chemistry student will have soon discovered the principles that affect the separation of components in a mixture while gaining invaluable laboratory experience.

CHAPTER TWO: EXPERIMENTAL WORK

A simple experiment was developed for the use of gas chromatography in the introductory chemistry laboratory. Students will be given pure single standard solutions to analyze. The student will then be given two "unknown" samples and asked to determine qualitatively which component(s) are present in his or her "unknown" samples.

The two gas chromatographs to be used for this experiment are identical Perkin Elmer Autosystems; each is equipped with a flame ionization detector, a 15-meter fused-silica capillary column with an inner diameter of 0.32mm and a Methyl Silicone film thickness of 1.0μm. Helium is the carrier gas used. The gas chromatographs are interfaced with computers for data acquisition and manipulation. The computers control the operation of the gas chromatographs. Strip-chart recorders are interfaced with the computers to facilitate the printing of data and chromatograms.

The first step toward the development of the gas chromatography laboratory experiment was to structure a start-up procedure from stand-by mode to fully operational and running mode (see Appendix 2-Laboratory Instructors Manual).

Much of the initial work done towards the development of the gas chromatography laboratory experiment, after the development of a start-up procedure, was the optimizing of the two instruments. The two instruments had to be optimized to ensure reproducible and comparable results were obtainable from each instrument. A simple method was developed for both

GCs. The method starts the GC oven at a temperature of 60°C for 1 min, ramps the temperature at 10°C/min to 100°C, then holds the temperature at 100°C for 2 min. Samples of various alkanes (butane, pentane, hexane, heptane, octane, and nonane) were injected into each instrument and the retention times were compared.

The flow rates for all of the gases used by the gas chromatograph had to be obtained and the flow rates adjusted accordingly to ensure that , in operation, the comparability between the two instruments were comparable. The helium, air, and split gas flow rates were obtained using a gas soap-bubble flow meter and the built-in stopwatch on the instrument. All gas flow rates were tested and set at the following levels:

Table 2-I Flow rates in mL/min

GAS	GC#1	GC#2
Split flow rate	100	100
Air flow rate	402	402
Hydrogen flow rate	40	40

The flow rates were obtained in triplicate to ensure accuracy.

A new syringe was used and much improved results were obtained from each instrument. The old syringe was not able to be cleaned adequately due to contaminants trapped inside it. The data that was acquired using the

new syringe was analyzed and a plot of the number of carbons vs. the logarithm of the corrected retention times was obtained. A straight-line plot was obtainable. The retention times that were obtained for each of the alkanes were corrected using the retention time of an "unretained" sample of butane from a cigarette lighter. The following formula was used to calculate the corrected retention times:

Formula #1 $$Rt_{corrected} = Rt_{analyte} - Rt_{standard}$$

$Rt_{analyte}$ is the retention time of the analyte. $Rt_{standard}$ is the retention time of the unretained butane gas. $Rt_{corrected}$ is the corrected retention time.

Table 2-II Data set used for Figure 2-I from run JW-101795-GC#1-J.

# Carbons	Rt(min)	Log(Rt-0.751)
5	0.882	-0.883
6	1.132	-0.419
7	1.682	-0.031
8	2.873	0.327
9	5.410	0.668

A computer program called "Graphical Analysis" by Vernier Software; Portland, Oregon was used for all of the following plots. A linear least-

squares fit was made for each plot using the software built into the graphing program.

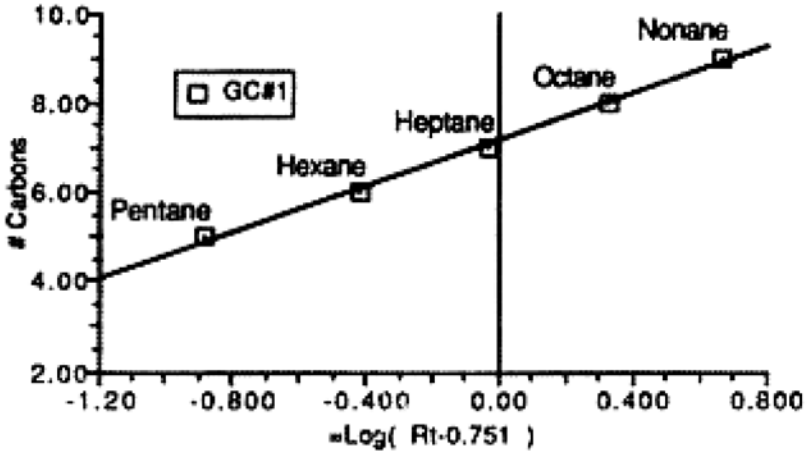

Figure 2-I # Carbons vs. Log($Rt_{corrected}$) from run JW-101795-GC#1-J.

Table 2-III Data set used for Figure 2-II from run JW-101795-GC#2-J.

# Carbons	Rt(min)	Log(Rt-0.7455)
5	0.877	-0.881
6	1.123	-0.423
7	1.655	-0.041
8	2.800	0.313
9	5.240	0.653

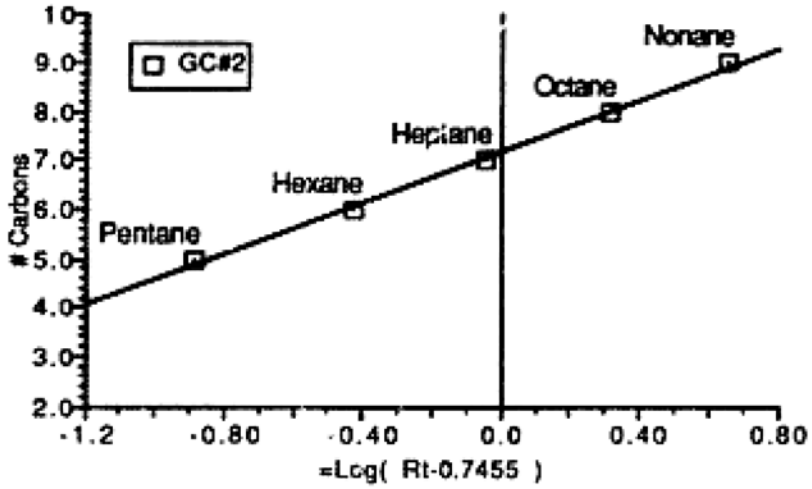

Figure 2-II # Carbons vs. Log($Rt_{corrected}$) from run JW-101795-GC#2-J.

See chromatograms JW-101795-GC#1-J and JW-101795-GC#2-J for references to the above data sets and Figures numbered 2-I and 2-II respectively (Appendix 3-Chromatograms).

Once straight-line plots were obtained for the analysis of various types of alkanes, focus of the project shifted to analysis of alcohols. The types of alcohols that were available to be analyzed were 1-propanol, 1-butanol, 1-pentanol, and 1-hexanol. The samples were injected, the corrected retention times were obtained using Formula #1, and a plot of the number of carbons vs. the Logarithm of the corrected retention times was made.

Table 2-IV Data set used for Figure 2-III from run JW-102695-GC#1-C.

# Carbons	Rt(min)	Log(Rt-0.751)
3	0.96	-0.674
4	1.363	-0.213
5	2.248	0.175
6	4.150	0.531

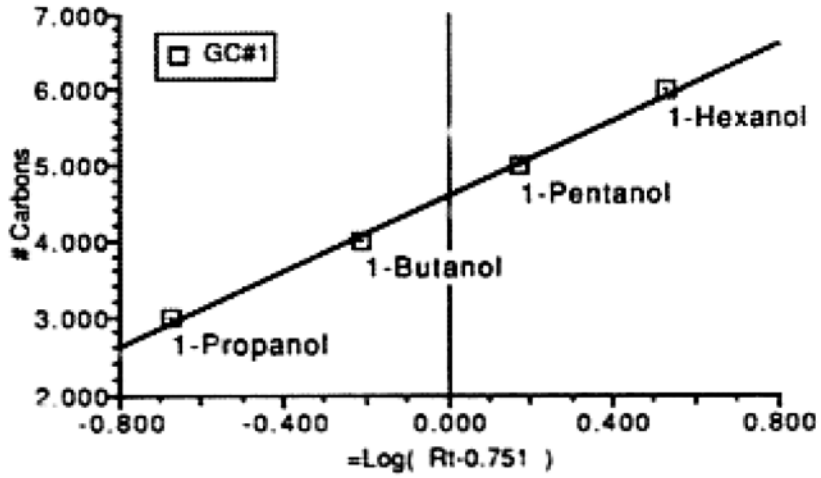

Figure 2-III # Carbons vs. Log($Rt_{corrected}$) from run JW-102695-GC#1-C.

Table 2-V Data set used for Figure 2-IV from run JW-102695-GC#2-C.

# Carbons	Rt(min)	Log(Rt-0.7455)
3	0.965	-0.621
4	1.373	-0.202
5	2.265	0.182
6	4.180	0.536

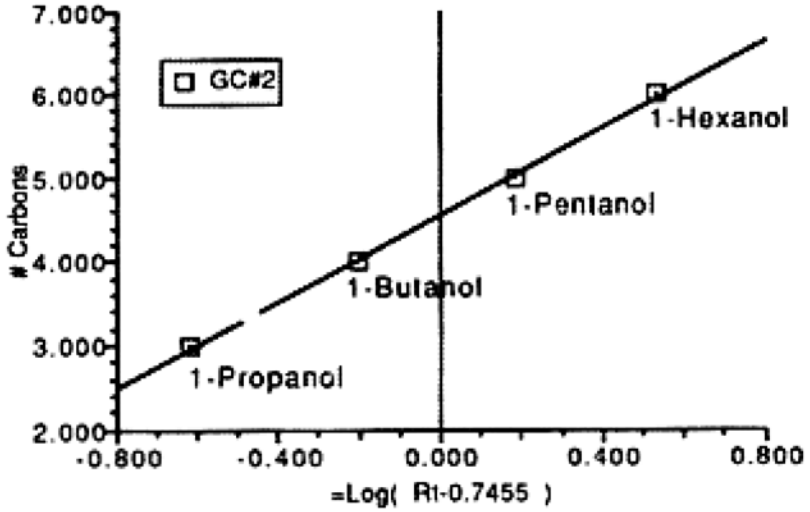

Figure 2-IV # Carbons vs. Log($Rt_{corrected}$) from run JW-102695-GC#2-C.

See chromatograms JW-102695-GC#1-C and JW-102695-GC#2-C for references to the above data sets and Figures numbered 2-III and 2-IV respectively (Appendix 3-Chromatograms).

Table 2-VI Comparison of statistics obtained from the n-alkane plots and alcohol plots of data acquired from GC #1 and GC#2.

GC #	Figure	Slope (# Carbon/Log(Rtcorrected))	Y-Intercept (# Carbons)	Correlation Coefficient
1	2-I	2.58+/-0.09	7.17+/-0.05	0.998
2	2-II	2.62+/-0.09	7.19+/-0.05	0.998
1	2-III	2.48+/-0.10	4.61+/-0.04	0.998
2	2-IV	2.59+/-0.06	4.56+/-0.02	0.999

Note that the slopes and Y-intercepts agree within the least significant figure, indicating that the two instruments give comparable results. The data also indicates that the two instruments stay comparable after optimization; the data for Figure 2-I and 2-II were collected nine days before the data for Figure 2-III and 2-IV.

Various branched alkanes (2-methyl butane, 2-methyl hexane, and 2-methyl heptane) were injected into the gas chromatographs and analyzed. The retention times of these analytes are reported in Table 2-VII and Table 2-VIII. It is apparent that the retention times of the branched alkanes are longer than the retention times of the non-branched alkanes. For example, the retention time of hexane is much shorter than that of 2-methyl hexane since hexane has a lower molecular weight and consequently higher vapor pressure than 2-methyl hexane. The retention time of n-heptane is slightly longer than that of 2-methyl hexane since both have the same formula, but

different structures.

Table 2-VII A comparison of the retention times of n-alkanes and branched n-alkanes analyzed on GC#1.

Analyte	# Carbons	Rt(min)	Notebook Reference
Butane	4	0.752	JW-101795-GC#1-I
2-methyl Butane	5	0.837	JW-103195-GC#1-B
Hexane	6	1.132	JW-101795-GC#1-J
2-methyl hexane	7	1.468	JW-103195-GC#1-F
Heptane	7	1.682	JW-101795-GC#1-J
2-methyl heptane	8	2.363	JW-103195-GC#1-E
Octane	8	2.873	JW-101795-GC#1-J

Table 2-VIII A comparison of the retention times of n-alkanes and branched n-alkanes analyzed on GC#2.

Analyte	# Carbons	Rt(min)	Notebook Reference
Butane	4	0.748	JW-101795-GC#2-I
2-methyl Butane	5	0.855	JW-103195-GC#2-B
Hexane	6	1.123	JW-101795-GC#2-J
2-methyl hexane	7	1.448	JW-103195-GC#2-F
Heptane	7	1.655	JW-101795-GC#2-J
2-methyl heptane	8	2.338	JW-103195-GC#2-E
Octane	8	2.800	JW-101795-GC#2-J

See chromatograms JW-101795-GC#1-(I and J), JW-103195-GC#1-(B, F, and E), JW-101795-GC#2-(I and J), and JW-103195-GC#2-(B, F, and E) for references to the above data sets in Tables 2-VII and 2-VIII respectively (Appendix 3-Chromatograms).

One of the goals of the project was to use "real-world" samples to pique the students' interest in the experiment. An ideal "real-world" sample would be one which is does not present a health risk (after it is "burned" in the flame ionization detector) and with which the students have contact in their everyday lives outside of the chemistry laboratory. The first thought was to use unleaded gasoline or a similar type of mixture in an environmental analysis scenario. The analysis of gasoline and paint-thinner compounds proved to be too complicated for use in the introductory chemistry laboratory. The gasoline and paint thinner samples that were analyzed gave chromatograms that were very complex and it was difficult to decipher which components were present based on the standards that were at my disposal and had been run previously. Therefore, gasoline and paint thinner were ruled out as possible samples to be analyzed in the introductory chemistry laboratory.

The search for practical and safe "real-world" samples that could be analyzed in the introductory chemistry laboratory continued. Dr. Leslie Hull suggested that it might be possible to perform simple identifications of various types of ketones and alcohols present in perfumes and cologne. The analysis of perfume could be based on a murder-mystery story line where the

determination of the student's unknown perfume sample would in turn link a "suspect" to some kind of crime. I obtained a number of perfume samples and began to analyze them. The perfume samples analyzed were: "Tuxedo", "Opium", "Sophia", "Aujard", and "Vanderbilt".

All of the perfume samples produced relatively uninteresting chromatograms. Each had one major peak with a short retention time that was consistent with that of some low-molecular weight alcohol. The other components present in the perfume were at such low concentrations that they produce very small peaks, most were barely above the baseline of the chromatogram. The baseline of the chromatogram was attenuated to facilitate the observation of peaks that were normally obscured by it. A number of peaks were detected after the attenuation of the signal. However, none of the peaks corresponded to any of the standards that had been analyzed previously.

In an effort to identify the components present in the perfume samples, they were all analyzed by a gas chromatograph equipped with a mass-spectrometer (GC-MS). The temperature program used for the analysis of the perfume samples on the GC-MS began at 70°C for 1 min, ramped at 20°C/min to 100°C, and then ramped at 20°C to 200°C. More peaks were observed for the perfume samples when run on the GC-MS using a temperature program which has a slightly higher final temperature then that used on the gas chromatographs in the general chemistry laboratory. The

chromatograms produced on the GC-MS indicated that the temperature program used on the gas chromatographs in the general chemistry laboratory was not high enough to move all of the components present in the perfume through the column in a timely manner.

The temperature program was changed on the GCs in the general chemistry laboratory. The temperatures and rates were increased to increase the rate of migration for the components present in the perfume samples. The increased temperature increased the number of peaks on the resultant perfume chromatograms. However, the identification of these "new" peaks would be too difficult and beyond the level of understanding of introductory chemistry laboratory.

