PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

Ministry of Superior Studies and Scientific Reasearch

UNIVERSITY CENTER OF EL OUED

INSTITUTE OF SCIENCE AND TECHNOLOGY

In partial fulfillment of the requirements for the degree of:

MASTER OF SCIENCE

Domain : Science and Technique
Field : Procedure Engineering
Specialty : Chemical Engineering

Presented by : MEFTAH Seif Eddine

Thesis:

APOINTING A DATABASE TO THE GC-2014 GAS CHROMATOGRAPH

Presented on the 1st of July, 2012

In front of Jury Composed of:

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2011-2012

What is written without effort is in general read without pleasure

—Samuel Johnson (1709–1784)
Johnsonian Miscellanies
Vol. ii, p. 309

ABSTRACT

As you know, Gas Chromatography equipped with flame ionization detector (GC/FID) has the ability to perform quantitative measurements, and Gas Chromatography equipped with a Mass spectrometer (GC/MS) has the ability to perform qualitative analysis.

In this work we provided a DATABASE for the Gas Chromatograph with flame ionization detector to enable it to perform qualitative analysis along with quantitation.

A mixture of complex volatile compounds was used to evaluate and compare different linear velocities of the carrier gas (N_2) and different temperature programs of column (DB-5) to determine the parameters used to optimize the separation.

After, a series of saturated aliphatic n-Alkanes ($C_{14} - C_{17}$) was injected into the gas chromatograph in the optimized operating conditions to determine their specific retention times and to apply in Kovat's equation and evaluate the quality of unknown compounds situated in that field of retention times.

الملخص

نعلم ان الكروماتوغرافية الغازية المزودة بكاشف التأين باللهب (GC-FID) لها قدرة على التحليل الكمي العالية جدا، اما الكروماتوغرافية الغازية المزودة بكاشف مطيافية الكتلة (GC-MS) لها قدرة على التحليل الكيفي.

في هذا العمل قمـــنا بمحاولة تزويد الكرومـاتوغرافية الغازية المزودة بكاشف التأين باللهب (GC-FID) بقاعدة بيانات و ذلك لكي تكون لها قدرة على التحليل الكمي و الكيفي معا.

تم استخدام مزيج من المركبات الطيارة بعديد من السرعات التدفق الخطية للغاز الحامل (N_2) و في درجة حرارة ثابتة ، و درجة حرارة مبرمجة للعمود (DB-5) قصد اختيار الشروط المناسبة التي تعطينا احسن فصل للمركبات في المزيج.

ثم تم حقن خليط من الألكانات النظامية C_{14} إلى C_{17} في الشروط المناسبة من اجل معرفة ازمنة الاحتفاظ لهذه المركبات و ذلك لتوظيفها في علاقة "كوفاتس" قصد التحليل الكيفي للمركبات التي تتواجد ازمنتها في تلك المجالات.

ACKNOWLEDGMENTS

{ قل إن صلاتي ونسكي ومحياي ومماتي لله رب العالمين لا شريك له
وبذلك أمرت وأنا أول المسلمين }

In the name of Allah, the Most Gracious and the Most Merciful

Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. For nothing would be possible without Him. Without the guidance of God, my Mother El Hadja NAIMA, my fiancé Nariman and all my friends I would not have persevered this far in my life. With their guidance, love, and continual support during both the good and bad times I have grown and now have a wealth of knowledge. For all they have given me I am eternally grateful.

CONTRIBUTORS

Special appreciation goes to my supervisor, Mr. MESBAHI Mohamed Adel, for his supervision and constant support. His invaluable help of constructive comments and suggestions throughout the experimental and thesis works have contributed to the success of this research.

I would also like to thank the other members of the Jury, Mr. BENMEYA Omar and Mr. LEOUINI Salah Eddine, and specially Mr. REBEI Abdel Karim for their advice and assistance through my years here at Tech, and providing me with the necessary knowledge to operate the GCsolution Software.

My acknowledgement also goes the laboratory manager Mr. TLIBA Ali for his special cooperation. Sincere thanks to all my friends that helped me obtain the products I needed for this research especially NEFEIDI Sadok, DADDA Abdelali, KIR Oussama, ABBADI Abd Razak and others for their kindness and moral support during my study. Thanks for the friendship and memories.

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PREFACE

Gas chromatography is a powerful means of performing qualitative and quantitative measurements of complex mixtures of volatile substances, for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water. GC is very accurate if used properly and can measure picomoles of a substance in a 1 µl liquid sample, or parts-per-billion concentrations in gaseous samples.

Due to their versatility and resolution, chromatographic separations of complex mixtures are used for many purposes in academia and industry. If anything, recent developments in the life sciences have increased the interest and need for chromatography be it for quality control, proteomics or the downstream processing of the high value products of modern biotechnology.

In this research you will read about the use of gas chromatography for qualitative analysis of complex mixtures and determining parameters used to optimize the separation, and the necessary instrumentation.

Qualitative analysis by GC is usually done either by coupling with a mass spectrometer (GC/MS) to determine structural data of the unknown, or by determining the retention time of the unknown but to identify it you will need cross to reference it with a DATABASE.

In order to investigate and provide a useful and reliable method for our analysis and because our instrument at the VTRS laboratory was never put to work, i conducted a series of experiments studying the ideal linear velocity of the carrier gas and temperature programing against isothermal chromatography to figure out which was best to use with the carrier gas (N_2) and column (DB-5) at hand. The experiment uses common industrial solvents, and the standardization was done by a series of n-Alkanes (C_{14} to C_{17}).

The equation of Kovats allowed us to use the database of Adams R.P. as reference to our standardization with the n-Alkane Standard, thus to be able to be used as a reference for future analysis and give adequate qualitative results.

PART I

INTRODUCTION TO CHROMATOGRAPHY

You will never do anything in this world without courage; it is the greatest quality of the mind next to honor.

—Aristotle (384 BC – 322 BC)

INTRODUCTION TO CHROMATOGRAPHY

Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture which contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time".

A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel [1].

1.1. Chromatography Theory

Chromatography is a separation method that exploits the differences in partitioning behavior between a **mobile phase** and a **stationary phase** to separate the components in a mixture. Components of a mixture may be interacting with the stationary phase based on charge, relative solubility or adsorption. There are two theories of chromatography, the plate and rate theories [1].

1.1.1. Retention

The retention is a measure of the speed at which a substance moves in a chromatographic system. In continuous development systems like HPLC or GC, where the compounds are eluted with the eluent, the retention is usually measured as the *retention time* R_t or t_R, the time between injection and detection. In interrupted development systems like TLC the retention is measured as the *retention factor* R_f, the run length of the compound divided by the run length of the eluent front:

$$R_f = \frac{distance\ moved\ by\ compound}{distance\ moved\ by\ eluent}$$

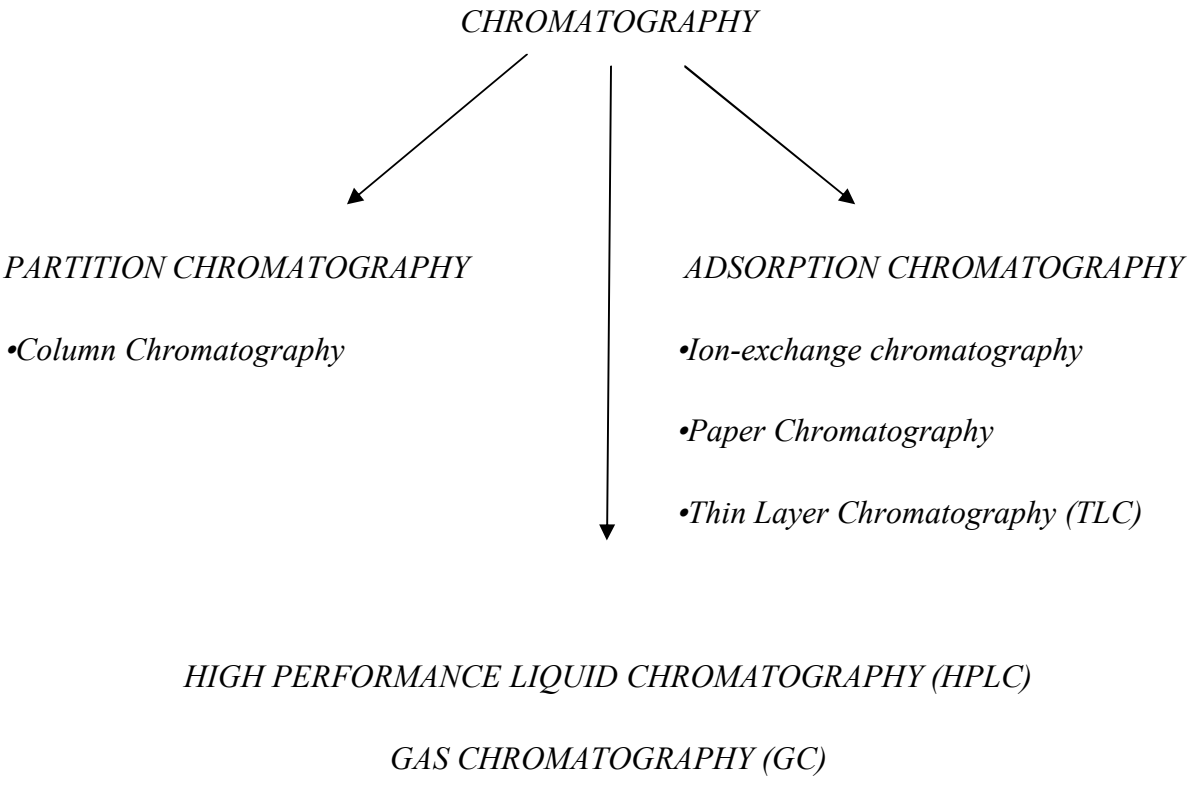
The retention of a compound often differs considerably between experiments and laboratories due to variations of the eluent, the stationary phase, temperature, and the setup. It is therefore important to compare the retention of the test compound to that of one or more standard compounds under absolutely identical conditions.

1.1.2. Plate theory

The plate theory of chromatography was developed by Archer John Porter Martin and Richard Laurence Millington Synge. The plate theory describes the chromatography system, the mobile and stationary phases, as being in equilibrium. The partition coefficient K is based on this equilibrium, and is defined by the following equation:

$$K = \frac{Concentration\ of\ solute\ in\ stationary\ phase}{Concentration\ of\ solute\ in\ mobile\ phase}$$

K is assumed to be independent of concentration, and can change if experimental conditions are changed, for example temperature is increased or decreased. As K increases, it takes longer for solutes to separate. For a column of fixed length and flow, the retention time (t_R) and retention volume (V_r) can be measured and used to calculate K [1].



1.2. Paper chromatography

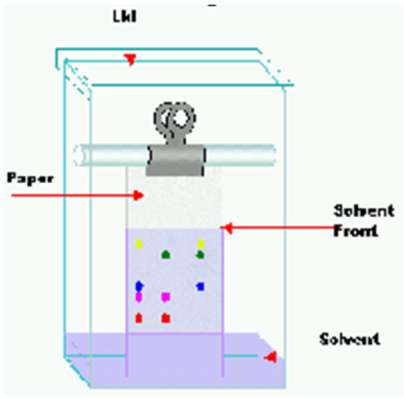


Figure 1.1: Schematic of Paper Chromatography Technique

In paper chromatography, chemical interactions with the paper make compounds travel at different rates. A small spot of solution containing the sample is applied to a strip of *chromatography paper* about one centimeter from the base. This sample is *adsorbed* onto the paper. This means that the sample will contact the paper and may form interactions with it. Any substance that will react with (and thus bond to) the paper cannot be measured using this technique. The paper is then dipped in to a suitable solvent (such as ethanol or water) and placed in a sealed container. As the solvent rises through the paper it meets the sample mixture which starts to travel up the paper with the solvent. Different compounds in the sample mixture travel different distances according to how strongly they interact with the paper. Paper chromatography takes some time and the experiment is usually left to complete for some hours.

The final *chromatogram* can be compared with other known mixture chromatograms to identify sample mixes. *Two-way paper chromatography* involves using two solvents and rotating the paper 90^{o} in-between. This is useful for separating complex mixtures of similar compounds.

1.3. Thin layer chromatography (TLC)

In *thin layer chromatography* or *TLC* the stationary phase consists of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat carrier like a glass plate, a thick aluminum foil, or a plastic sheet.

The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different adsorbents. TLC is a standard laboratory method in organic chemistry. Because of its simplicity and speed TLC is often used for monitoring chemical reactions and for the qualitative analysis of reaction products.

TLC plates are made by mixing the adsorbent with a small amount of inert binder like calcium sulfate (gypsum) and water, spreading thick slurry on the carrier, drying the plate, and activation of the adsorbent by heating in an oven. The thickness of the adsorbent layer is typically around 0.1 - 0.25 mm for analytical purposes and around 1 - 2 mm for preparative TLC [2].

Several methods exist to make colorless spots visible:

- Often a small amount of a fluorescent dye is added to the adsorbent that allows the visualization of UV absorbing spots under a blacklight ("UV_{254}").
- Iodine vapors are a general unspecific color reagent.
- Specific color reagents exist into which the TLC plate is dipped or which are sprayed onto the plate.

Once visible, the spots can be quantified by way of calculating their R_f values. These values should be the same regardless of the extent of travel of the solvent, and in theory are independent of a single experimental run. They do depend on the solvent used, and the type of TLC plate.

1.4. Column Chromatography

Column chromatography is frequently used by organic chemists to purify liquids (and solids). An impure sample is loaded onto a column of adsorbent, such as silica gel or alumina. An organic solvent or a mixture of solvents (the eluent) flows down through the column. Components of the sample separate from each other by partitioning between the stationary packing material (silica or alumina) and the mobile elutant.

In column chromatography, the stationary phase is packed into a glass tube to form a cylinder or **column** of granules. Solvent or buffer can flow freely between the granules Stationary phase may be silica gel or ion exchange resin or a variety of other substances that may have particular affinity for amino acid molecules.

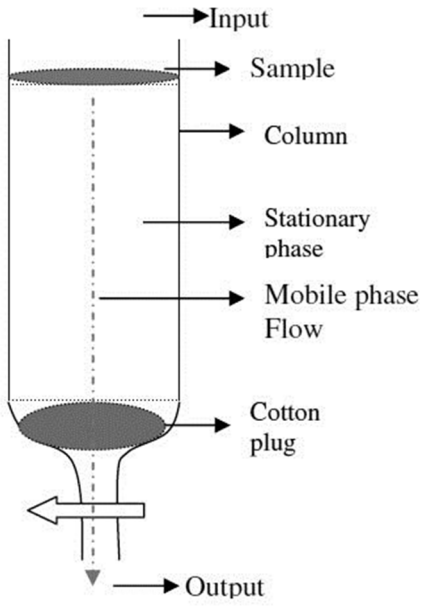


Figure 1.2 Column Chromatography

The sample is applied with care as a layer on top of the stationary phase. Then solvent is added and flows through the column. Samples molecules move while they enter the flowing solvent.

The stationary phase is polar compounds are attracted to the polar column packing by hydrogen bonding or dipole-dipole attractions The more polar component interacts more strongly with the stationary phase. Polar compounds are move slowly. Non-polar compounds are going to come off the column first, while the polar compounds are going to come off the column last.

Usually, one starts will a less polar solvent to remove the less polar compounds, and then slowly increase the polarity of the solvent to remove the more polar compounds. Molecules with different polarity partition to different extents, and therefore move through the column at different rates. The eluent is collected in fractions [2].

1.5. Ion exchange chromatography

Ion exchange chromatography is commonly used in the purification of biological materials. There are two types of exchange: cation exchange in which the stationary phase carries a negative charge, and anion exchange in which the stationary phase carries a positive charge. Charged molecules in the liquid phase pass through the column until a binding site in the stationary phase appears. The molecule will not elute from the column until a solution of

varying pH or ionic strength is passed through it. Separation by this method is highly selective. Since the resins are fairly inexpensive and high capacities can be used, this method of separation is applied early in the overall process [3].

1.6. High performance liquid chromatography (HPLC)

Figure 1.3 *Modern HPLC system*

High performance liquid chromatography, frequently referred to simply as *HPLC*, is a form of column chromatography used frequently in biochemistry. The analyte is forced through a column by liquid at high pressure, which decreases the time the separated components remain on the stationary phase and thus the time they have to spread out within the column, leading to broader peaks. Less time on the column then translates to narrower peaks in the resulting chromatogram and thence to better selectivity (it's easier to differentiate one peak from another) and sensitivity (tall, narrow peaks can be easier to discriminate from noise than shorter, broader peaks). Solvents used include any miscible combination of water or various organic liquids (the most common are methanol or acetonitrile). Often, a gradient over time in the solvent composition passing through the column is used to separate analyte mixtures, as a function of how well the changing solvent composition differentially mobilizes the analyte [2].

1.7 Gas Chromatography

Gas Chromatography (GC) is defined as a physical technique for the separation of volatile and semi-volatile compounds by passing a mobile carrier gas over a stationary phase. Separation occurs as analytes partition in and out of the stationary phase while being carried through the column by the moving mobile phase. The major factor in separation is due to analytes having different affinities for the stationary phase [3].

PART II

GAS CHROMATOGRAPHY

Science is written in this greatest book, which stays open for us ceaselessly before our eyes but no-one can understand it if he does not first learn to understand the language and the letters in which it is written.

—Galileo Galilei (1564-1642)

Saggiatore (Opere VI).

Gas chromatography

Chromatography, in one of its several forms, is the most commonly used procedure in contemporary chemical analysis and the first configuration of chromatography equipment to be produced in a single composite unit and made commercially available was the gas chromatograph. Gas chromatography was invented by A. J. P. Martin who, with R. L. M. Synge, suggested its possibility in a paper on liquid chromatography published in 1941. Martin and Synge recommended that the liquid mobile phase used in liquid chromatography could be replaced by a suitable gas. The basis for this recommendation was that, due to much higher diffusivities of solutes in gases compared with liquids, the equilibrium processes involved in a chromatographic process would be much faster and thus, the columns much more efficient and separation times much shorter. So the concept of *gas chromatography* was envisioned more than fifty years ago, but unfortunately, little notice was taken of the suggestion and it was left to Martin himself and his coworker A. T. James to bring the concept to practical reality some years later in 1951, when they published their epic paper describing the first gas chromatograph

The first published gas chromatographic separation was that of a series of fatty acids, a titration procedure being used, in conjunction with a micro burette, as the detector. The micro burette was eventually automated providing a very effective in-line detector with an integral response. After its introduction by James and Martin, the technique of GC developed at a phenomenal rate, growing from a simple research novelty to a highly sophisticated instrument, having a multi-million dollar market, in only 4 years. The gas chromatograph was also one of the first analytical instruments to be associated with a computer which controlled the analysis, processed the data and reported the results.

A more sophisticated form of the gas chromatograph was constructed by James and Martin and described by James in 1955. The instrument was a somewhat bulky device with a straight packed column, 3 ft. long, that was held vertically and thermostatted in a vapor jacket. Initially, the detector was situated at the base of the column and consisted of the automatic titrating device, the separation was presented as a chromatogram in the form of a series of steps, the height of each step being proportional to the mass of solute eluted. The apparatus was successfully used to separate some fatty acids, but the limited capability of the device to sense only ionic material motivated Martin to develop a more versatile detector, the Gas Density Balance.