The focus of the identification of the components in the perfumes samples changed to simply identifying which perfume sample was being analyzed. The unknown perfume would be analyzed by GC and the resultant chromatogram would compared to that of a "standard" perfume sample run earlier. Since the perfumes samples when analyzed each yielded a somewhat complex and distinctive chromatogram which serves as a "fingerprint" to distinguish between the perfumes.

This "fingerprint" method of sample identification would illustrate to the students that it is possible to identify very complex sample mixtures (based on the samples complexity) and also that simple five-peak chromatograms are not generally the types of chromatograms that the chemist sees in "real-world" analyses. The use of perfume samples would

give the student a look at the chemical complexity of an everyday, taken-for-granted product. The student would realize the connection between the chemical laboratory and the household products they use.

The use of "fingerprint" identification as a method to distinguish between different perfumes was now the main focus of the gas chromatography laboratory experiment. I needed more information about forensic chemistry (crime science) in order to be able to build a story around the perfume samples. The perfume had to be related in some way to a crime or mystery plot.

While conducting a literature search, I came across a short paragraph about the identification of flammable hydrocarbons that were used in arson crimes by gas chromatography "fingerprinting" method (17). The paragraph stated that the most widely used flammable hydrocarbon mixtures used by arsonist to set fires were gasoline, paint thinner, diesel fuel, and lighter fuel. This interested me since I had started the development of the gas chromatography experiment trying to use gasoline as a sample. Ironically, in the beginning of the development of the experiment, I had excluded gasoline as a possible sample for the experiment because of its complexity and now because of its complexity was considering it a possible sample.

I thought that it would be much easier to develop an interesting story-line around the gasoline using an arson scenario since this technique of identification of flammable hydrocarbons is actually used in "real-world" forensic chemistry laboratories. I decided to research the possibility of

identifying flammable hydrocarbons used as an accelerants in arson fires.

I had already analyzed some gasoline and paint thinner, so I took a look at the chromatograms again. This time I looked at the whole chromatogram and not the specific peaks. Each chromatogram was distinct from the others. I obtained new samples of gasoline, paint thinner and lighter fuel and analyzed them by gas chromatography using the higher temperature program. Again, each chromatogram was distinctive from the others. This proved that they could be used as samples in the general chemistry laboratory.

All of the preliminary work on the GCs was utilized in the development of a laboratory experiment where the students are required to analyze both an unknown n-alkane solution mixture and an unknown sample of either charcoal lighter fluid, unleaded gasoline, or paint thinner.

CHAPTER THREE: RESULTS AND DISCUSSION

The GC experiment that was developed (see Appendix 1-Student Laboratory Manual) is divided up into five sections as follows: (a) pre-laboratory questions, (b) examining the relationship between a sample's structure and it's boiling point, (c) analysis of an unknown sample that was collected "at the scene of a crime", (d) analysis of an unknown mixture of n-alkanes, and (e) post-laboratory questions.

Experiment Itinerary

A. Students read and study the laboratory experiment and answer the pre-laboratory questions before coming to lab.

B. The laboratory instructor leads the whole class in a brief (15 minute) discussion on the principles and theory behind gas chromatography. The topics of the discussion should include the different types of intermolecular forces, the molecular weight and boiling point relationship, vapor pressures, and chromatographic separations.

C. A group of students, from side one of benches one and two, will obtain their unknown and known standards samples, in sealed vials with rubber septum caps, then meet at the gas chromatographs while the other two benches of students work on the first section of the experiment. The students at the GCs will get a brief tour of the instrument from the laboratory instructor. The instructor will point out the following parts of the instrument: gas supply, gas regulator, sample injection port, flame ionization detector exhaust port, oven and the column. The laboratory instructor will

demonstrate the proper technique for injecting a sample into the instrument and starting an analysis (the tour and injection lesson should take about 5 minutes). The students will then analyze their unknown samples and known standard solutions. The students will make one injection each and photocopy the resultant chromatograms for the other students at their bench. Each analysis will take 11 minutes to complete, so it will take about 52 minutes for eight students (four students per instrument) to analyze all of their samples.

D. Once the first group of students have completed the analysis of all their samples, the second (side one of bench three and side two of bench two), and then third (side two of bench three and side two of bench one) group of students will perform the analysis of their samples on the GCs. The first bench is to return to their lab bench, share their data with other students at their bench, and work on the first section of the experiment. The total estimated time for 24 students to complete the experiment is three hours.

E. The students will then answer the post-laboratory questions and write a laboratory report on the experiment.

Pre-Laboratory Work

The first section of the GC experiment is a group of pre-laboratory questions designed to get the students thinking about the concepts and principles upon which the experiment is based. According to Bloom's taxonomy of cognitive development the pre-laboratory questions are mostly low level I (knowledge: recall), II (comprehension: use of a specific rule in a

typical way), and III (novel application: use of learned concept in a situation novel to the student)(19). There are four questions which focus on the concepts of vapor pressures, normal boiling points, and intermolecular forces. The students are then required to graph the boiling point vs. molecular weight for a homologous series of n-alkanes (C_1-C_9). This plotting exercise will illustrate to the students the relationship that as the molecular weight increases so does the boiling point of a compound in a homologous series (See Table 3-I and Figure 3-I).

Table 3-I Molecular weights and boiling points for homologous series of n-alkanes (18).

Compound	Formula	Molecular Weight(grams)	Boiling Point(°C)
Methane	CH_4	16.04	-164
Ethane	CH_3CH_3	30.07	-88.63
Propane	$CH_3CH_2CH_3$	44.11	-42.07
Butane	$CH_3(CH_2)_2CH_3$	58.12	-0.5
Pentane	$CH_3(CH_2)_3CH_3$	72.15	36.07
Hexane	$CH_3(CH_2)_4CH_3$	86.18	68.95
Heptane	$CH_3(CH_2)_5CH_3$	100.21	98.42

Nonane	$CH_3(CH_2)_7CH_3$	128.26	150.798

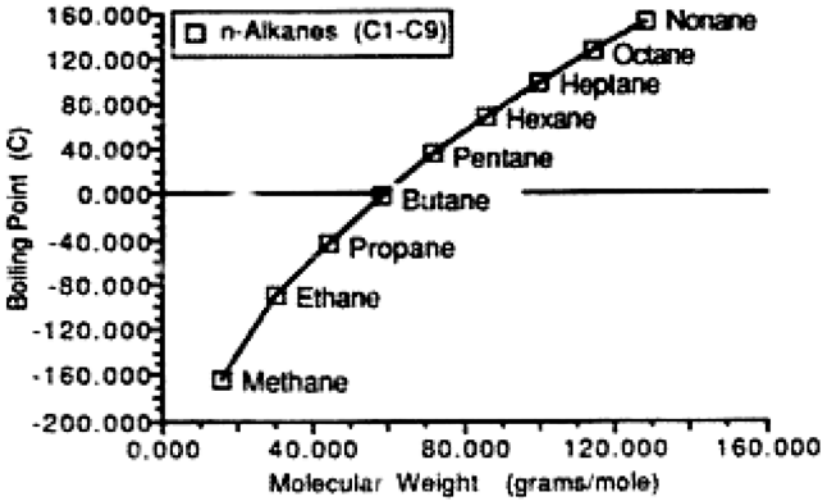

Figure 3-I Molecular Weight vs Boiling Point
n-alkanes

The pre-laboratory questions and the construction of the molecular weight vs. boiling point plot are to completed and studied by the students before coming to the laboratory. This will allow the students to immediately discuss the concepts and relationships upon entering the laboratory.

<u>Intermolecular Forces and the -OH Functional Group</u>

In the second section of the experiment the students will further examine the relationship between a sample's structure and its boiling point by answering more questions similar to the pre-laboratory questions. The students will also add another data set to the molecular weight vs. boiling

point plot they have already constructed. The new data set the students will plot is the boiling points of the following alcohols: 1-methanol, 1-ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, and 1-nonanol. The students will compare the boiling points of the above alcohols to those of the n-alkanes (See Table 3-II and Figure 3-II). This plot will illustrate the that the -OH functional group increases the intermolecular forces and, as a result, a substance's boiling point will also increase.

Table 3-II Molecular weights and boiling points for homologous series of alcohols (18).

Compound	Formula	Molecular Weight(grams)	Boiling Point(°C)
Methanol	CH_3OH	32.04	64.96
Ethanol	CH_3CH_2OH	46.07	78.50
Propanol	$CH_3(CH_2)_2OH$	60.11	97.40
Butanol	$CH_3(CH_2)_3OH$	74.12	117.25
Pentanol	$CH_3(CH_2)_4OH$	88.15	137.30
Hexanol	$CH_3(CH_2)_5OH$	102.18	158.00
Heptanol	$CH_3(CH_2)_6OH$	116.21	176.00
Octanol	$CH_3(CH_2)_7OH$	130.23	194.45
Nonanol	$CH_3(CH_2)_8OH$	200.37	213.50

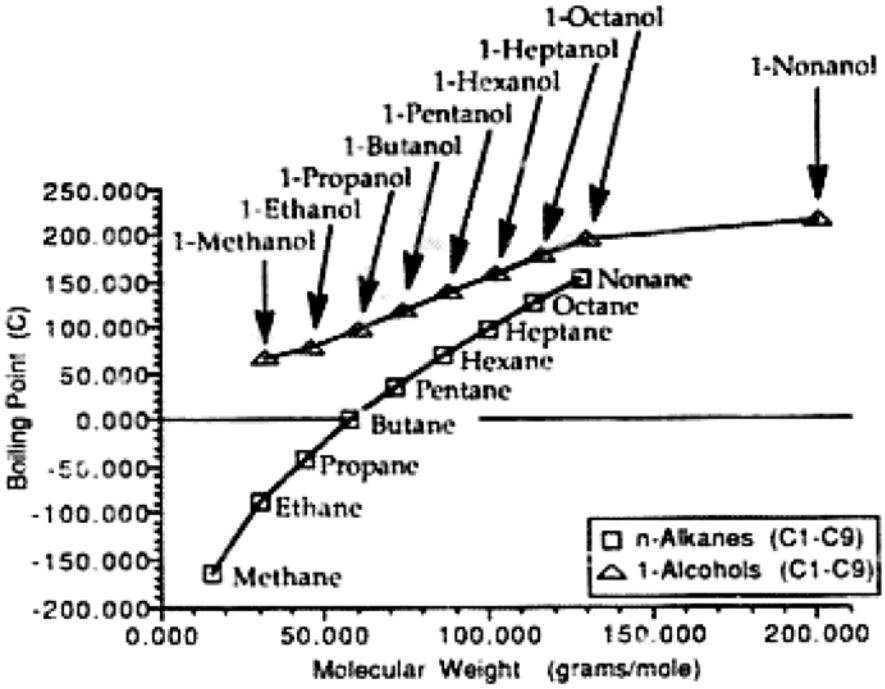

Figure 3-II Molecular Weights vs Boiling Point
n-alkanes and 1-alcohols

The second section was added to the laboratory as a way to increase the amount of time the students are on-task and engaged. This section provides the student with another opportunity to think about the important concepts of the experiment and it also can be completed at anytime during the experiment. The students are to work on this section when they are not working on the gas chromatograph instrument, or analyzing data.

Analysis of a "Forensic" Sample

The third section of the experiment is an analysis of an unknown sample that was "collected at the scene of the crime". Students will be given an unknown sample that consists of either gasoline, paint thinner, o. lighter fuel to make a "fingerprint" identification in comparison with previously run mixtures whose identities are known. The chromatograms of the known mixtures will be posted in the laboratory by the instructor. This section of the experiment is designed to pique the students' interest in the experiment by adding the elen ent of discovery and mystery.

The students will be told that the unknown sample was "collected at a crime scene" where arson is suspected and will then discover which of the three flammable hydrocarbons was used as the accelerant in the fire. The students will have to recognize patterns in the chromatograms to be able to distinguish between them. The chromatograms for the three accelerants are very distinct, the students will quickly discover which flammable mixture of hydrocarbons was used by the arsonist.

The task of solving a crime will keep the students interested and focused on the experiment; this determination of flammable hydrocarbons is used in "real" forensic chemistry laboratories. The students will realize the importance and power of using science to solve "real-life" problems. This part of the experiment will also illustrate to the students the connection between the chemicals in the laboratory and in their households.

Analysis of n-Alkane Samples

The fourth activity is another GC analysis, this time of an unknown

mixture of n-alkanes. The components are identified by straight-forward chromatogram comparisons with standard chromatograms also analyzed by the students. The third section of the experiment had the students look at the chromatogram as a whole, whereas this section is designed to have the students study the individual peaks of the chromatograms more closely. The student will be required to now look at the individual "trees in the forest" instead of looking at the "forest" as a whole. The student will discover the concept of retention time and the factors that affect it. This section will provide the student with a concrete example of intermolecular forces at work and also the relationship between a substance's molecular weight and its boiling point.

Each laboratory bench will be given two different known single n-alkane standard solutions and one unknown mixture that consists of as many as six n-alkanes. The students will compare the retention times of the peaks on their chromatograms with those of the pure single n-alkane standards they will also analyze to determine the components of their unknown n-alkane mixture. Since each bench will analyze only two of the six possible n-alkanes in their unknown, the students will have to consult other benches to determine which components are present in their unknown sample. The students will list the retention time of each standard n-alkane on the blackboard to help disseminate the information. This will promote cooperation among the students in the laboratory.

Post-Laboratory Work

The fifth section of the GC experiment is a group of post-laboratory questions designed to challenge the student by expanding on the knowledge gained during the course of the experiment. The seven questions in this section ask the student about the concepts of intermolecular forces, experimental technique, and experimental error. According to Bloom's taxonomy the post-laboratory questions are mostly higher order (above level III)(19). These questions are level IV (analysis: students required to break down the materials into parts to make concepts more explicit), level V (synthesis: putting together parts to form a whole pattern or structure of ideas not clearly there before), and level VI (evaluation: students must make judgments about the validity of material and methods for a given purpose).

The main source of error for the experiment is sample injection. Good sample injections are crucial to obtaining reliable results. The students must be instructed in the proper injection technique since they will have only one chance to inject a sample. The only way to improve student injections is to have the students practice their injection technique but there is insufficient time in the lab to do this in a single lab period.

Pilot Mini-Experiment Results

I was able to test the GC experiment with the help of sophomore chemistry major Doug Tanner. Doug was given a copy of the laboratory experiment to study the day prior to his attempting to perform a shortened version of the experiment. Doug was given three pure n-alkane standard solutions (pentane, hexane, and nonane), an unknown sample "that was

collected at the scene of a crime" (charcoal lighter fuel), and an unknown n-alkane mixture that consisted of two of the three pure n-alkane standards (pentane and nonane).

I illustrated the proper injection technique to Doug and allowed him to handle the syringe to become comfortable with it. Doug then analyzed his three single n-alkane standard solutions and noted the resultant retention times. He then analyzed his unknown n-alkane mixture. By comparing the re'ention times of the peaks on his chromatograms, Doug quickly identified the components of his mixture as pentane and nonane. Doug then analyzed an unknown "arson accelerant" sample. Doug obtained a chromatogram and compared it to the chromatograms of gasoline, charcoal lighter fuel, and paint thinner. Doug determined that his unknown sample was charcoal lighter fuel. Doug was able to analyze three standards and identify both of his unknown samples correctly in about 55 minutes. The analysis time of 55 minutes for five samples, by one student, is consistent with the estimated 52 minutes for four samples, by four different students. The 52 minute estimate is based on four 11 minute GC runs with time between each injection allowing the students to get organized and acquainted with the instrument and the syringe.

Future Applications

Future applications of the gas chromatograph in the general chemistry laboratory are unlimited. The versatility of the GC allows a range of samples to be analyzed. The use of "real-world" samples coupl·d with an interesting

application of the experimental technique gets students interested and engaged in the experiment. A number of different kinds of samples could be incorporated into the GC experiment, such as perfume or environmental samples. In a simple variation on the experiment described here, perfume samples could be used as unknown samples, with a mystery scenario developed around the identification of a suspect, based on the perfume or cologne that he or she wears. Samples with environmental implications such as ground water and wastewater analysis will motive the students to learn more about chemistry and gas chromatography. The use of a different sampling technique, such as manual head space sampling, could also be incorporated into the GC experiment.