The gas density balance was the first detector with a truly catholic response that was linearly related to the vapor density of the solute and consequently its molecular weight. The gas density balance had a maximum sensitivity (minimum detectable concentration) of about 10^{-6} g/ml at a signal to noise ratio of two. This detector inspired the invention of a wide range of detectors over the next decade providing both higher sensitivity and selective response.

The modern gas chromatograph is a fairly complex instrument mostly computer controlled. The samples are mechanically injected, the analytical results are automatically calculated and the results printed out, together with the pertinent operating conditions in a standard format. However, the instrument has evolved over many years although the majority of the added devices and techniques were suggested or describe in the first three international symposia on gas chromatography held in 1956, 1958 and 1960 [4] .

Since then, development has continued making the best use of the extreme sensitivity, versatility, the possibilities of automation and the ease with which new analyses can be developed. Because separation of compound mixtures on the column occurs while they are in the gaseous state, solid and liquid samples must first be vaporized. This represents, without hesitation, the greatest constraint of gas phase chromatography and weighs against it, since its use is limited to the study of thermo stable and sufficiently volatile compounds. However, the applications are numerous in all domains and the development of high speed or multidimensional gas chromatography make this technique even more attractive. It’s very great sensitivity permits detection of quantities of the order of pictograms for certain compounds.

2.1 Components of a GC installation

A gas chromatograph is composed of several components within a special frame. These components include the injector, the column and the detector, associated with a thermostatically controlled oven that enables the column to attain high temperatures (Figure 2.1 and 2.2). The mobile phase that transports the analytes through the column is a gas referred to as the carrier gas. The carrier gas flow, which is precisely controlled, enables reproducibility of the retention times [5].

Analysis starts when a small quantity of sample is introduced as either liquid or gas into the injector, which has the dual function of vaporizing the sample and mixing it with the gaseous flow at the head of the column. The column is usually a narrow-bore tube which coils around itself with a length that can vary from 1to over 100 m, depending upon the type and the contents of the stationary phase. The column, which can serve for thousands of successive injections, is housed in a thermostatically controlled oven. At the end of the column, the mobile phase (carrier gas) passes through a detector before it exits to the atmosphere.

In GC there are four operational parameters for a given stationary phase: L, length of the column, μ, velocity of the mobile phase (which affects the theoretical efficiency N), T, temperature of the column and β, phase ratio, which affects the retention factor k. The operating conditions of the chromatograph allow modifications in terms of T and μ and therefore affects both the efficiency of the column and the retention factors.

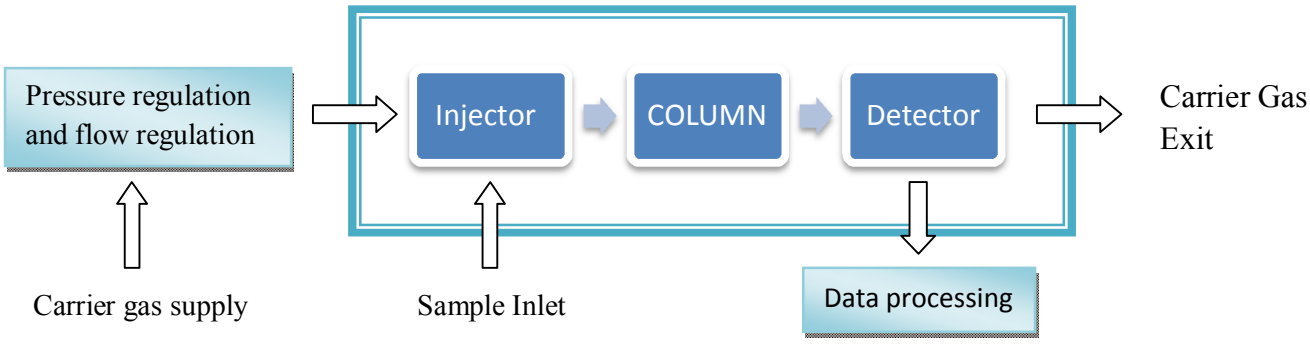


Figure 2.1 *Schematic of a Gas Chromatograph.*

Figure 2.2 *A commercial gas chromatograph (Shimadzu GC – 2014 courtesy of the VTRS laboratory) with a flame ionization detector.*

2.2 Carrier Gas and Flow Regulation

The mobile phase is a gas (helium, hydrogen or nitrogen), either drawn from a commercially available gas cylinder or obtained, in the case of hydrogen or nitrogen, from an on-site generator, which provides gas of very high purity. The carrier gas must be free of all traces of hydrocarbons, water vapor and oxygen, because all of these may deteriorate polar stationary phases or reduce the sensitivity of detectors. For these reasons the carrier gas system includes filters containing a molecular sieve to remove water and a reducing agent for other impurities. The nature of the carrier gas has no significant influence upon the values of the partition coefficients K of the compounds between the stationary and mobile phases, owing to an absence of interaction between the gas and solutes. By contrast, the viscosity of the carrier gas and its flow rate have an effect on the analytes' dispersion in the stationary phase and on their diffusion in the mobile phase, and by consequence upon the efficiency N and the sensitivity of

detection (Figure 2.3). Hydrogen is the carrier gas of choice. If this gas cannot be used for safety reasons, helium may be substituted [5].

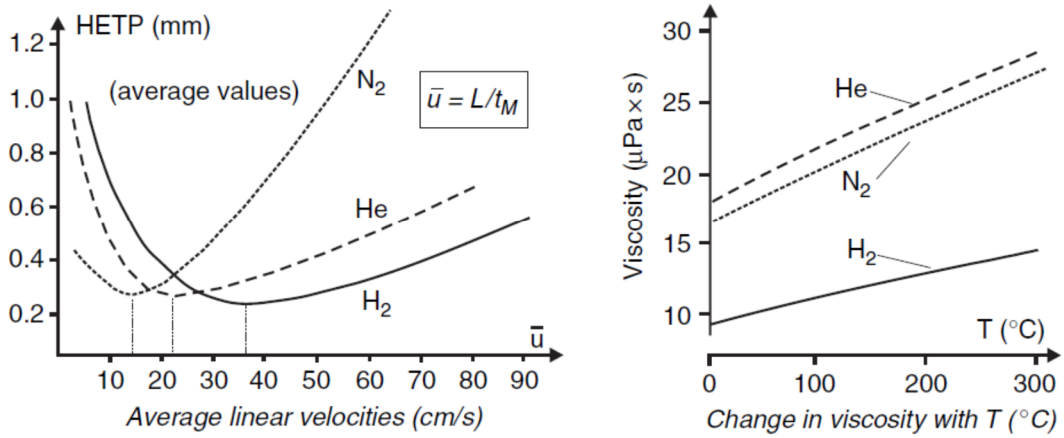


Figure 2.3 *Efficiency as a function of the linear velocity of the carrier gas – viscosities of carrier gases.*

These are typical Van Deemter curves showing that hydrogen, of the three gases studied under the same conditions, allows a faster separation, conveying a greater flexibility in terms of the flow rate, which is very useful for temperature programming. Note the increase in the viscosity of these gases with the temperature and that helium is more viscous than nitrogen at the same temperature.

2.3 Sample introduction and the injection chamber

2.3.1 Sample Introduction

The most common injection method is where a micro syringe is used (Figure 2.4) to inject a very small quantity of sample in solution (e.g. 0.5 µL), through a rubber septum into a flash vaporizer port at the head of the column [4].

Figure 2.4 *Typical syringes used in GC (SGE 5 µl courtesy of VTRS laboratory).*

2.3.2 Injectors

The injector, which is the sample's entrance to the chromatograph, has different functions. Besides its role as an inlet for the sample, it must vaporize, mix with the carrier gas and bring about the sample at the head of the column. The characteristics of the injectors, as well as the modes of injection, differ according to column type. The use of an automatic injection system can significantly enhance measurement precision [6].

Direct vaporization injector

For packed and megabore columns, which typically use a flow rate of about 10 mL/min, direct vaporization is a simple way to introduce the sample. Any model of this type comprises a metal tube with a glass sleeve (called the insert). It is heated to the average boiling temperature of the compounds being chromatographed. The needle of the micro-syringe containing the sample pierces the septum, made of silicone rubber, which closes the end of the injector. The other end, also heated, is connected directly to the column (Figure 2.5). Once the entire liquid plug has been introduced with a syringe it is immediately volatilized and enters the column entirely within a few seconds, swept along by the carrier gas.

Split/splitless injector

For capillary columns able to handle only a small capacity of sample, even the smallest volume that it is possible to inject with a micro-syringe (0.1 µL), can saturate the column. Special injectors are used which can operate in two modes, with or without flow splitting (also called split or splitless).

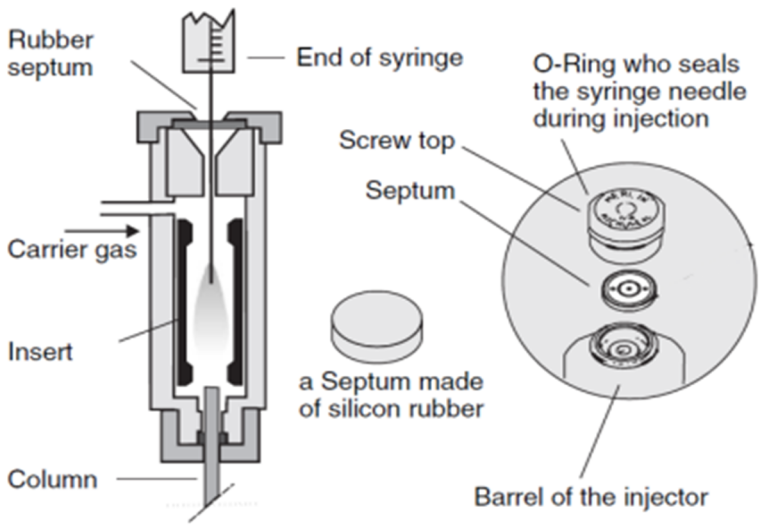


Figure 2.5 *Direct vaporization injector used for packed columns.*

Typical schematic of a model employing a septum. Depending upon the function required a wide range of inserts exist [7].

In the split mode a high flow rate of carrier gas arrives in the vaporization chamber where it mixes with the vapors of injected sample (Figure 2.5). A vent valve, regulated at between 50-100 mL/min, separates this flow into two fractions, of which the largest portion is vented from the injector, taking with it the majority of the sample introduced. The split ratio typically varies between 1 : 20 and 1 : 500. Only the smallest fraction, containing an amount of sample equal to the ratio of division, will penetrate into the column.

$$Split\ ratio = \frac{(split\ outlet\ flow\ rate + column\ outlet\ flow\ rate)}{column\ outlet\ flow\ rate} \qquad (2.1)$$

When working with capillary columns, this type of injector is also used for very dilutes samples in the splitless mode. In this mode a smaller volume of solution is injected very slowly from the micro-syringe during which bleeding valve is maintained in a closed position for 0.5 to 1 minute in order that the vaporized mixture of compounds and carrier solvent are concentrated in the first decimeter of the column. The proper use of this mode of injection, which demands some experience, requires a program that starts with a colder temperature in order that the solvent precedes the compounds onto the column. The re-opening of valve 2 provides an outlet for an excess of solvent-diluted sample. Some less volatile compounds are eliminated and that can interfere with the results of the analyses.

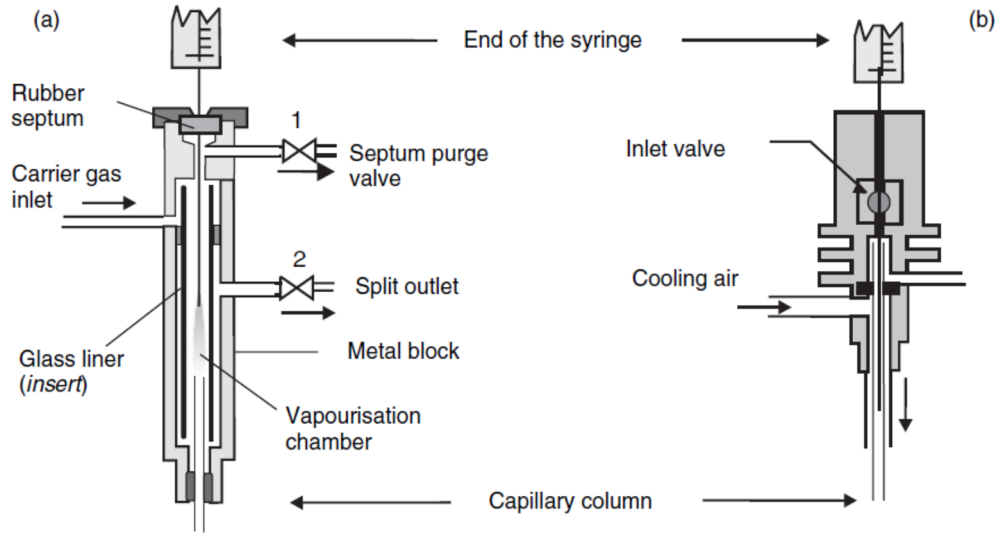


Figure 2.6 *Injectors.*

(a) Above left, injection chamber. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). A proportion of carrier gas (1) flows upward and purges the septum, another (2) exits through the split outlet (a needle valve regulates the split) and finally a proportion passes onto the column [5].

(b) Above right, cold injection onto the column.

Cold on-column injection

This is not a vaporization technique. The sample is deposited directly into the capillary column ('cold on-column' or COC). A special micro-syringe, whose needle (steel or silica) is of 0.15mm diameter, is necessary for penetrating the column which is cooled to 40 ^{o}C before being allowed to return to its normal operating temperature. This procedure, useful for thermally labile compounds or high boiling compounds, is difficult to master without the aid of an autosampler. It is known not to discriminate between compounds of different volatilities.

2.4 Thermostatically Controlled Oven

The gas chromatograph comprises an oven with sufficient volume to hold one or two columns easily and which can heat up to more than 400 ^{o}C. A weak thermal inertia permits a rapid but controlled temperature climb (gradient able to attain100 ^{o}C/ min). The temperature must be controlled to within 0.1 ^{o}C in order to get reproducible separations in isothermal or temperature programmed modes [5].

2.5 Columns

There are two column types, which differ in their performance: packed columns and capillary columns. For packed columns the stationary phase is deposited or bonded by chemical reaction onto a porous support. For capillary columns a thin layer of stationary phase is deposited onto, or bound to the inner surface of the column [12].

2.5.1 Packed Columns

These columns, less commonly used today, have diameters of 3.18 and 6.35mm and a length of between 1 - 3m (Figure 2.7). Manufactured from steel or glass, the internal wall of the tube is treated to avoid catalytic effects with the sample. They can withstand a carrier gas flow rate

within the range 40-100 mL/min. They contain an inert and stable porous support on which the stationary phase can be impregnated or bounded [12].

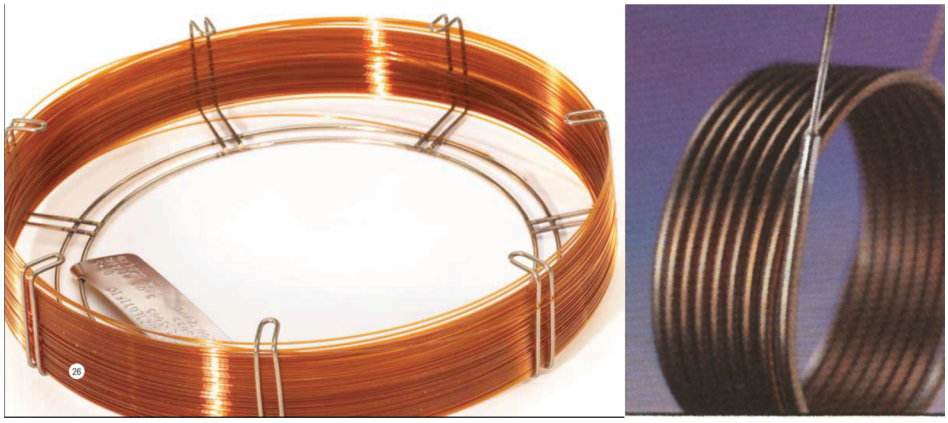


Figure 2.7 *Examples of packed (right) and capillary (left) columns.*

Although the performance of packed columns is more modest than capillary columns, they are still usually employed for many routine analyses. Easy to manufacture and with a large choice of stationary phases available, they are not however, well adapted to trace analyses.

2.5.2 Capillary Columns (open tubular)

They are usually made of the highest purity *fused silica* obtained by the combustion of tetrachlorosilane ($SiCl_4$) in an oxygen-rich atmosphere. The internal diameter of the tube used for these columns varies from 100 to 530 µm, its thickness is 50 µm and the length is of 12 to 100 m. These columns are rendered flexible by the application of a polyimide outer coating, a thermally stable polymer (T_{max} = 370 ^{o}C) or a thin aluminium film. They have the advantages of physical strength and can be wound into coils around a lightweight metallic circular support. Some manufacturers offer columns made from a metal capillary (aluminium, nickel or steel) which tolerates high operating temperatures of the order of 450 ^{o}C, providing that the stationary phase is stable enough. The internal surface of the column is usually treated to favor a regular bonding for the stationary phase. This could be a chemical treatment (HCl at 350 ^{o}C), or the deposit of a thin layer of alumina or silica gel depending on the technique used to bond the stationary phase. The thickness can vary between 0.05 and 5 µm. These are WCOT (*wallcoated open tubular*) or PLOT (*porous layer open tubular*) columns depending upon the nature of the stationary phase employed. Covalent bonding via Si–O– Si–C allows organic compounds to be bound to the silica surface. Columns are particularly stable and can be rinsed periodically with solvents which enable them to recover their initial performance quality [12].

The* '530*μ*m'*column, Constituted from a capillary of 0.53mm internal diameter with length varying from 5 to 50 m, these columns are designated, depending on the supplier, by their principal characteristic. They require a carrier gas flow rate of at least 5 mL/min and can be as high to 15 mL/min, close to that used in packed columns. However, the resolution and performance of these columns are lower than that of capillary columns of smaller diameter. It is possible to replace a packed column with a '530' column on the older chromatographs, while retaining the same injectors, detectors and flow rates. Their main advantage over packed columns is their lack of bleeding, i.e. the progressive loss of the stationary phase with time. These columns are rarely packed columns.

In order to deposit a film of known thickness, a method consists to fill the column with a solution of stationary phase of known concentration (e.g. 0.2 per cent in ether) so that the desired thickness is obtained after evaporation of solvent. This layer can then be reticulated by peroxide or by γ irradiation. The procedure is similar to the application of a dye on a surface that has been treated beforehand to obtain a firm attachment.