This new general chemistry laboratory experiment incorporates relevant "real-world" samples, methods and techniques. This experiment reflects the excitement of chemistry and its use in solving "mysteries" while giving the general chemistry student hands-on training using state-of-the-art instrumentation. The end result will be a more meaningful laboratory experience for the general chemistry student, which will serve to cognitively challenge and interest the student in the field.

APPENDIX I

Experiment 8

Analysis of an Unknown Mixture by Gas Chromatography

PURPOSE OF EXPERIMENT: To study the principles of gas chromatography by analyzing an unknown mixture of n-alkanes and use gas chromatography to solve a mystery.

The need for scientists to separate solutions into their individual components is extremely important in chemistry laboratories around the world. The most widely used analytical technique to separate materials is called chromatography, which was invented by a Russian botanist, Mikhail Tswett, in the first part of the century. Tswett found that when he passed extracts of green leaves through a glass column packed with finely divided calcium carbonate the solutions separated as colored bands on the column. Because Tswett's separations involved colors, he called this new technique chromatography (Greek *chroma* meaning color and *graphein* meaning to write).

Modern chemists use an instrument known as a **Gas Chromatograph** which utilizes the principles of chromatographic separations to isolate and identify related components of complex mixtures. The gas chromatograph is a relatively simple instrument with great versatility. Small sample volumes

and tremendous separation power are the source of the gas chromatograph's great utility in almost all fields of chemistry. Gas chromatography is used in pharmaceutical, research, environmental, and forensic crime laboratories.

A gas chromatograph consists of five basic parts; carrier gas supply, sample inlet device, chromatography column, detector, and recording device with data manipulation capabilities. A schematic drawing of a typical gas chromatograph is presented in Figure 1. Notice that the sample inlet, column, and detector are all contained in a thermostat. The function of the thermostat is to regulate the temperature at which the analysis is to be run. Gas chromatographic analysis is extremely temperature-dependent.

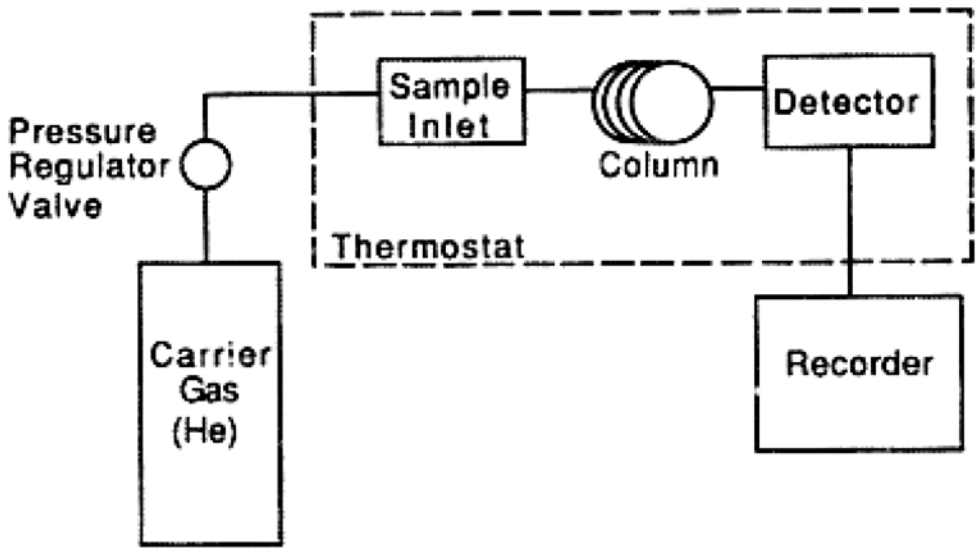

Figure 1 Gas Chromatograph Schematic Drawing

A small amount of sample is introduced via a syringe into a heated injection port where it is immediately vaporized. An inert gas, such as

Helium, called the **mobile phase** or **carrier gas**, sweeps the vaporized sample into a **capillary column** which contains the **stationary phase**. A capillary column consists of a tube packed with a support material, such as fused silica. The support material is coated with a one-micrometer thick nonvolatile **liquid phase**, such as methyl silicone, which is the stationary phase. Each component of the sample then distributes itself in a characteristic manner between the gaseous phase and the stationary liquid phase. The rate of migration for the components is determined by the time the sample spends in the gaseous mobile phase compared to the time the sample spends in the liquid stationary phase. An individual component will undergo many thousands of transfers between the stationary and mobile phase as it passes through the column during analysis. Gas chromatography column dimensions vary in length and diameter. Column lengths can be as short as ten meters or as long as forty meters. The inside diameter of the column is usually about 0.20 to 0.40 millimeters. The column is coiled to allow it to fit inside a thermostat called the **oven**.

If the analysis conditions are well chosen, the components of the mixture are all separated and each passes from the column through a detector at a different time. The degree of separation of the components is determined ultimately by their molecular structures. The characteristic manner in which a sample distributes itself between the mobile phase and the stationary phase in the column is highly temperature dependent. The temperature of the column is adjusted to obtain the proper vapor pressures for the components

to be separated. **Vapor pressure** is a measure of the tendency for the molecules of a given substance to escape from the liquid phase and enter the gas phase at a given temperature. The vapor pressure of a component is determined by the strength of intermolecular forces. The vapor pressure will be low if the intermolecular forces are strong between a component and the stationary phase. Conversely, the vapor pressure will be high if the intermolecular forces between a component and the stationary phase are weak.

Because an individual component of a substance can migrate through a column only when it is in the moving gaseous phase, the component's vapor pressure will determine the rate that it will migrate through the column. For example, a component with a high vapor pressure (and weak intermolecular forces) will migrate through a column faster than a component with a low vapor pressure (and strong intermolecular forces). Increasing the analysis temperature will increase the rates of migration through the column for all the components of a mixture because all of the components of a mixture will have higher vapor pressures at higher temperatures. Conversely, lowering the analysis temperature will decrease the rates of migration through the column for all the components of a mixture.

Temperature programs are used to facilitate the separation of both high- and low-vapor pressure components in a mixture by gradually increasing the temperature (at a pre-set rate) of the column during analysis. Components that have high vapor pressures are separated at lower

temperature, while the components with low vapor pressures are moved at faster rates through the column at the higher temperatures. Temperature programing increases the sensitivity of the instrument while shortening the time needed to separate components with low vapor pressures.

The presence of the individual components in the effluent stream is detected by a **flame ionization detector (FID)**. The flame ionization detector is based on the formation of ions when the sample passes through a hydrogen flame. The components are "burned" in the detector as they elute from the column. The resulting ions are converted into a signal which is transferred to a computer and a printer. The flame ionization detector will give a signal for any substance that "burns" in the flame. Therefore, the flame ionization detector is insensitive towards noncombustible gases such as H_2O, CO_2, SO_2, and NO_2. The main drawback of using the flame ionization detector is that the sample is destroyed during the analysis.

The continuous detection of the effluent gas stream results in a **chromatogram** as shown in Figure 2. The shape of the peaks typically corresponds to that of the normal Gaussian distribution curve unless there is a distortion due to instrumental or operational factors. The average amount of time from sample injection for each of the components to **elute** from the column is called the **retention time**, t_R. Retention times are a measure of the amount of time that each component is retained by the stationary phase. The

chromatogram that is produced from an analysis provides the chemist with both qualitative and quantitative results. Retention times are used to identify the components in the sample by comparison to the retention times of known standards run under the same analysis conditions. The area under each peak is proportional to concentration and can be used to determine relative amounts of each component in a mixture.

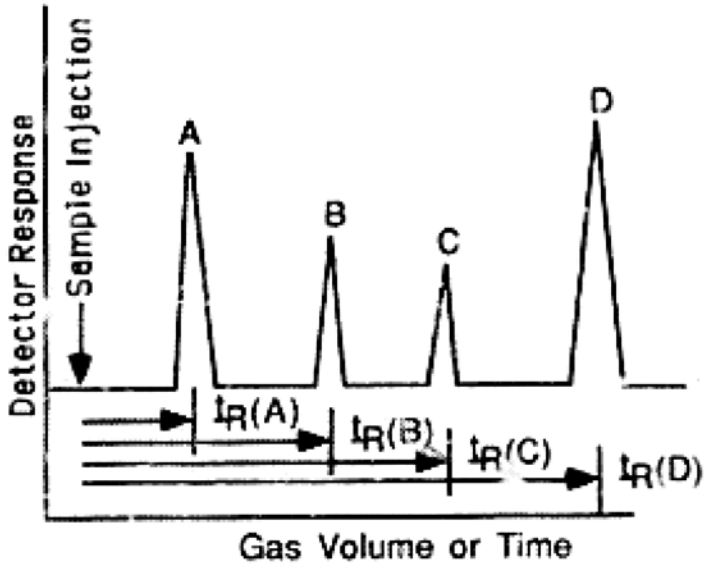

Figure 2 Elution Chromatogram

EXPERIMENTAL PROCEDURE

(Study this section and the **PRE-LABORATORY QUESTIONS** before coming to the laboratory. You will work in partners and in groups involving all members of your lab bench. **Wear safety goggles when performing this experiment.**)

A. Examine the relationship between a sample's structure and its boiling point (Record your data for this section in the corresponding parts of the data sheets at the end of the experiment.)

In this experiment you will discover the relationship between a component's structure and its boiling point.

Use the data from PRE-LABORATORY QUESTIONS to answer the following questions.

1. Describe the relationship between molecular weight and boiling point for the n-alkanes you plotted.

2. Water has a molecular weight of 18.0 g/mol and a boiling point of 100^{o}C. Is this data consistent with the relationship you just described? Explain.

3. What kinds of intermolecular forces are there between the n-alkane molecules? What kinds of intermolecular forces exist between the water molecules?

4. What effect will the addition of an alcohol (-OH) functional group on the n-alkane have on the boiling point? Explain.

5. Confirm your answer to question number 4 by looking up the boiling points of the following alcohols in the Handbook of Chemistry and Physics: 1-Methanol, 1-Ethanol, 1-Propanol, 1-Butanol, 1-Pentanol, 1-Hexanol, 1-Heptanol, 1-Octanol, and 1-Nonanol and comparing them to boiling points for appropriate n-alkanes given in the PRE-LABORATORY QUESTIONS.

Plot the boiling points vs. molecular weights for the various alcohols on the same graph you constructed for the PRE-LABORATORY QUESTIONS and submit this plot with your report.

B. Analysis of an unknown sample that was collected "at the scene of a crime" (Record your data for this section in the corresponding parts of the data sheets at the end of the experiment.)

The gas chromatograph to be used for this experiment is a Perkin Elmer Autosystem equipped with a flame ionization detector, a 15-meter fused-silica capillary column with an inner diameter of 0.32 mm and a Methyl Silicone film thickness of 1.0 μm. The gas chromatograph is interfaced with a computer for data acquisition and manipulation. Helium is used as the carrier gas and is regulated at about 7 mL/min. The sample injection port is kept at a constant temperature of 200°C. The temperature

program used for the analysis begins at 70°C for 1 minute, ramps at 20°C/min to 100°C for 1 min, and then at 20°C/min to 160°C for 3 min. The same temperature program is used throughout the experiment. The flame ionization detector is kept at a constant temperature of 250°C.

Each side of a lab bench is to obtain one capped vial from the lab instructor which contains an unknown sample that was collected "at the scene of a crime".

In this section of the experiment, you will learn how to use a syringe to inject a sample into the gas chromatograph (GC), and qualitatively determine the identity of an unknown solution that was collected "at the scene of a crime" by comparing its chromatogram with that of standards analyzed previously.

Highly flammable mixtures are often used to accelerate fires in arson crimes. Often the arsonist is careless, and leaves the container (plastic or metal) that was used to contain the flammable mixture at the arson scene. The debris and residues that are left after the container and its contents have burned can be brought back to the chemistry laboratory to be analyzed. Once in the laboratory, the debris is placed into a new container (a new, unlined paint can), sealed, and heated to 60-110°C to separate the hydrocarbon vapors from the debris. A sample of the air above the debris is removed by a syringe and analyzed by gas chromatography. This technique can often distinguish

between hydrocarbons in charcoal lighter fluid, gasoline, and paint thinner (the three most widely used accelerants in crimes involving arson).

The chromatograms of flammable mixtures are very complex and serve as **"fingerprints"** that are used to distinguish between accelerants. Once the chemist identifies the accelerant, it may be possible to link a suspect to the arson.

Have one pair of students at your lab bench inject a 0.25 µL representative sample of the unknown sample that was collected at a crime scene into the injection port on the top of the gas chromatograph using a 1.0 µL syringe that has been rinsed with the proper solvent and conditioned with the solution to be analyzed. The laboratory instructor will demonstrate the proper sample injection technique. Immediately after the sample is injected, press the green "Run" key on the key pad of the instrument to start the acquisition of data. The chromatogram is displayed on the computer until the analysis is complete at which time a hard copy of the chromatogram will be printed. Make a photocopy of the resulting chromatogram for every member at your lab bench.

Determine which accelerant was used in a recent fire where arson is suspected by comparing the chromatogram of the unknown samples that were collected at the crime scene with the chromatograms of charcoal lighter fluid, gasoline, and paint thinner that are posted in the laboratory.

C. Analysis of an unknown mixture of n-alkanes (Record your data for this section in the corresponding parts of the data sheets at the end of the experiment.)

In this section of the experiment, you will learn how to use a syringe to inject a sample into the gas chromatograph (GC), and quantitatively determine the components of an unknown solution by comparing the retention times (t_R) given by the chromatogram. You will also see how a component's structure and boiling point determine the rate of migration through the chromatography column.

Each side of a lab bench is to obtain three capped vials from the lab instructor. One vial contains a mixture of unknown n-alkane components (which may contain as many as six n-alkanes for identification) and the other two vials contain pure single n-alkane standard solutions.

Have one pair of students at your lab bench inject a 0.25 µL representative sample of the unknown n-alkane mixture into the injection port on the top of the gas chromatograph using a 1.0 µL syringe that has been rinsed with the proper solvent and conditioned with the solution to be analyzed. The laboratory instructor will demonstrate the proper sample injection technique. Immediately after the sample is injected, press the green "Run" key on the key pad of the instrument to start the acquisition of data. The chromatogram is displayed on the computer until the analysis is

complete at which time a hard copy of the chromatogram will be printed. Make a photocopy of the resulting chromatogram for every member at your lab bench. Have different pairs of students at your bench do the same with each of the two pure single n-alkane solutions. Each sample is injected and analyzed twice per bench (once for each side of a bench). Each student is to inject a sample into the gas chromatograph.

List on the blackboard the identity of each of your standards, their retention times, and note the instrument that was used to determine the retention times (GC#1 or GC#2). Compare the chromatograms of the n-alkane unknown mixtures with those of single pure n-alkane solutions to determine the components of your unknown. Since each bench will analyze two known single n-alkane solutions, you will have to consult other lab benches to identify the components that are present in your unknown n-alkane mixture.

NOTE: Copies of all chromatograms must be submitted with final laboratory report.

PRE-LABORATORY QUESTIONS

1. How is a substance's vapor pressure related to its boiling point?

2. What is the definition of normal boiling point?

3. Solid carbon dioxide (CO_2) sublimes at room temperature. What does this indicate to you about solid carbon dioxide's vapor pressure? Explain.

4. Answer the following questions with "increase", "not change", or "decrease".

   (a) If the intermolecular forces in a liquid increase, the normal boiling point of the liquid will _____.

   (b) If the intermolecular forces in a liquid increase, the vapor pressure of the liquid will _____.

   (c) If you increase the surface area of the liquid, the vapor pressure of the liquid will _____ _____.

PRE-LABORATORY QUESTIONS

5. a. Look up the boiling points and the molecular weights of the following n-alkanes: methane, ethane, propane, butane, pentane, hexane, heptane, octane, and nonane.

b. Obtain a good piece of graph paper (with at least 10 divisions per inch), and construct a plot of boiling point vs. molecular weight of the n-alkanes using the data from part (a). Use boiling point as the y-axis, and use molecular weight as the x-axis. Connect the points of the graph.