2.6 Stationary Phases

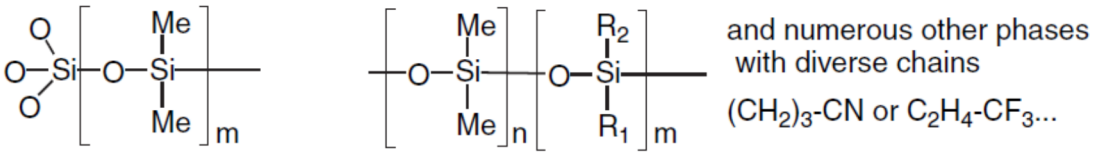
For packed columns, for which impregnation techniques are very simple, over 100 stationary phases of various types have been proposed in the literature. On the other hand, for bonded phase capillary columns the choice of stationary phase is limited because the generation of the film at the surface of the column requires a different principle than impregnation. The current phases correspond in principle to two families: the polysiloxanes and the polyethylene glycols. Each category can be the object of minor structural modifications.

The stationary phases described below are the more classical types, while in the catalogues, phases especially adapted to particular applications can be found.Among them are phases for the separation of sulfur products, chlorinated pesticides,permanent gases, aldehydes, polycyclic aromatic hydrocarbons (PAH), etc [12].

2.6.1 Polysiloxanes

Polysiloxanes (also known as silicone oils and gums) are based upon a repetitive backbone that consists of two hydrocarbon chains per silicon atom (Figure 2.8). There are about 20 different compositions of alkyl or aryl chains (methyl or phenyl) to which can be incorporated further functional groups (e.g. cyanopropy, trifluropropyl). Monomers combined in variable proportions also convey changes in the properties of stationary phases (polarity, extended

stability from −50 to300 / 325°C, for the dimethylpolysiloxanes, depending on the column). Owing to their very broad temperature range these phases are the most widely used. A well-known phase which is used as a reference, since it is the only one that is perfectly defined, is squalane, which on the McReynolds scale has a polarity of zero. This saturated hydrocarbon ($C_{30}H_{62}$) is derived from squalene, a terpenoid natural extract from shark's liver (also present in the sebum of the skin). On this stationary phase, which can be used between 20 and 120°C (following either deposition or impregnation), the compounds are eluted in increasing order of their boiling temperatures, the retention time being inversely proportional to the analyte vapor pressure. Diverse bonded phases based upon polyalkylsiloxanes, almost apolar, can be used as a replacement for squalane [12].



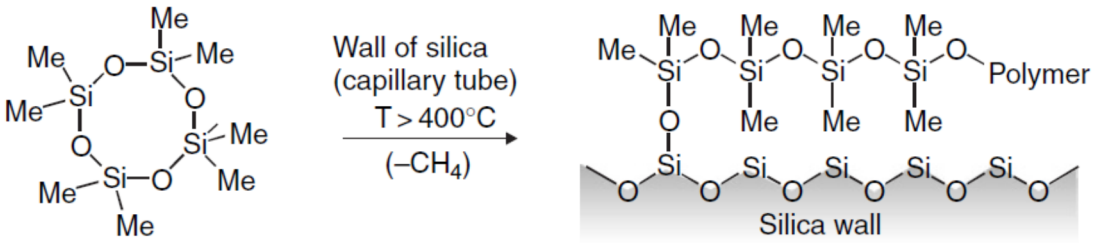
ex. R1 and R2 = Ph m = 95% and n = 5%

Figure 2.8 *Bonded polysiloxanes.*

2.6.2 Polyethyleneglycols (PEG)

The best known representative of this family is Carbowax. These polar polymers (M_r = 1500 to 20 000 – for the Carbowax 20M) can be used for deposition, impregnation or as bonded phases (40 < T < 240/260 °C, depending on column diameter and film thickness).

Method of formation of a bonded phase



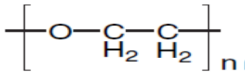
Polyethyleneglycols  "carbowax "

Figure 2.9 *Structure of polyethylene glycols.*

An inventory of all the compositions of this type of phase, used either for impregnation or bonding, would be lengthy. Treatment of the internal wall of a silica column with tetradimethylsiloxane will obtain a stationary phase bounded, polymerized and later reticulated. (The bonding resembles the fixing of indelible colors in order to create a brightly tinted fabric: the color contains an active site with which is able to attach itself, for example, to the alcohol functionality of cellulose on cotton fibers) [12].

2.6.3 Chiral stationary phases

These are generally polysiloxane basic phases mixed with 10 to 20 per cent by weight of β-cyclodextrin (polysaccharide) (Figure 2.10). This type of column is suitable for purifying racemic mixtures. If an organic compound, for example, comprises an asymmetric carbon the R and S enantiomers will not have the same affinity for the charged stationary phase in cyclodextrin, which also is expressed as two characteristic peaks. Therefore a chemical compound as a pure race mate will yield two peaks equal in size, each corresponding to an enantiomer.

Some columns accept high temperatures up to 450 °C (e.g. DEXSIL 400 or PETROCOL). Amongst the applications here is the analysis of the triglycerides of fatty tissues and simulated distillation as in the petrol industry. The latter replaces the conventional distillation, which can take up to 100 hours per analysis [12].

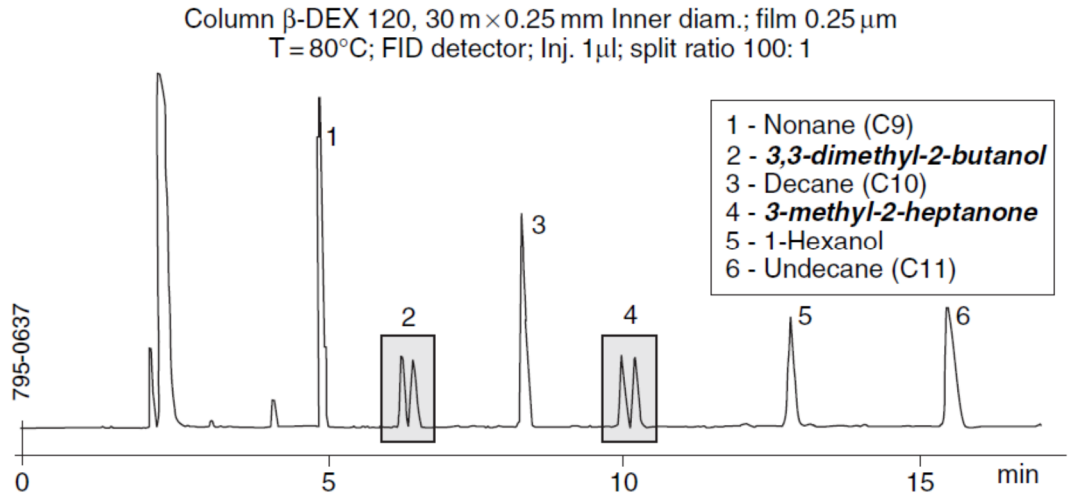


Figure 2.10 *Example of a separation with a chiral phase which contains cyclodextrins.*

The use of a chiral column to separate a racemic mixture of compounds leads to a splitting of the chromatogram signals as can be seen clearly for alcohols, 2 and 4. This chromatogram in isothermal mode, allows the calculation of retention indexes for the separated compounds (adapted from a Supelco illustration).

2.6.4 Solid stationary phases

These phases are constituted from a variety of adsorbent materials: silica or alumina deactivated by mineral salts, molecular sieves, porous glass, and graphite. Capillary columns made by deposition of these materials in the form of a fine porous layer are called PLOT. They are employed to separate gaseous or highly volatile samples. Columns containing graphitized carbon black have been developed for the separation of N_2, CO, CO_2 and very light hydrocarbons. The efficiency of these columns is very high.

Historically, silica gel, a thermo stable material and insensitive to oxygen, was one of the first compounds to serve as a solid stationary phase for GC columns. Today solid phases have become much more elaborate.

To compare or predict the behavior of capillary columns it is useful to calculate the phase ratio $\beta = V_M/V_S$ (Figure 2.11). Calling d_C the internal diameter of the column and d_f the film thickness deposited on its inner surface, an approximate calculation leads to:

$$\beta = \frac{V_M}{V_S} = \frac{d_c}{4d_f} \qquad (2.2)$$

If the compounds to be separated are volatile, a column with a small phase ratio should be chosen $\beta < 100$ and vice versa. A column of 320µm with a stationary phase of 1µm film thickness leads to a β ratio of 80 while for a column of 250µm with a film thickness of 0.2µm, β=310. Recalling that $K = k\beta$, it transpires that k, for a given compound and a given stationary phase, will increase if β decreases. The β parameter, which is accessible from the physical characteristics of the column, allows the calculation of K, the distribution factor. Values are generally very large (e.g. 1000) owing to the nature of the mobile phase which in this case is a gas [12].

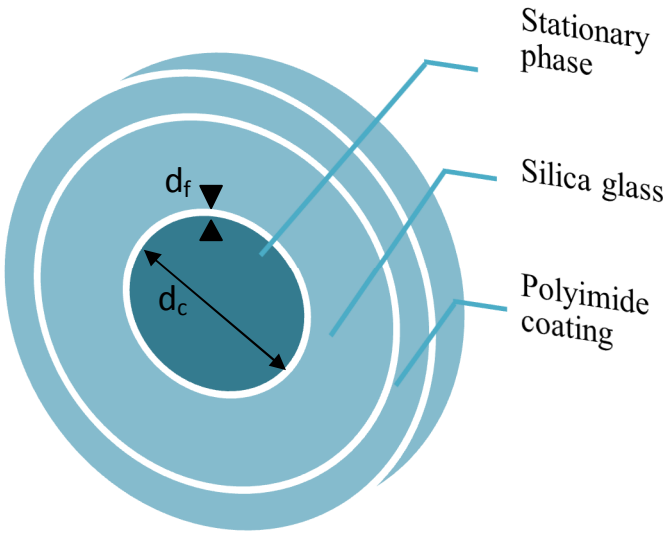


Figure 2.11 *Capillary column sections*

2.7 Principal gas chromatographic detectors

Some detectors are universal; that is they are sensitive to practically every compound that elutes from the column. On the other hand, there are discriminating (selective) detectors that are sensitive only to specific compounds, yielding a much uncomplicated chromatogram. The ideal situation to quantify an analyte would be to have a detector which sees only this analyte. They can be also categorized as destructive or non-destructive of the analytes [9].