POST-LABORATORY QUESTIONS

1. What would be the effect on your results if you did not press the "RUN" key immediately after the injection of the sample into the injection port of the instrument?

2. What types of intermolecular interactions occur between the stationary phase, which is methyl silicone, and the n-alkane components of the mixture? Explain.

3. What types of intermolecular interactions occur between the stationary phase and helium (the mobile phase)? Explain.

POST-LABORATORY QUESTIONS

4. Explain which member of each of the following pairs of compounds you would expect to have the higher boiling point: (a) hexane and 1-hexanol; (b) heptane and 2-methyl heptane; and (c) 2-methyl heptane and octane. In each case, tell what intermolecular forces are involved.

5. A student compares a complicated chromatogram obtained for a mixture in the laboratory with a reference chromatogram of a pure compound. In each chromatogram there is a peak at t_R = 2.38 min.

Can the student conclude that the unknown mixture contains the reference compound;

(a) without performing additional experiments? Explain.

(b) with additional experiments? Describe these experiments.

POST-LABORATORY QUESTIONS

6. A student pushes "RUN" 30. seconds after one injection. All other data is collected properly.

(a) How will this affect the separation of the components in a mixture?

(b) How will this affect the retention times? (increase, no change, decrease)

(c) How will this affect the identification of the components in the sample being analyzed?

Explain your answers to each part of this question.

7. A student obtains the following data for 5 replicate injections of the same sample:

Run	t_R (minutes)
1	3.23
2	3.61
3	3.04
4	3.93
5	3.88

Explain these results in terms of possible experimental error.

APPENDIX II: LABORATORY INSTRUCTORS MANUAL

INSTRUMENT USED IN EXPERIMENT

The two gas chromatographs (GCs) used for this experiment are Perkin Elmer Autosystems. Each gas chromatograph is identically equipped with a 15-meter fused-silica capillary column with an inner diameter of 0.32 mm and a methyl silicone film thickness of 1.0 μm, and a flame ionization detector. The gas chromatograph is interfaced with a computer for data acquisition and manipulation. Helium is used for the carrier gas and is regulated at about 7mL/min. The sample injection port is kept at a constant temperature of 200^{O}C.

A 1.0-μL syringe equipped with a Chaney adaptor and guide assembly is used for the injection of samples into the gas chromatograph. The Chaney adaptor eliminates subjective error by providing a mechanical method to assure the operator repetitive sample volumes. The guide assembly aids in preventing plunger bending and prevents accidental removal of the plunger from the syringe.

INSTRUMENT START-UP PROCEDURE

The following is a start-up procedure for the two GCs used in the general chemistry experiment. The procedure starts with the instrument turned completely off.

1. Check the septum in the injector port and change if the septum

appears torn or damaged. The septum should be checked **before** the instrument is turned on, because the injection port which holds the septum will be heated to 150°C after the instrument is turned on. The septum will have to be changed frequently. **Note: Changing the septum may affect the gas flow rate and may affect the compatibility between the two instruments. If you make any changes to one GC, you should make the same changes to the other.**

2. Check each of the gas tanks and replace if below 100 psi. **Note: Changing the Gas tanks may affect the gas flow rate and may affect the compatibility between the two instruments. If you make any changes to one GC, you should make the same changes to the other.**

3. Turn on the gas supply both on the gas tank and on the gas regulators (air tank, helium tank, and hydrogen tank).

4. Turn on the instrument. The ON/OFF switch is on the front, lower right of the instrument.

5 Turn on the computer. The ON/OFF switch is on the back left, middle of the computer.

6. Turn on the printer. The ON/OFF switch is on the top right of the printer.

The instrument is now turned on. The instrument must warm up to the proper temperature prior to the ignition of the flame ionization detector (FID). Wait until the instrument display on the key pad reads "READY"

before proceeding.

LIGHTING THE FLAME IONIZATION DETECTOR (FID)

7. Autozero the instrument. Press the "AUTOZERO" button on the key pad on the front, upper right of the instrument. The baseline signal in mV will be displayed. Press the "SET" button on the key pad to autozero the instrument. Once the FID has been lit a noticeable increase in the mV signal will be noticed by the analyst.

8. To ignite the FID:

1. Bleed some hydrogen into the FID by turning on the hydrogen gas valve on the top, back left of the instrument. The hydrogen gas valve is the lower of the two knobs.

2. After about ten to twenty seconds place the FID ignitor on the top of the FID port. The FID ignitor looks like a car cigarette lighter and is found under the hinged panel on the top right of the instrument.

3. Once the ignitor is positioned above the FID port, press down on the ignitor slightly to make the heating filament glow and slowly turn on the air by turning the knob on the instrument above the hydrogen gas valve. A "popping" noise should be heard and a noticeable increase in the mV signal from the instrument should be observed. The FID is now lit. If there is no "popping" noise or noticeable increase in the mV signal repeat the start-up procedure from direction number 6. Please note that the filament will not glow if the ignitor is not pressed down and, therefore, it will not be possible to

ignite the FID.

The instrument is now ready to analyze samples.

SAMPLE ANALYSIS METHODS

The name of the methods used in this experiment are "baseline" and "n-alkane". The "baseline" method is to be run before the analysis of samples (i.e. before the lab begins). This method is designed to clean out the column. The temperature program for the "baseline" method starts at 75°C, then ramps at 20°C/min to 200°C. The program runs for 10 minutes and no chromatogram will be printed at the end of the analysis.

After a baseline run has been completed, the method must be changed from "baseline" to "n-alkane". The "n-alkane" method is used for the analysis of all samples in this experiment. The temperature program used for the "n-alkane" method begins at 70°C for 1 minute, ramps at 20°C/min to 100°C for 1 min, and then at 20°C/min to 160°C for 3 min. The same temperature program is used throughout the experiment. The flame ionization detector is kept at a constant temperature of 250°C. The "n-alkane" method runs for 11 min after which time a chromatogram will be printed.

The "baseline" method should be run after the analysis of samples has been completed (i.e. after the lab ends).

CHANGING METHODS

Using the computer mouse, click on the word "METHOD" on the menu bar on the top of the screen. A menu will appear. Double click on the word "EDIT". A method directory will appear, double click on the method you want to use. Now, click on the word "INSTRUMENT" on the menu bar on the top of the screen. A menu will appear. Double click on the words "SET-UP". Now, click on the word "ACTIVATE" on the menu bar on the top of the screen. A menu will appear. Double click on the word "ACTIVATE". The method will now be activated and the instrument will configure itself to the settings stored in the method. A "NOT READY" message will appear on the status screen of the instrument while it is equilibrating to the proper temperature. The instrument status screen on the instrument will read "READY" once the instrument has reached temperature equilibrium.

SAMPLE INJECTION

In this experiment, 0.25-µL sample volumes are used. The Chaney adaptor on the syringe is set at 0.50 µL. The syringe should be rinsed with an appropriate solvent (acetone) at least three times before coming into contact with the sample solution. The syringe should then be rinsed two or three times with sample solution. The syringe is now conditioned and ready for the injection of the sample.

(Note: do not touch the sample injection port because it will be at 200°C.)

Pierce the rubber septum on the top of the sample container and draw up 0.5 µL of sample solution into the syringe by pulling up the plunger. Lower the plunger down to the 0.25-µL mark on the syringe barrel. Now, draw 0.25 µL of air into the syringe by pulling up the plunger to the 0.5-µL mark on the syringe barrel. The injection of air and sample into the GC results in better-looking chromatograms. Position the syringe above the injection port with both hands (it is important that the syringe be totally vertical to ensure that the sample is properly injected and that the syringe does not get damaged during the injection). Lower the syringe and carefully pierce the septum with the needle.

(Note: if you feel strong resistance from the syringe while trying to inject a sample, stop and remove the syringe. Do not try to force the injection, it may result in a damaged syringe!)

Once the syringe has pierced the septum and the syringe has been lowered as much as possible, rapidly lower the plunger to inject the sample and quickly remove the syringe needle from the injection port.

The sample must be injected all at once and enter the column as a single "plug". If the sample is not injected as a single "plug" tailing will result. Tailing is when the shape of the chromatogram peak does not appear to be symmetric.

Once the syringe is removed from the injection port the green "RUN"

key on the instrument key-pad must be pressed to start the collection of data. The chromatogram will be presented on the computer screen during the analysis. After the analysis is complete, the chromatogram will be printed automatically.

The instrument will reset and equilibrate itself to the pre-set analysis conditions specified in the method. You must wait for the "READY" message to be displayed on the instrument status screen to inject another sample.

SHUT-DOWN PROCEDURE

After all analysis is completed the following shut-down procedure should be followed:

1. Run "Baseline" method to clean out column.
2. Turn off the hydrogen gas valve on the instrument.
3. Turn off the air gas valve on the instrument.
4. Turn off the computer.
5. Turn off the printer.
6. Turn off the instrument.
7. Turn off the gas from all tanks by turning the smallest knob on the regulator and the main valve of the gas tank (at top of tank).
8. Clean up the area around the instrument.

EXPERIMENT

1. Conduct a brief discussion of the principles of separation of the components (intermolecular forces) in a mixture using the gas chromatograph. The students should already have a nodding acquaintance with this from the pre-lab activity they were assigned. The students should come to class with a plot of boiling point vs. molecular weight for n-alkanes (C_1-C_9) which they will use for PART A of the experiment.

2. Have students obtain standard and unknown sample solutions in capped septum vials. Each side of a bench will be analyzing two pure single n-alkane solutions, one unknown n-alkane mixture that consists of from three to six different n-alkanes, and one unknown sample that was "collected at a crime scene were arson is suspected". The "arson accelerant" sample is either gasoline, paint-thinner, or lighter fuel. The chromatograms for gasoline, paint-thinner, and lighter fuel should be obtained and posted in the laboratory prior to the students entering the laboratory. Note the small sample volumes that are required for this experiment.

3. Have side one of benches one and two meet at the two gas chromatographs by the computers while the other benches work on the first section of the experiment. Give a brief "tour" of the instrument pointing out the location of the following components: computer, printer, instrument key pad and "RUN" key (needed to start the acquisition of data), sample injection port (**Note: the sample injection port is hot, instruct the students not to touch**

it!), flame ionization detector exhaust port, oven, and column.

4. Show the students the syringe to be used for the injection of samples into the GC. The syringe is a normal 1.0-μL syringe equipped with a Chaney adaptor and guide assembly is used for the injection of samples into the gas chromatograph. The syringe with the Chaney adaptor may seem cumbersome and awkward to the students at first, so have them "handle" the syringe before it is their turn to inject a sample.

5. Illustrate proper injection techniques. See sample injection technique above.

6. Have students perform the analysis of their unknowns and standard solutions. The students are to photocopy the resultant chromatograms for all of the students at their laboratory bench.

7. After the first round of the students have analyzed their samples have the second group of students (from side two of bench two and side one of bench three), and then finally, have the third group of students (from side two of bench one and bench three) perform their experiment. Have the students who are not working on the GCs do PART A of the experiment and/or work with benchmates on their data for PARTS B and C.

APPENDIX THREE: LITERATURE CITED

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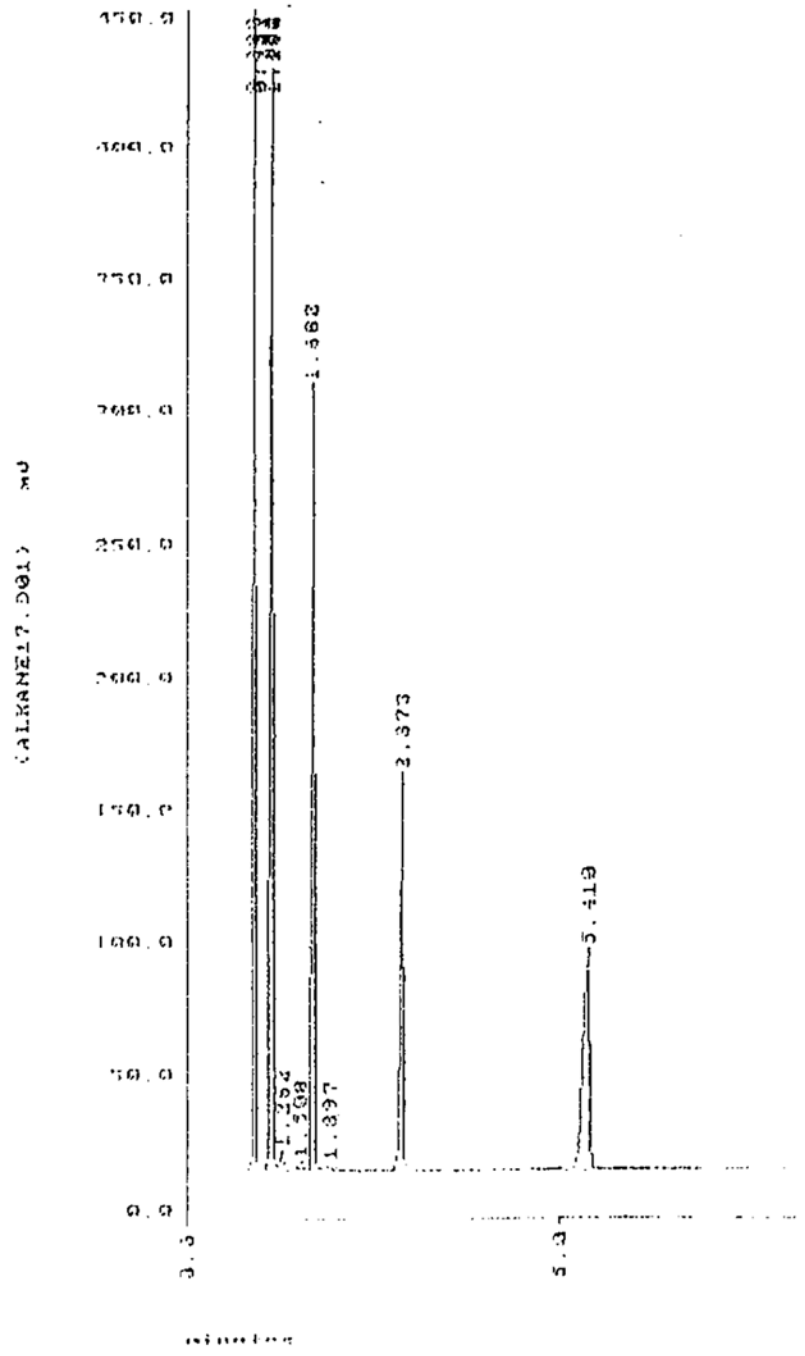
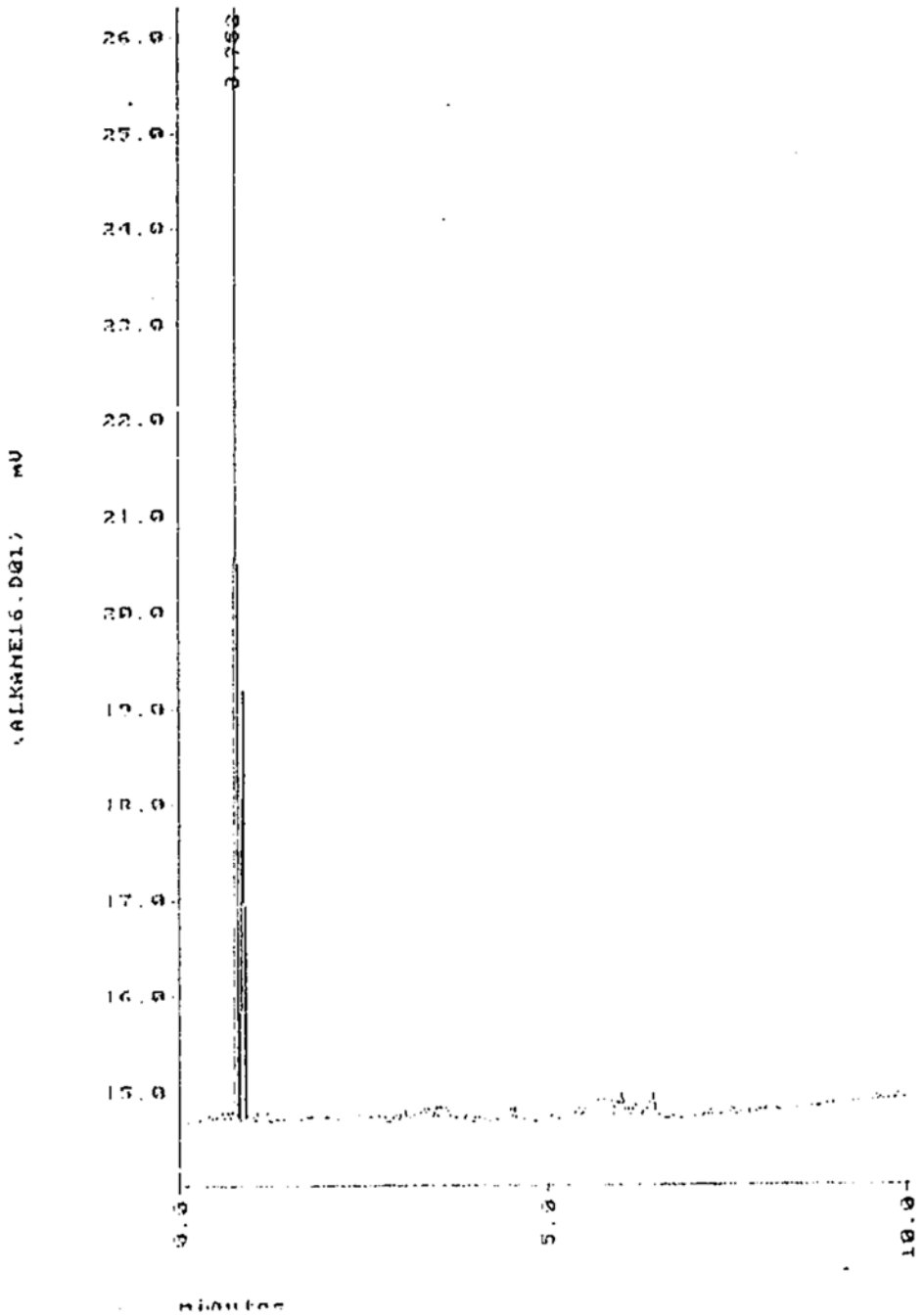
JW-101795-GC#1-J

0.5 µL alkane mixture



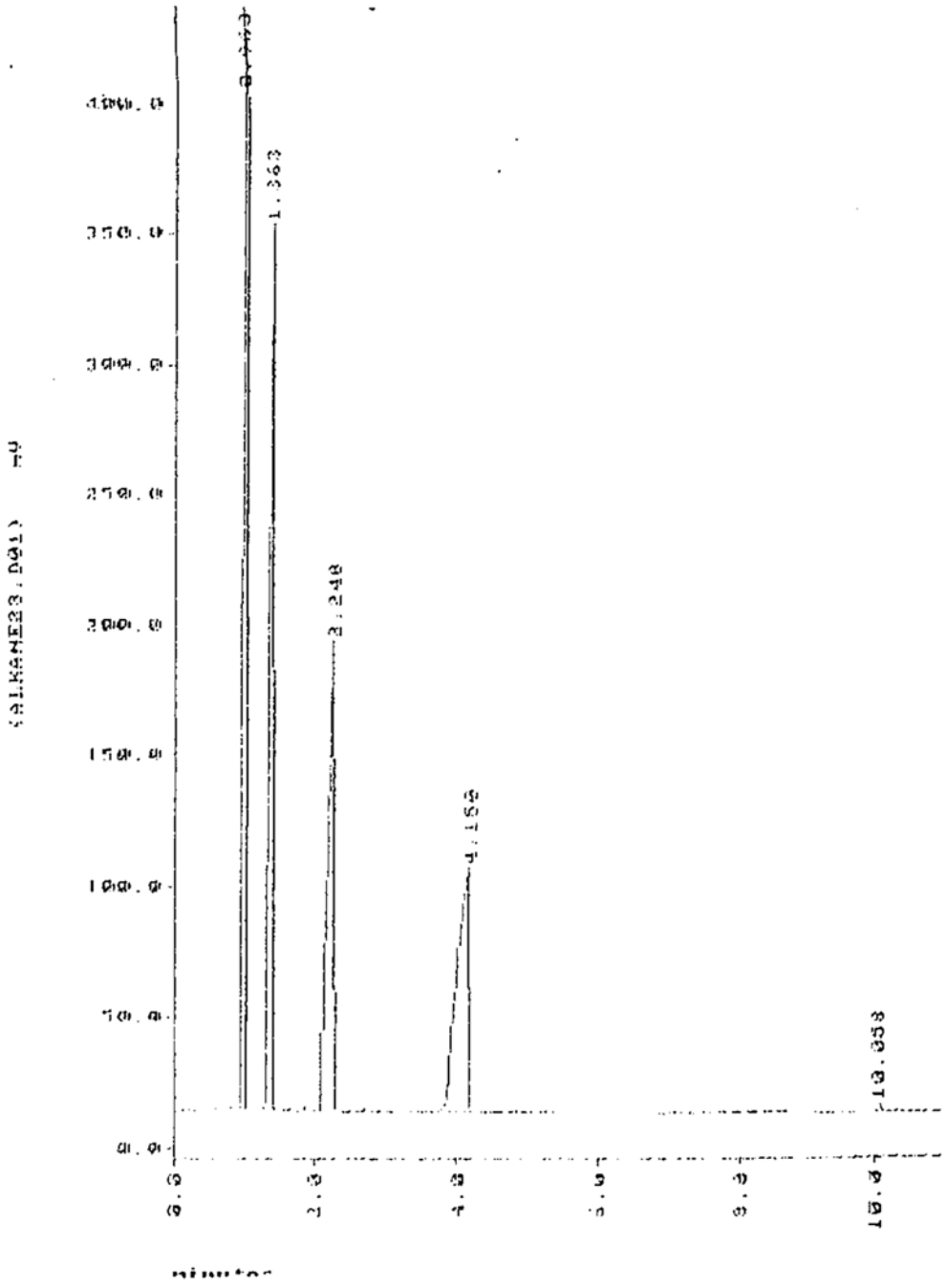
JW-101795-GC#1-I

1.0 µL butane



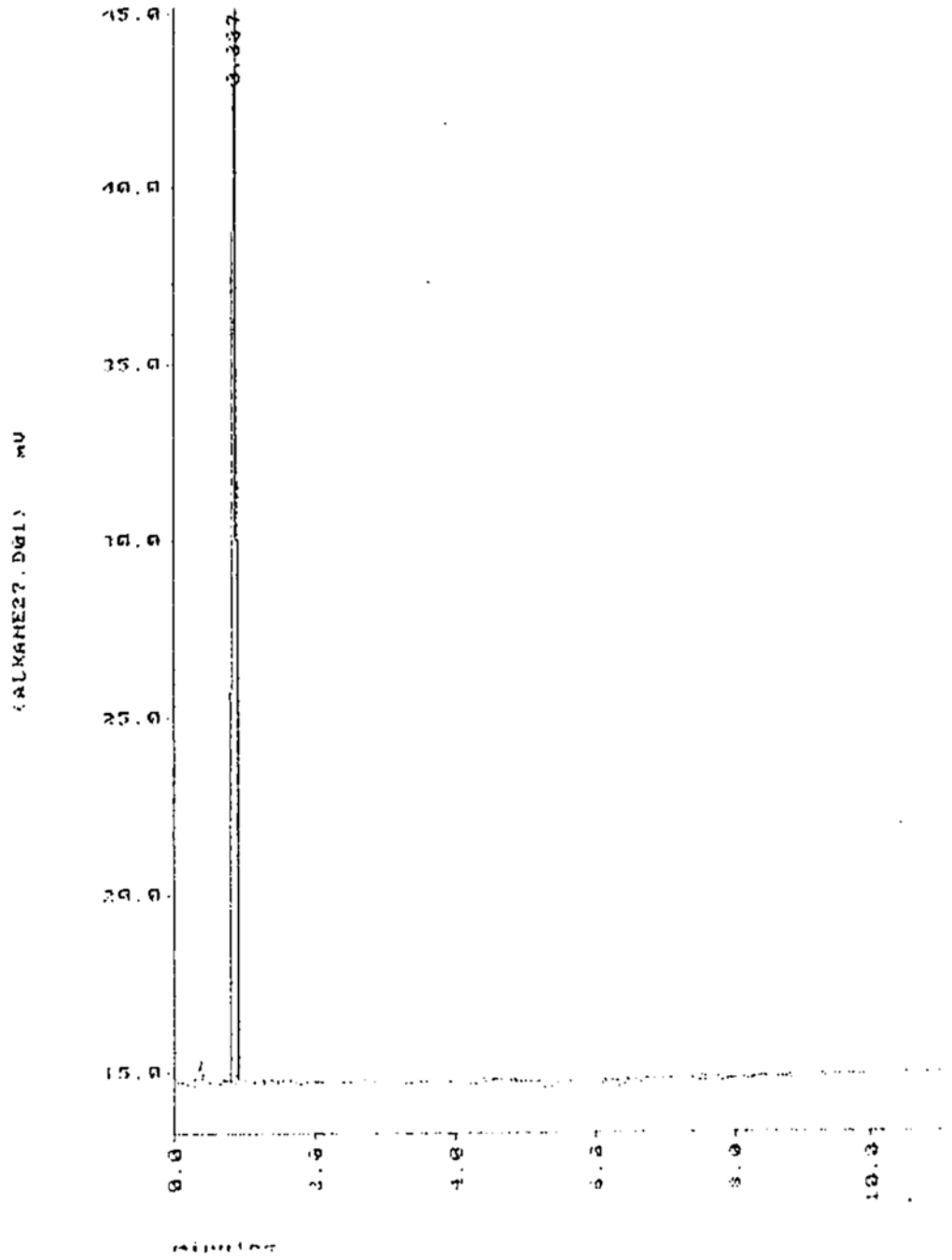
JW-102695-GC#1-C

1.0 µL alcohol mixture



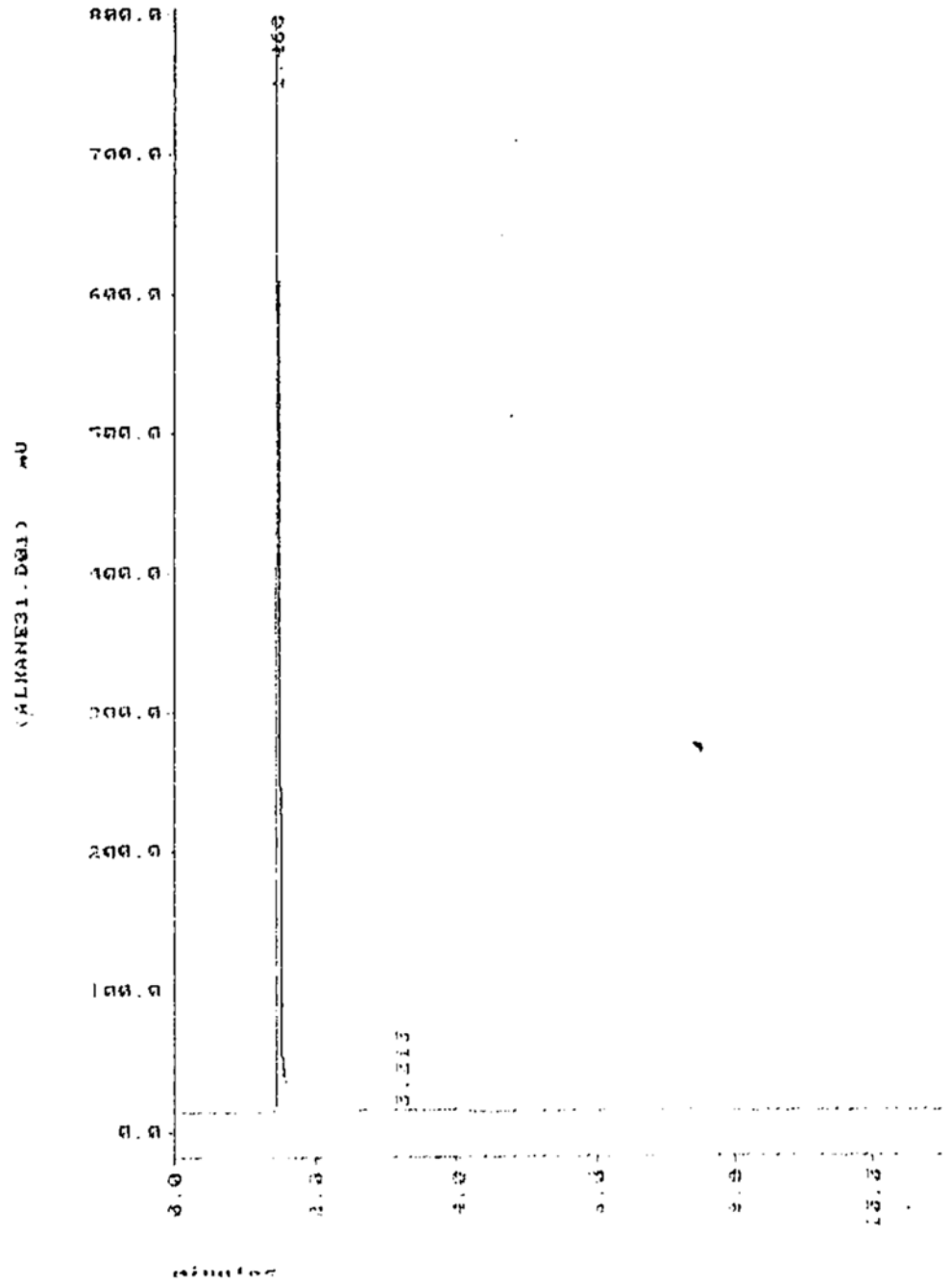
JW-103195-GC#1-B

0.5 µL 2-methyl butane



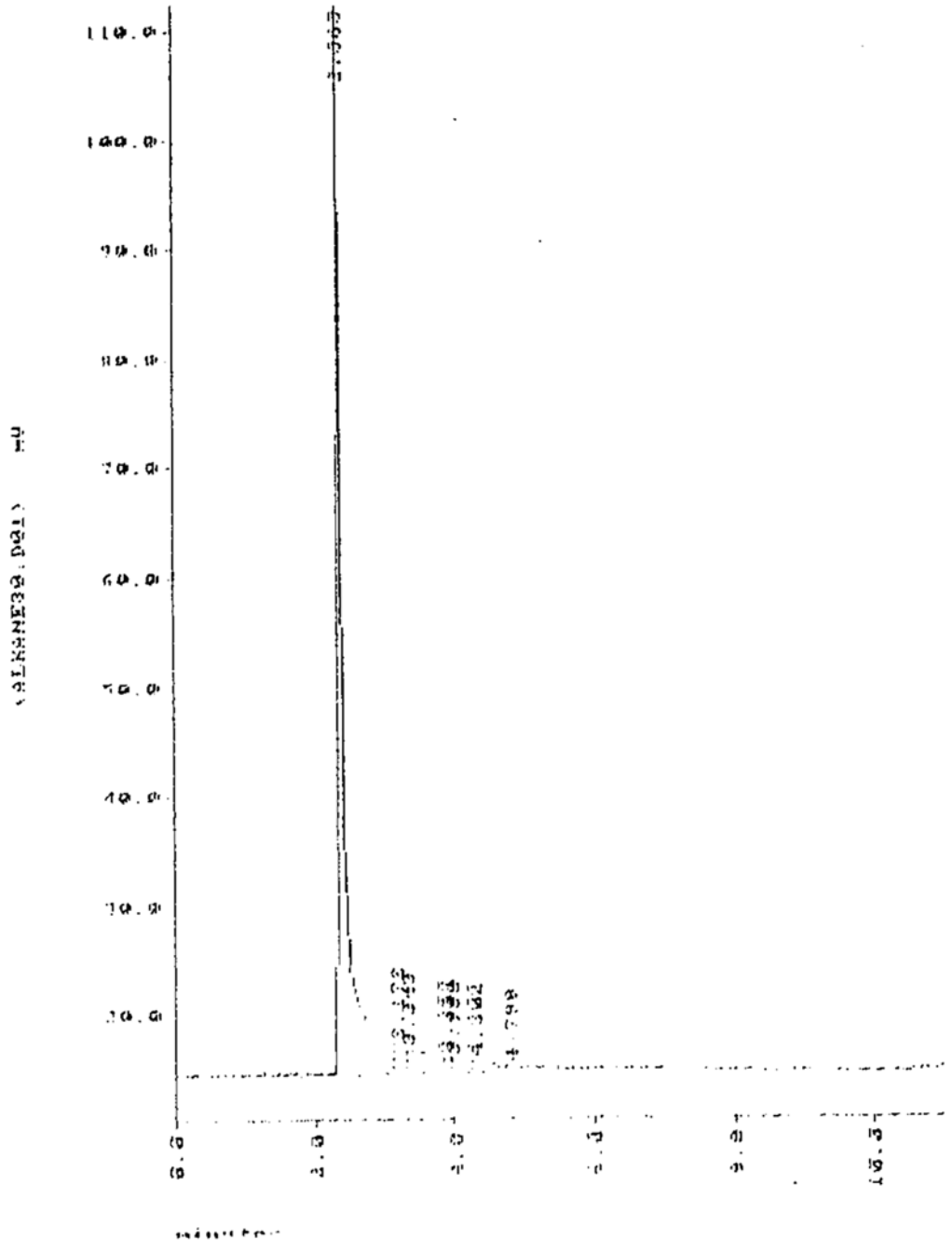
JW-103195-GC#1-F

0.5 μL 2-methyl hexane