Detectors are classified into two groups depending on whether they lead only to single information such as the retention time and those which yield, besides retention time, structural information of the analyte concerned. For this reason, some gas chromatographs are equipped with two or three detectors linked in series. Nonetheless, the response of all detectors is dependent on the molar concentration or on the mass of analyte in the carrier gas.

2.7.1 Thermal conductivity detector (TCD)

This general purpose and non-destructive detector, in use since the early days of GC, has for a long time remained a mainstay of the technique. Miniaturization has led to it being used as much for packed columns as for capillary columns of moderate sensitivity (400 pg/mL carrier gas) when compared with other detectors, it possesses nevertheless a very large linear range (six orders of magnitude). Its operating principle relies on the thermal conductivity of gas

mixtures as a function of their composition. The detector incorporates two identical thermistors, resembling minuscule filaments, placed in two tiny cavities of a metal block thermostatically maintained at a temperature above that of the column (Figure 2.12) [9].

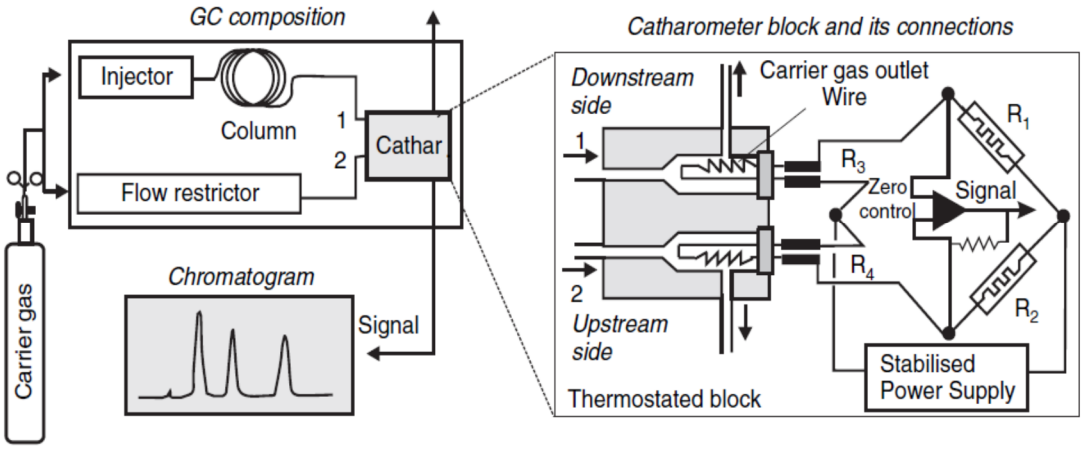


Figure 2.12 *Thermal conductivity detector.*

Left, schematic showing the carrier gas passage.

Right, the cross-sectional scheme of the metal block with its operating principle, based on an electrical Wheatstone bridge assembly (equilibrated when $R_1/R_2 = R_3/R_4$).

Both thermistors are located within the path of the carrier gas. One is flushed by the carrier gas evolving the column, while the other is flushed by a part of the carrier gas entering the injector.

In the steady state temperature equilibrium is established between thermal conductivity of the carrier gas and electrical current through the filament. When a solute elutes there is a change in the mobile phase composition, which in turn modifies its thermal conductivity. The thermal equilibrium being disrupted, this results in a variation of the resistance of one of the filaments which is proportional to the concentration of the compound in the carrier gas.

2.7.2 Flame ionization detector (FID)

Considered as almost universal for organic compounds this is effectively the detector *par excellence*, of GC. The gas flow issuing from the column passes through the flame of a small burner fed by a mixture of hydrogen and air. The detector destroys the organic compound present whose combustion results in the release of ions and charged particles responsible for the passage of a very weak current (10−12 A) between two electrodes (pd of 100 to 300 V).

One end of the burner, held at ground potential, acts as a polarization electrode while the second electrode, called the collector, surrounds the flame rather like a collar. An electrometer amplifies the signal to a measurable voltage (Figure 2.13) [10].

For organic compounds the intensity of the signal is considered to be proportional to the *mass flow* of carbon, excepted in the presence of hetero elements, such as the halogens. Thus the area under the peak reflects the mass of the compound eluted (dm/dt integrated between the beginning and end of the peak will give the total mass m). The sensitivity is expressed in Coulombs/g of carbon. The detection limit is in the order of 2 or 3 pg/s and the linear dynamic range attains 108.

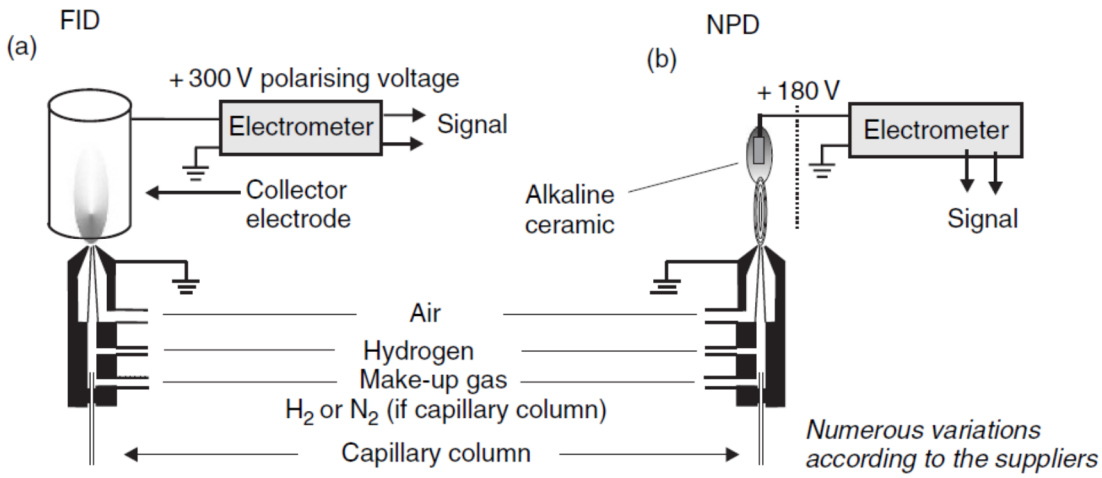


Figure 2.13 *FID detector (a) and NPD detector (b).*

The electrometers used with detectors allow the measurement of very small intensities. The response of these mass flow dependent detectors are unaffected by make-up gas.

An FID detector is not affected by variations in flow rate which can lead to errors with TCD.

In order to evaluate the presence of a volatile organic compound in polluted air (the VOCs), there exist portable instruments essentially housing a flame ionization detector that allows the measurement of the carbon content of the atmosphere examined, without chromatographic separation [10].

2.7.3 Nitrogen phosphorus detector (NPD)

Compared with the FID, this thermionic detector has a smaller flame in which the catalytic decomposition of compounds containing nitrogen (N), or phosphorus (P) yields, fairly specifically, negative ions which are received by a collector electrode. As it appears on the

representation (Figure 2.13), it comprises a small ceramic cylinder doped with an alkaline salt (e.g. rubidium sulfate). A voltage is applied to maintain a small plasma (800 ^{o}C) through the combustion of an air/hydrogen mixture. In these conditions the nitrogen present in air does not yield ions. Detector sensitivity is typically between 0.1 and 0.4 pg/s for nitrogen- or phosphorus-containing analytes, with a linear range of five orders of magnitude.

2.7.4 Electron capture detector (ECD)

This selective detector is considered to be excellent for trace analysis when analytes contain halogen atoms or nitro groups. A flow of nitrogen gas which has been ionized by electrons generated from a low energy - radioactive source (a few mCi of 63Ni) passes between two electrodes maintained at a voltage differential of around 100V (Figure 2.14). At equilibrium, a base current I0 is generated, mainly due to free and very mobile electrons. If molecules (M), containing an electrophore such an halogen (F, Cl, Br), cross the zone between the two electrodes, they capture thermally excited electrons to form heavy negative ions, which by consequence are much less mobile, leading to a decrease in the signal [10].

$$N_2 \xrightarrow{\beta^-} N_2^+ + e^-$$
$$M + e^- \longrightarrow M^-$$
$$M^- + N_2^+ \longrightarrow M + N_2$$

The measured intensity decreases exponentially by following a law of type $I = I_0 \exp[-kc]$. The linear range is of about four decades with nitrogen as the carrier gas. The presence of a radioactive source in this detector means that it must be licensed (maintenance visits and regular area testing). This non-destructive detector, well suited for compounds with high electron affinity, is mainly used for analyses of chlorinated pesticides.

Make-up gas. To provide maximum performances, the three detectors described above should be served with a gas flow of at least 20 mL/min, which is far superior to that within capillary columns. This flow rate is attained by mixing, at the outlet of the column, a make-up gas either identical or different (such as nitrogen), from the carrier gas.