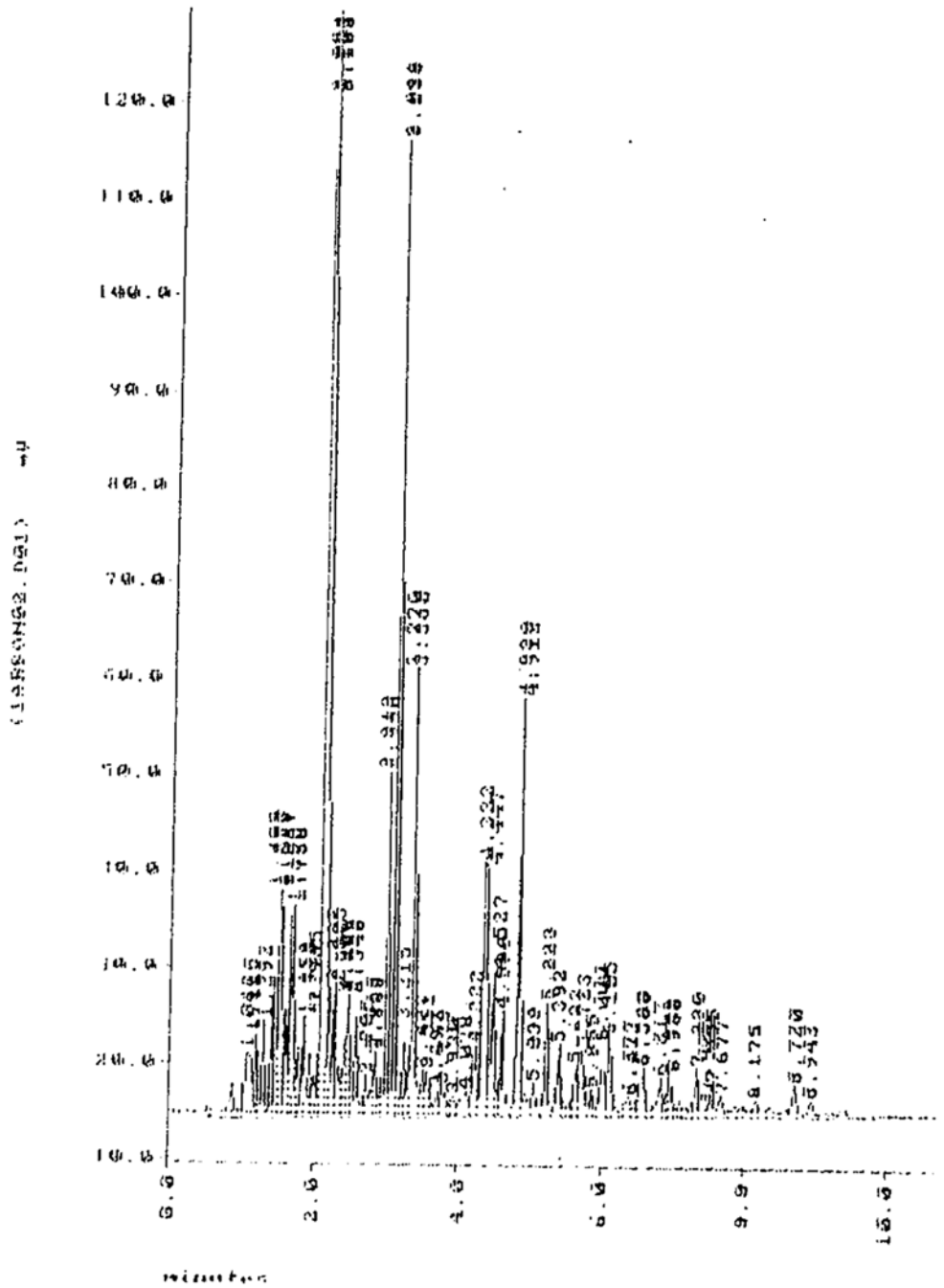
JW-103195-GC#1-E

0.5 μL 2- methyl heptane



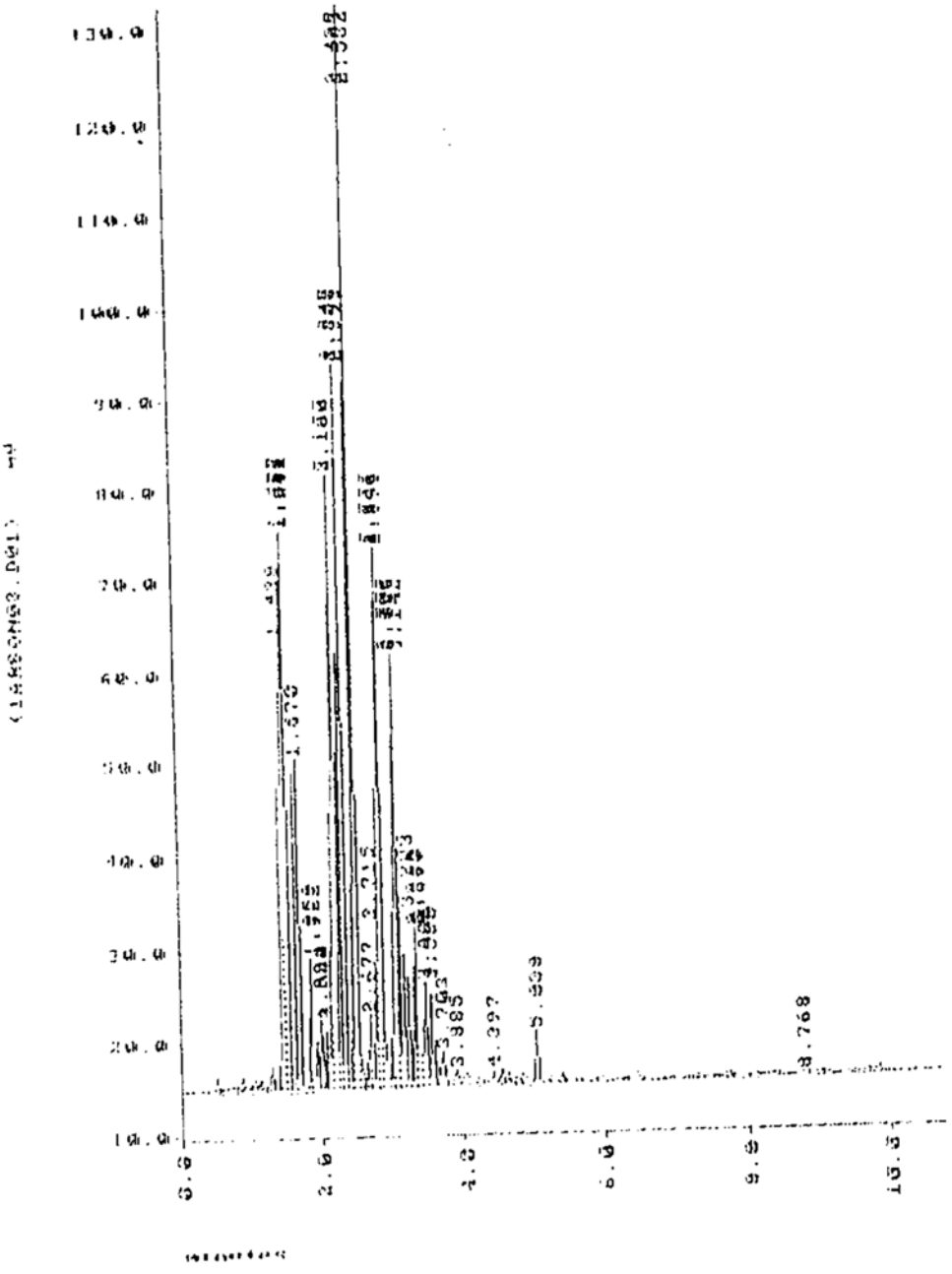
JW-021396-GC#1-A

0.25 µL unleaded gasoline



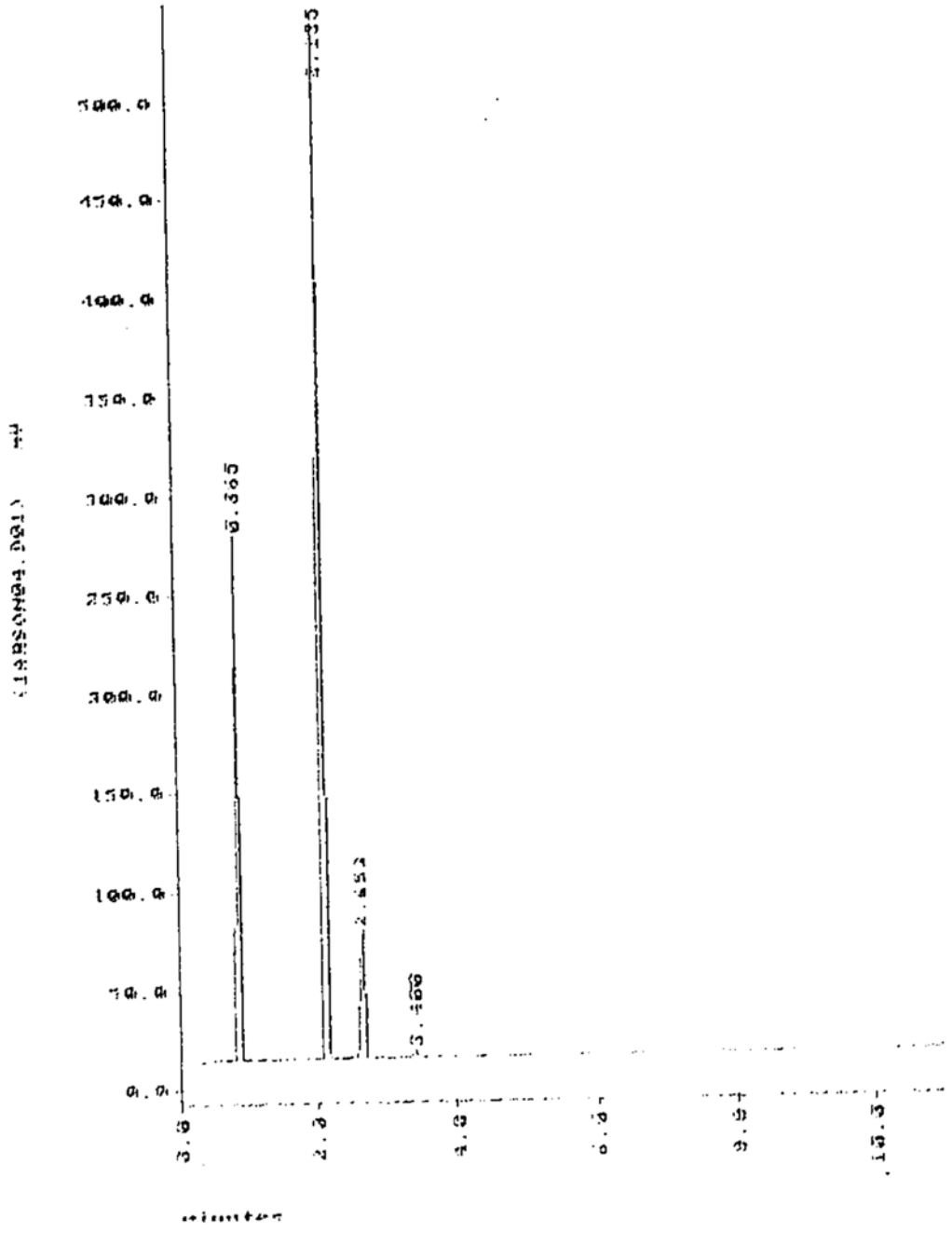
JW-021396-GC#1-B

0.25 µL charcoal lighter fuel



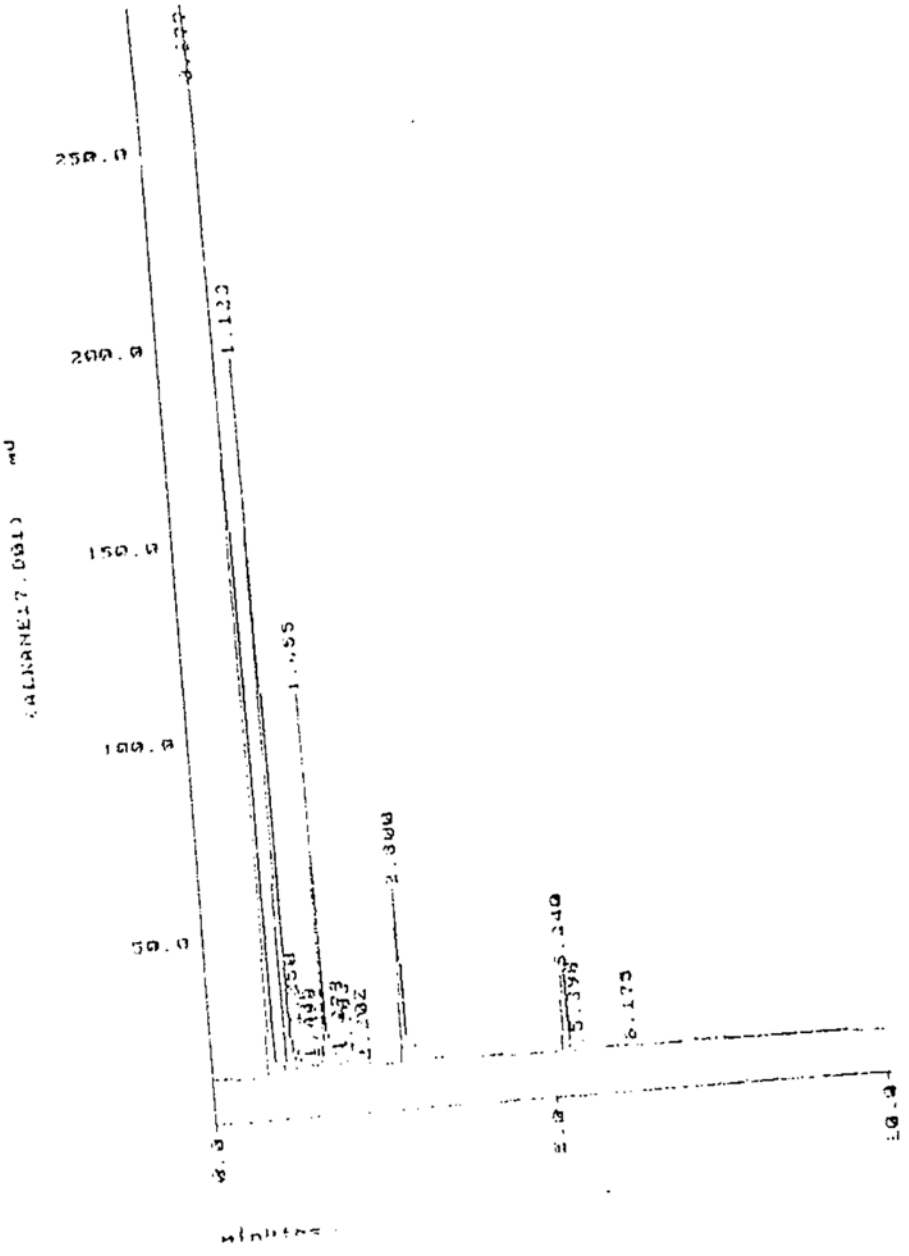
JW-021396-GC#1-C

0.25 µL paint thinner



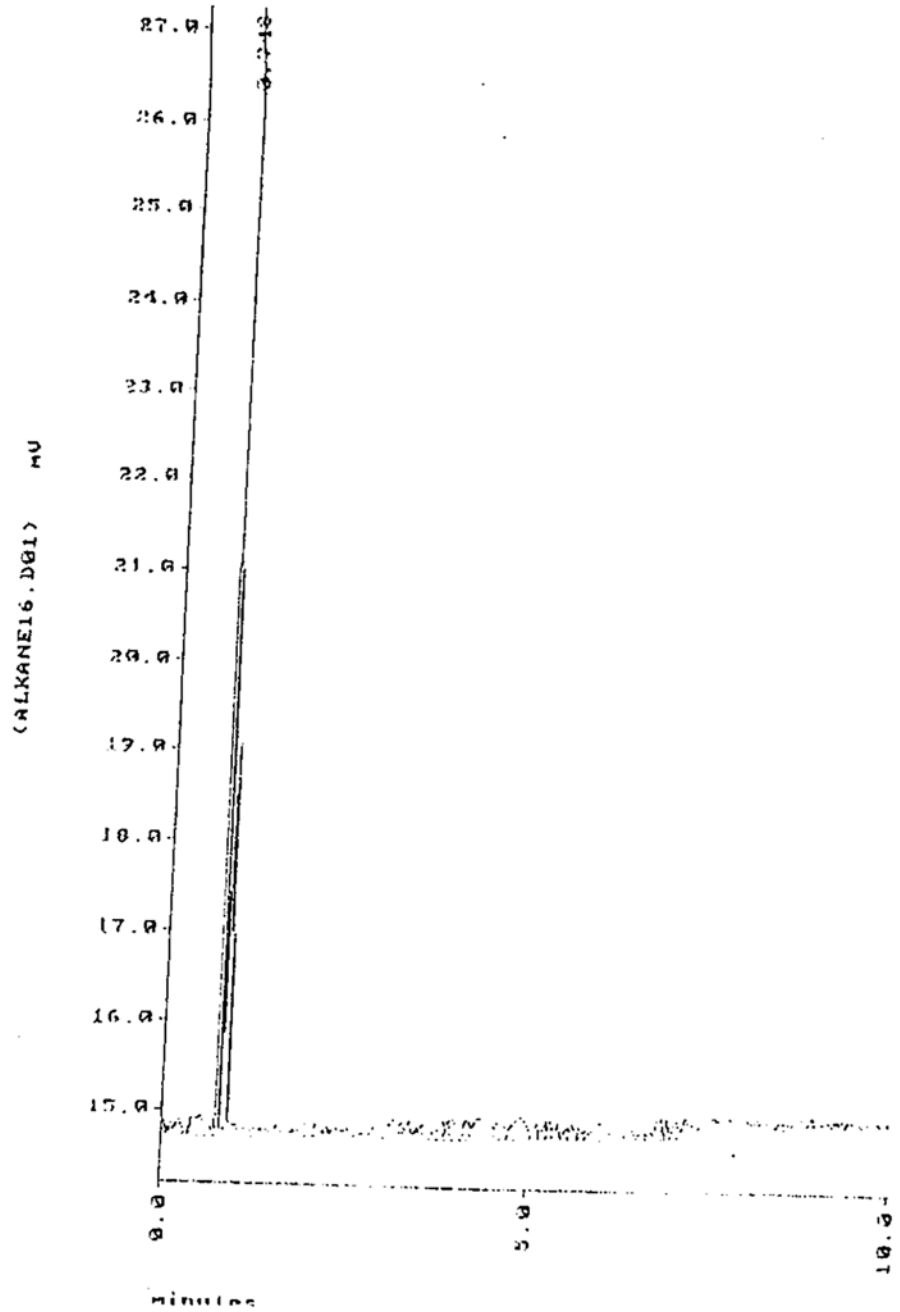