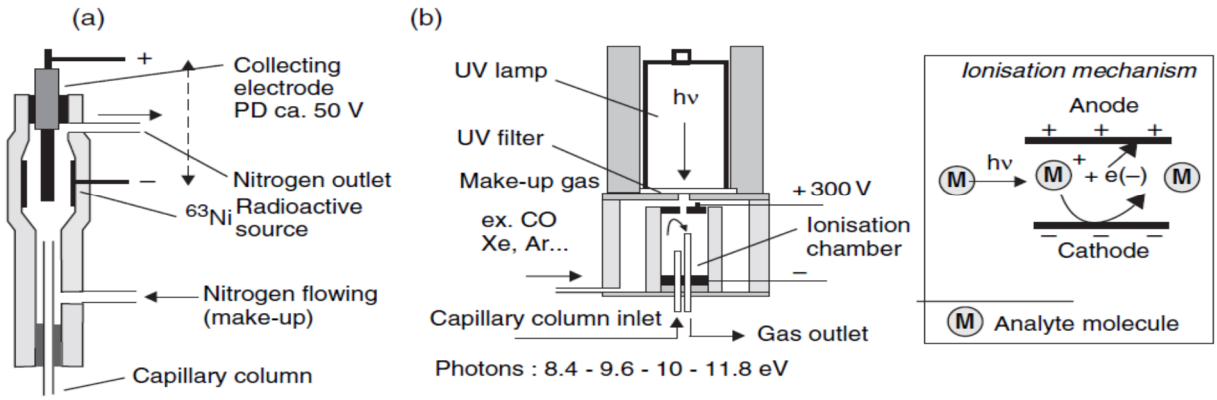


Figure 2.14 *Electron capture detector (ECD)* (a) *and photo-ionization detector (PID)* (b).

The ECD must be installed in an well ventilated position owing to it containing a radioactive source. The PID contains a UV source from which the photons are emitted, having a pre-selected energy, using a filter which prevents undesired carrier gas ionization ($M+h\nu \rightarrow M^{+}+e^{-}$).

Examples of filters: LiF at 11.8 eV, MgF_2 at 9.6–10 eV, sapphire at 8.4 eV. On contact with the electrodes the molecules return to uncharged state, ionization being therefore reversible. The use of the make-up gas provides an optimal flow .

2.7.5 Photo-ionization detector (PID)

This detector is fairly selective but it has only a narrow range of application, convenient for hydrocarbons as well as for S or P derivatives. The operating principle consists to provoke ionization of the analytes by irradiation with a UV lamp emitting photons of high energy (of 8.4 to 11.8 eV). The photo-ionization occurs when the energy of the photon is greater to that of the first ionization of the compound (Figure 2.14). A photon of 9.6 eV can, for example, ionize benzene (PI_l=9.2eV) but not isopropanol (PI_l=10.2eV). Electrons are collected by an electrode linked to an electrometer.

This detector can function at more than 400 ^{o}C and is not destructive since the ionization is reversible and affects only a small fraction of the molecules of each compound passing through. ECD and PID detectors are examples of class-specific detectors often used in trace environmental analysis [10].

2.8 Detectors providing structural data

None of the detectors previously described yield any information as to the nature of the compound eluted. At most they are selective. Compounds identification proceeds with the use of an internal calibration based on retention times or requires the knowledge of retention indexes. When the chromatogram is very complex, a confusion of identity could occur. To counteract this, several complementary detectors could be associated, or a detector able to convey structural information based on spectroscopic data, or elemental composition of the analytes. The retention time and specific characteristics for each compound could then be known. These detectors lead to stand-alone analysis techniques for which the results depend only on the ability of the column to separate properly the constituents of the sample mixture.

2.8.1 Atomic emission detector

The compounds coming out from the column enter into microwave plasma whose temperature is sufficient to create excited atoms. Thus, each element present in the solute eluted emits a characteristic spectrum, which permits its identification [9].

2.8.2 Other detectors

A mass spectrometry detector (MSD) which consists of a low resolution mass spectrometer can be placed to the outlet of the column. A fragmentation spectrum of each eluted compound is obtained. From the total ionic current (TIC), a chromatogram can be traced which represents all of the compounds eluted.

At the outlet of a capillary column, either in series or in parallel and depending upon whether any of them destroy the sample, several detectors can be installed. Here, three chromatograms of an injected mixture are obtained from each detector. Note that the sensitivity varies significantly from one detector to another.

By choosing a particular ion (*selective ion monitoring, or SIM*), a selective chromatogram can be produced. Although this method in some cases leads to a more modest sensitivity than with classic detectors, for many types of analyses it has become essential, notably in the context of environmental studies. Nevertheless it requires the use of high performance columns (*ID*=0,1–0,2mm) with very low bleeding. Similarly with an *infrared detector*, the IR spectrum can be graphically collated while with an *ultraviolet detector,* the corresponding UV spectrum of each compound eluted can be drawn. This is the domain of

coupled 'hyphenated' methods, widely used for trace analyses. The modes of detection mentioned above can be used jointly in the same installation equipped with a capillary column[9].

2.9 Fast chromatography

Conventional chromatography is a slow method of analysis. The retention times are often longer than an hour when separating components of a complex mixture. To reduce these times, influence can be brought to bear upon several parameters. The most obvious requires the use of a shorter column and so as not to lose efficiency, the diameter of the capillary column should be reduced. The stationary phase should be a thin film (01 µm) and the column must operate with a steep programmed temperature gradient (e.g. 100 ^{o}C/ min), now possible with modern GC instruments. Detector-response time also plays a significant role in achieving the best peak fidelity.

If all the compounds in an existing GC run are too much separated, presenting large vacant spaces between peaks on the chromatogram, then the strategies described above should produce faster analysis times. This leads us to the domain of *fast chromatography* of volatile compounds which uses columns of very different design, for example incorporating resistant outer coverings so that the temperature (200 ^{o}C/20 s) might be increased more sharply. This also avoids to the oven a long time to cool down and get ready for the next run. As a result the retention times are reduced significantly (Figure 2.15). This type of *fast chromatography* sometimes called *high-speed GC* finds its principal use in control analyses.

The detector must be able to store almost immediately the rapid variations in concentration at the moment of each analyte's elution. For detection by mass spectrometry there is good reason to be attentive to the speed of the sweep of the m/z ratio; a slow sequential sweep may lead to a situation in which the concentration in the ionization chamber is not the same from one end of the recording to the other.

There are now portable micro-chromatographs available weighing only a few kg for the rapid analysis of gases and volatile products. Although containing a reservoir of the carrier gas to assure their autonomy they are not bulky (Figure 2.16).

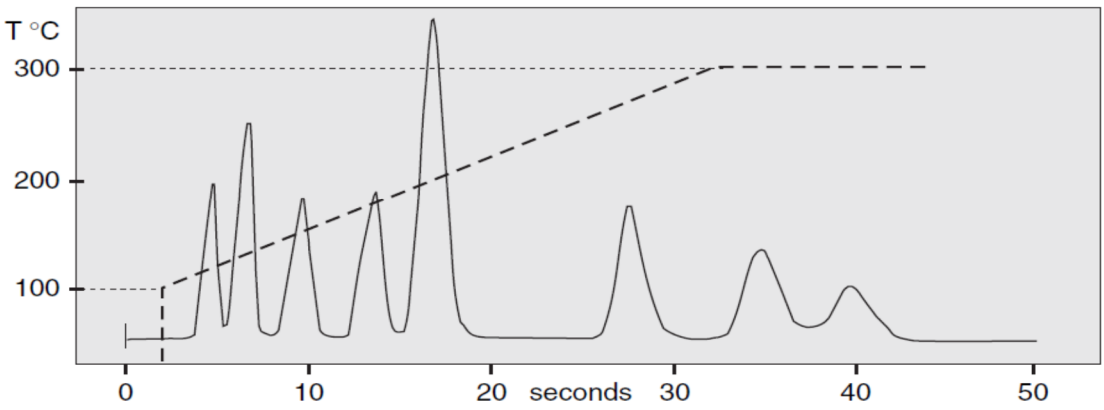


Figure 2.15 *Fast chromatography*.

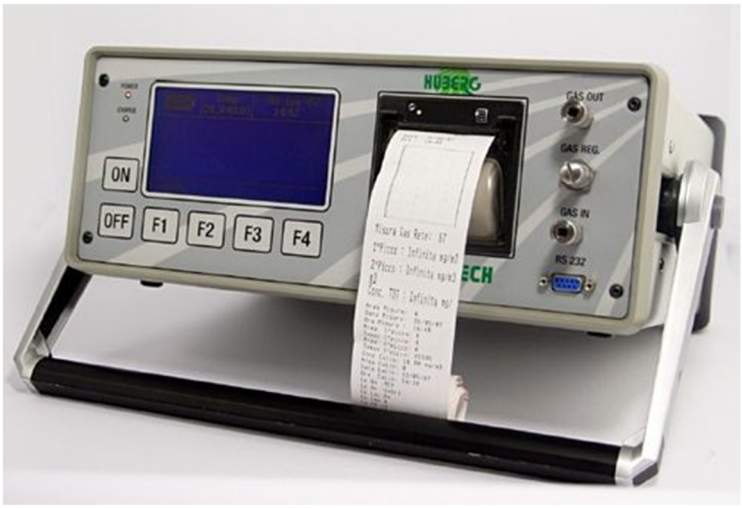


Figure 2.16 *Portable gas chromatograph*.

A lightweight (6.6 kg), battery operated, isothermal GC. This miniature analytical engine using a capillary column and a photoionization detector is conceived for the analysis of gas and other volatile compounds (VOCs). Below is an example of chromatogram obtained with such an instrument.

Certain components are obtained by micro-machining on silicon-chips. A short capillary column (5 m) is inserted into the outer metal sleeve, which is able to tolerate a rapid increase in temperature such as a gradient of 20°C/s. The efficiency (N) remains fairly poor though the temperature gradient allows an optimization of the selectivity between the compounds [5].

2.10 Multi-dimensional chromatography

In this technique, that has been used for a long time, the whole (or a fraction) first column effluent is analyzed through a second column having different selectivity. This allows the

resolution of analytes, which are not separated at the end of the first column. Called GC×GC or 2D-GC, the installation must comprise two detectors and an injection valve between the two columns positioned in series (Figure 2.17).