JW-101795-GC#2-J

0.5 μL alkane mixture



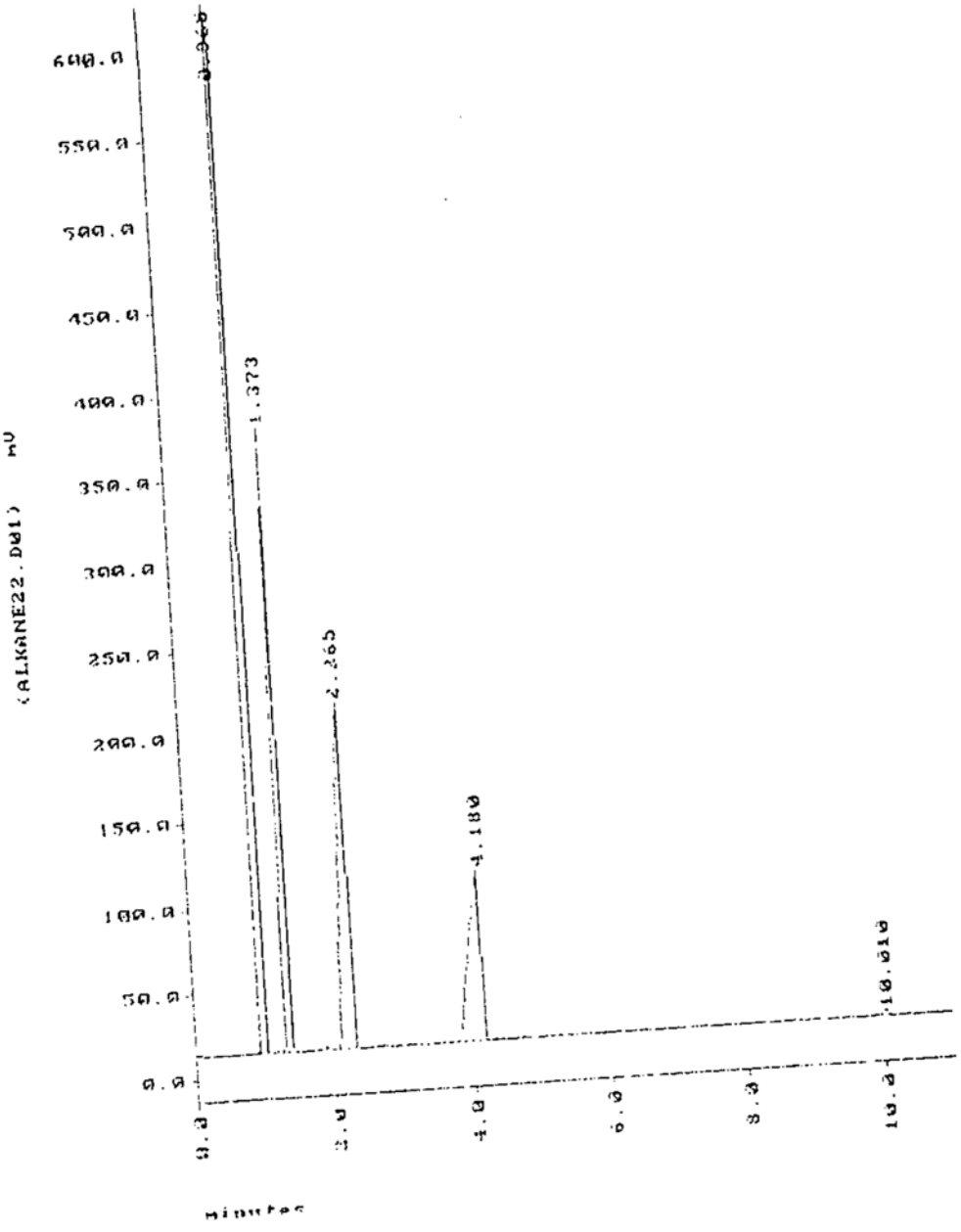
JW-101795-GC#2-I

1.0 µL butane



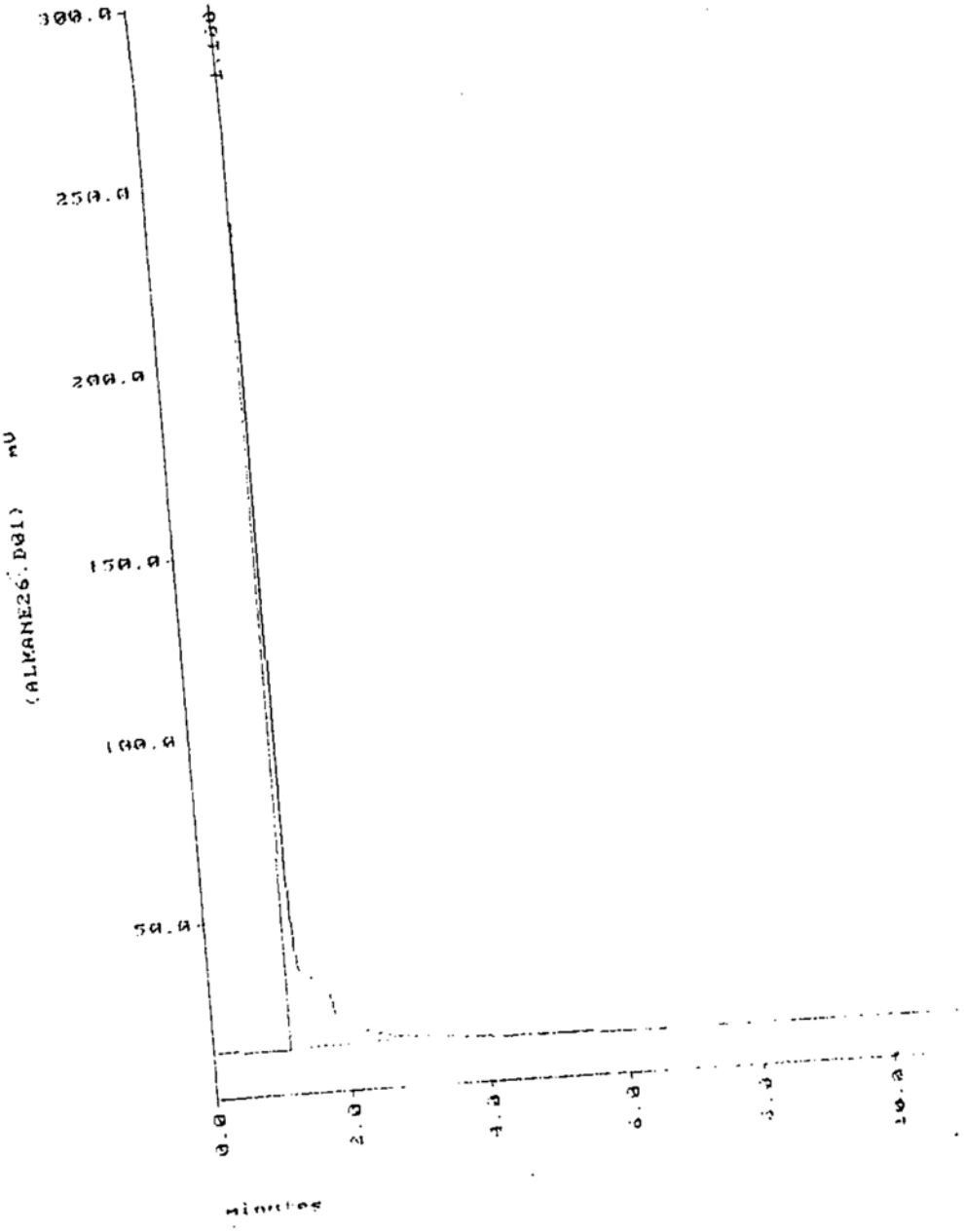
JW-102695-GC#2-C

1.0 µL alcohol mixture



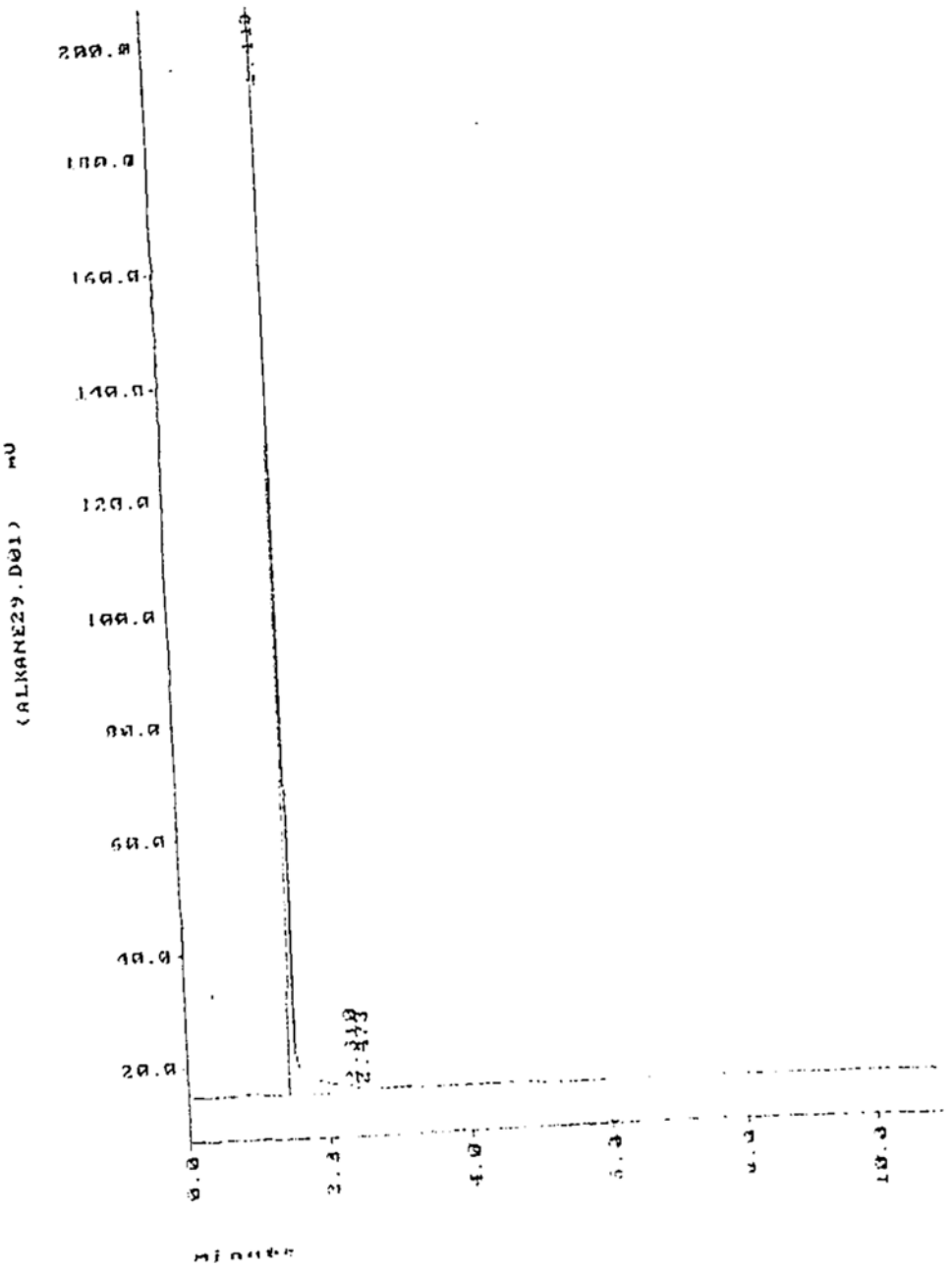
JW-103195-GC#2-B

0.5 μL 2-methyl butane



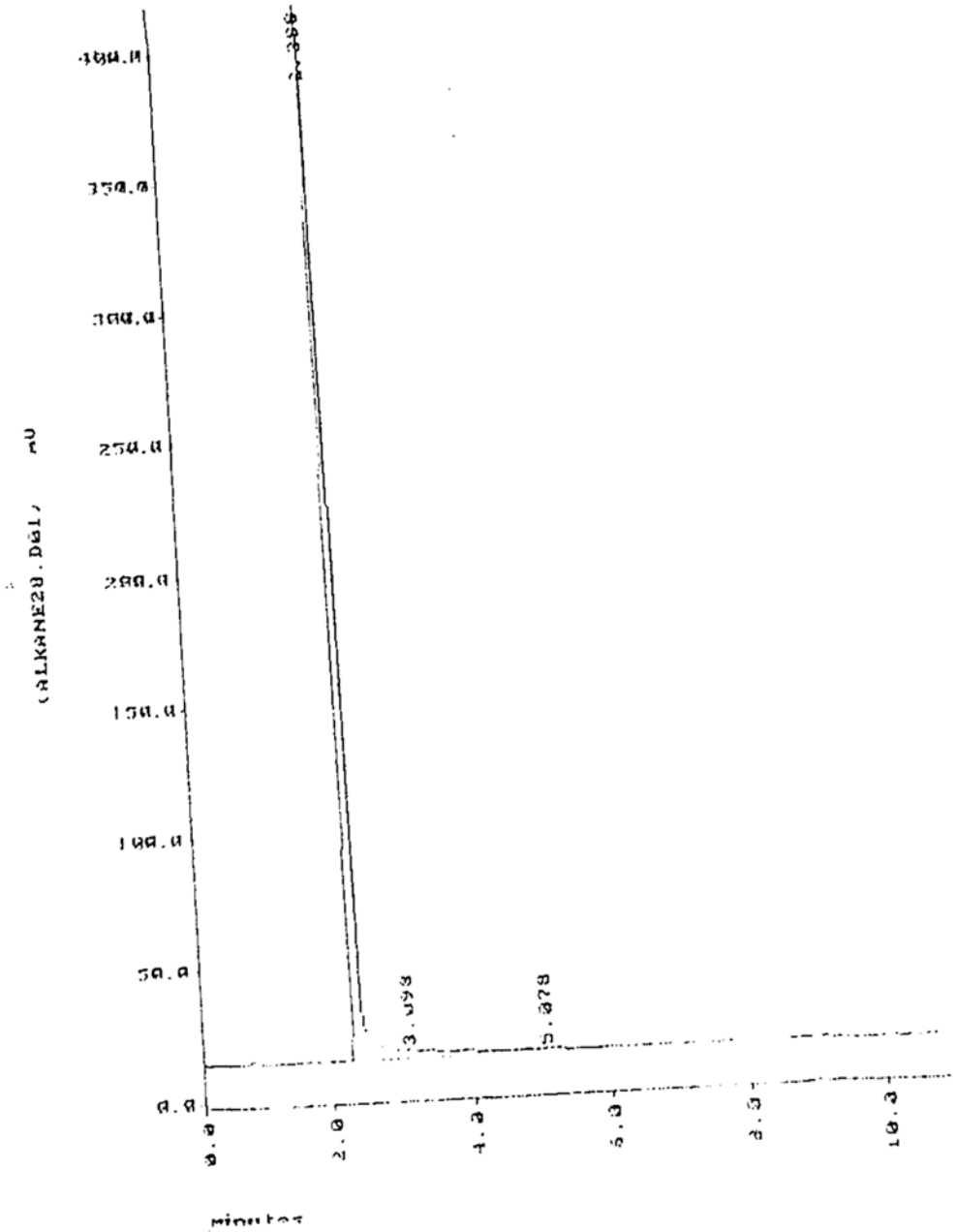
JW-103195-GC#2-F

0.5 μL 2-methyl hexane



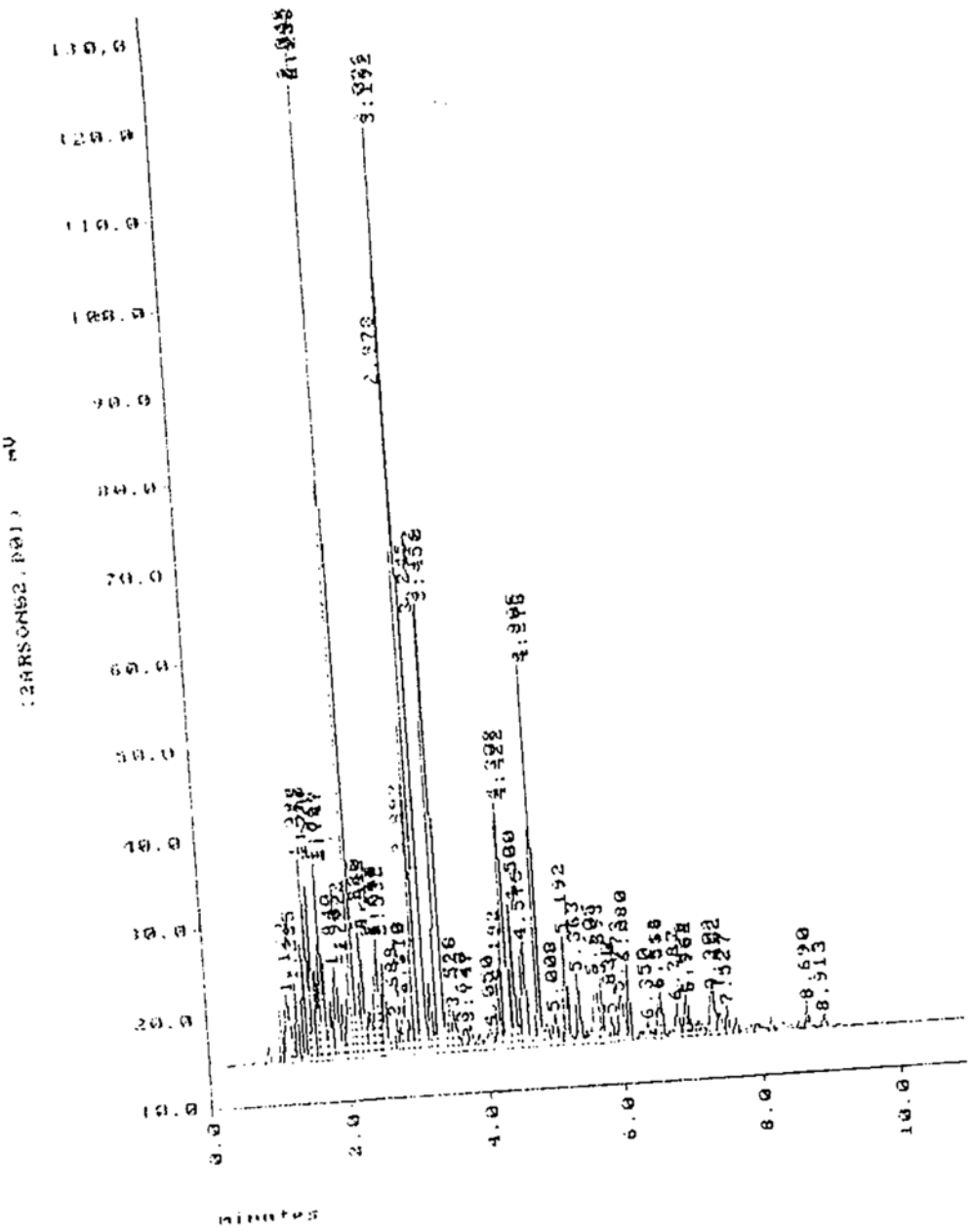
JW-103195-GC#2-E

0.5 μL 2- methyl heptane



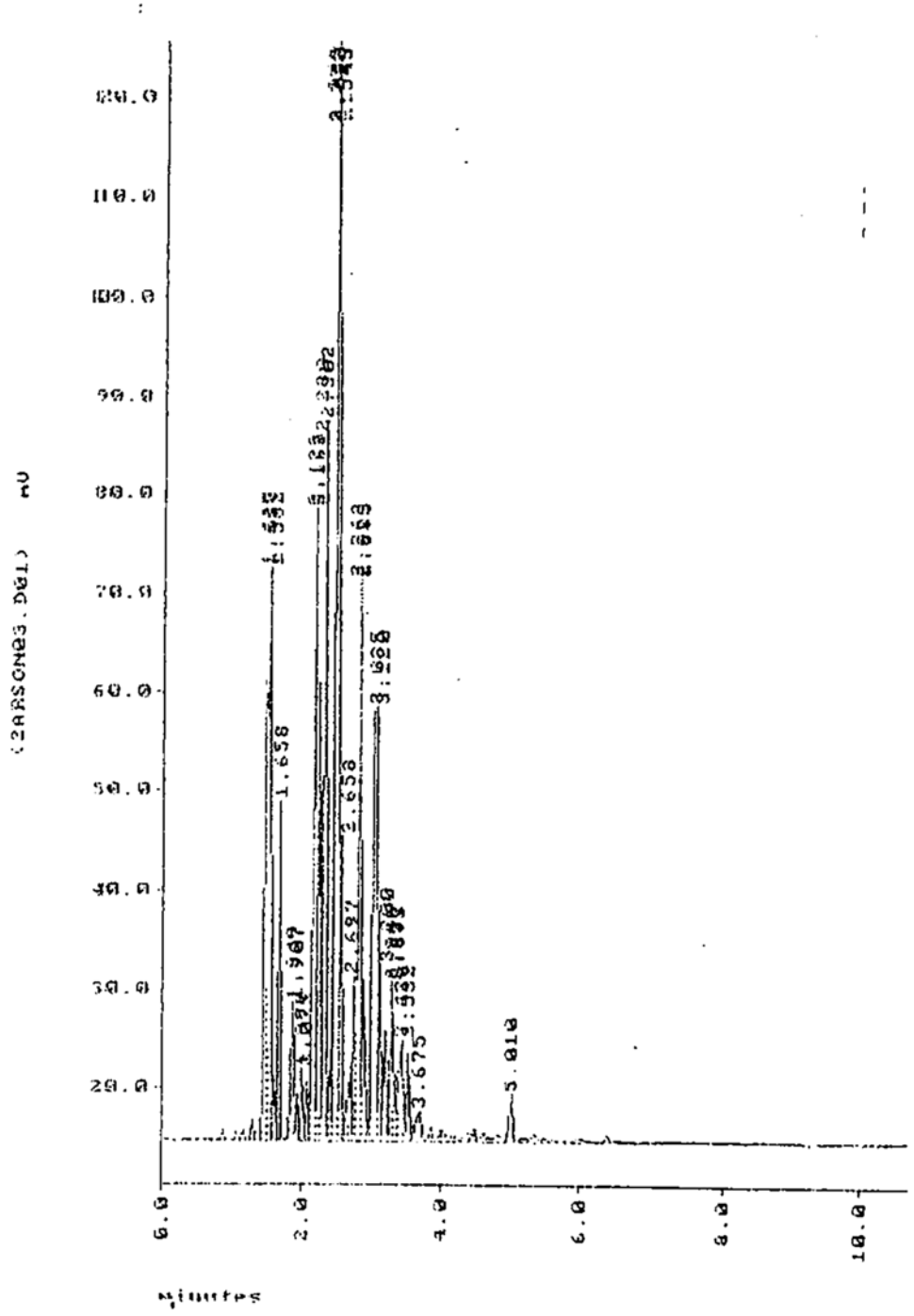
JW-021396-GC#2-A

0.25 µL unleaded gasoline



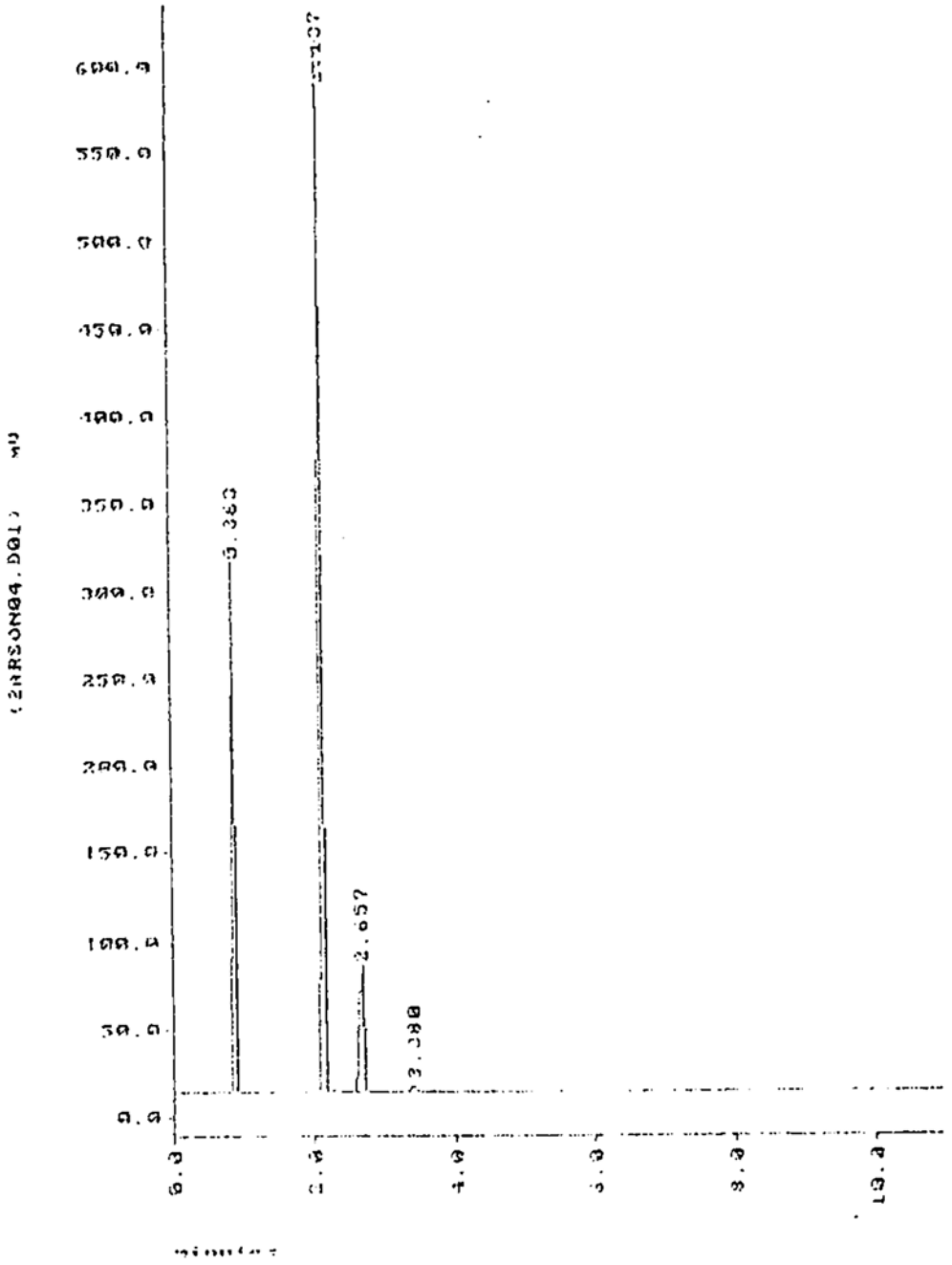
JW-021396-GC#2-B

0.25 µL charcoal lighter fuel



JW-021396-GC#2-C

0.25 µL paint thinner



PREVIOUS

DOCUMENTS

IN POOR

ORIGINAL

CONDITION