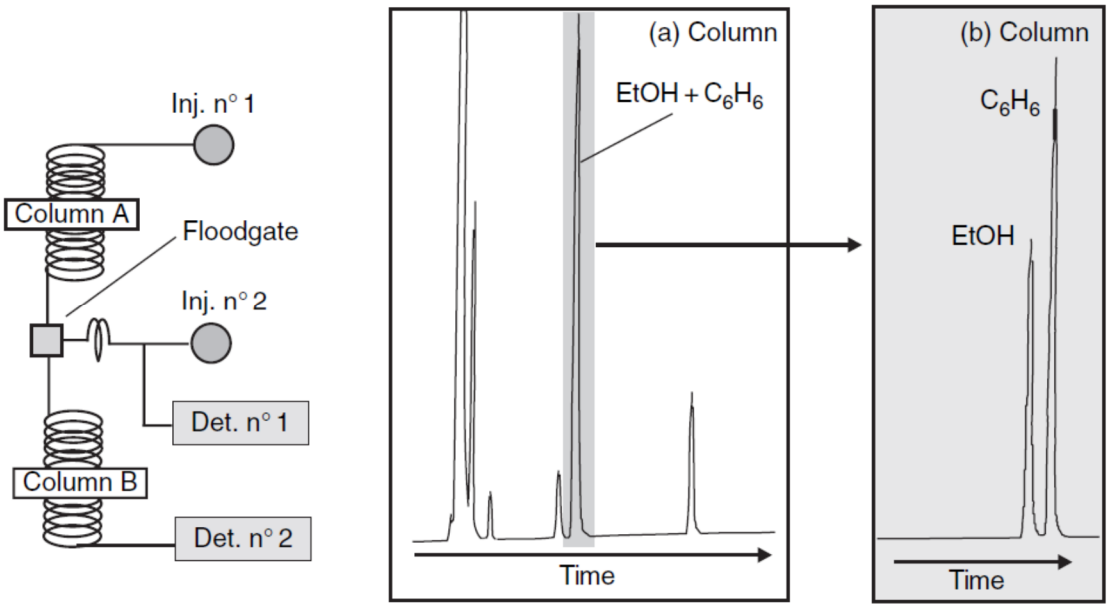


Figure 2.17 *GC × GC setup or two-dimensional chromatography.*

The arrangement of two columns each associated with a detector and an injection valve introduced between them. In the example shown in (a), a polar column cannot separate ethanol and benzene. The corresponding fraction is therefore re-injected onto (b), a non-polar column, in which the separation is affected.

2.11 Retention indexes and stationary phase constants

These parameters have been developed to pursue at least three objectives:

- To identify a compound by a more general characteristic than its retention time under pre-defined controlled conditions. As a result, a *system of retention indexes* has been developed which is an efficient and cheap means by which to avoid certain identification errors.
- To follow the evolution over time of a column's performance.
- To classify all stationary phases in order to simplify the choice of the column best adapted to a particular kind of separation problem. The chemical nature of the phases and polarities do not allow prediction of which column will be optimal for a given

separation. For this, the behavior of stationary phases with respect to several reference compounds should be examined, preparing the way toward *stationary phase constants*.

Wehrli and Kovats introduced the concept of the retention index to help confirm the structure of the organic molecules. This method utilizes a series of normal alkanes as a reference base instead of one compound as in the relative retention method. Identification can be assisted with the use of the retention index I [4].

In practice, the retention index is simply derived from a plot of the logarithm of the adjusted retention time versus carbon number times 100. To obtain a retention index, the compound of interest and at least three hydrocarbon standards are injected onto the column. At least one of the hydrocarbons must elute before the compound of interest and at least one must elute after it. A plot of the logarithm of the adjusted retention time versus the Kovats index is constructed from the hydrocarbon data. The logarithm of the adjusted retention time of the unknown is calculated, and the Kovats index is determined from the curve.

Many factors can influence the Kovats index, with temperature, flow, and column variation. However, the Kovats index is the preferred method of reporting retention data.

2.11.1 Kovats' straight line relationship

The retention index related to a given stationary phase is determined as follows. When a mixture containing compounds belonging to a homologous series of n-alkanes is injected onto a column maintained under isothermal mode, the resulting chromatogram is such that the logarithm of the adjusted retention times $t'_{R(n)}$ increases linearly with the number n of carbon atoms present in the corresponding n-alkane (Figure 2.18).

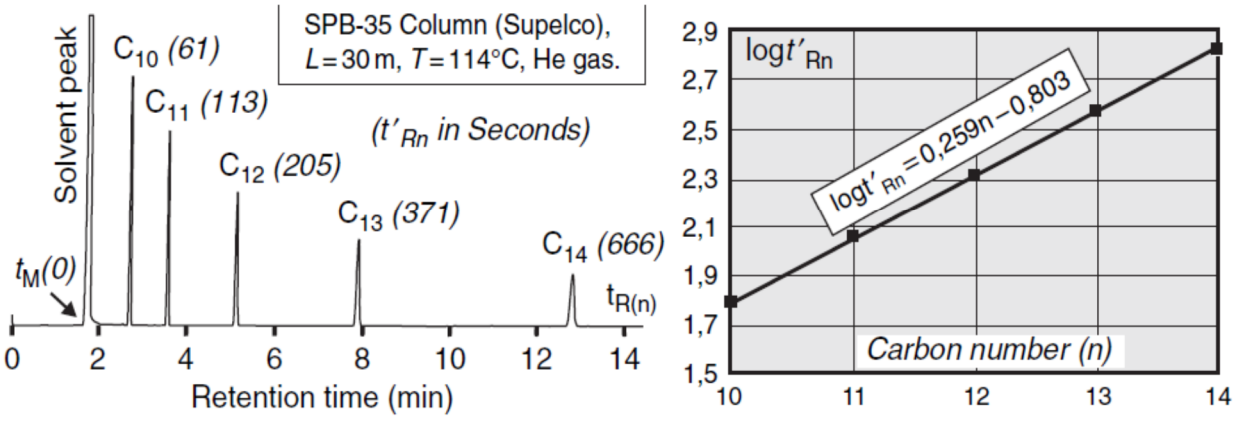


Figure 2.18 *Kovats' straight line graph.*

Left, isothermal chromatogram for a series of five n-alkanes (C_{10} -C_{14}).

Right, the corresponding plot of the Kovats' relationship for the chosen stationary phase and for the pre-determined analytical conditions [5].

On a graphical representation, the carbon number n versus $\log t'_{R(n)}$ usually yields a series of well lined up points according to the following semi-empirical mathematical relationship:

$$\log t'_{R(n)} = a\,n + b \qquad (2.3)$$

The adjusted retention time $t'_{R(n)}$ corresponds to the retention time t_R of an alkane having n atoms of carbon, minus the dead time t_M; a and b are numerical coefficients. The slope of the graph obtained depends on the overall performance of the column and the operating conditions of the chromatograph.

This expression (2.3) follows on from another linear relation seen in thermodynamics linking the variation in free energy and the equilibrium constant K;

$$\Delta G = -RT \ln K ;$$

Recall equation (2.2)

Since $K = k\,\beta;$

Therefore $t'_R = K\,t_M / \beta;$

Then $\log t'_R$ will increase as $\ln K;$

$$\ln t'_R = \ln K + (\ln t_M / \beta) \text{ [5].}$$

2.11.2 Kovats' retention index (or indice)

A compound (X) is now injected onto the column without changing the tuning of the instrument. The resulting chromatogram will enable I_x, the Kovats' retention index, to be calculated for X and the specific column employed: this is equal to 100 times the equivalent number of carbon atoms n_x of the 'theoretical alkane' having the same adjusted retention time than X. Two methods can be used to find n_x:

- The first is based on the Kovats' relationship previously obtained (Figure 2.18). This leads to a calculation of n_x (therefore I_x).
- The second gives a good estimation of I_x from the adjusted retention time of the two n-alkanes (n and n + 1C) that bracket compound X on the chromatogram:

1. Applied in isothermal temperature programing:

$$I_x = 100n + 100\left(\frac{\log t'_{R(X)} - \log t'_{R(n)}}{\log t'_{R(n+1)} - \log t'_{R(n)}}\right) \qquad (2.4)\ [14]$$

2. Applied in gradient temperature programing:

$$I_x = 100n + 100\left(\frac{t_{R(X)} - t_{R(n)}}{t_{R(n+1)} - t_{R(n)}}\right) \qquad (2.5)\ [14]$$

In contrast to the Kovats' regression line, the retention index depends only on the stationary phase and not on the column dimensions or the flow rate of the carrier gas. Due to this the *retention time* is converted into a *relative retention time* independent of experimental conditions, but normalized to a series of paraffins. In practice, to ensure of the experimental conditions for the two injections are uniform, compound X and the n−alkanes mixture are co-injected (Figure 2.19).

The chromatogram that gives the Kovats' relationship for a given stationary phase, can also serve to evaluate the performances of a column. For this, the separation number also known as the *trennzahl number* (TZ) is calculated. The two retention times occurring in these relationships relate to two successive alkanes differing by one carbon number (n and n+1) or to two compounds of a similar type. The separation number indicates how many compounds could be baseline separated reasonably well by the column in the interval of retention time of these two reference compounds. The alkanes whose elution times are either side of that of the compound being analyzed are chosen [11].

$$\mathrm{TZ} = \frac{t_{R2} - t_{R1}}{(w_{1/2})_1 + (w_{1/2})_2} - 1 \qquad (2.6)$$

$w_{1/2}$ *: Baseline width at half.*

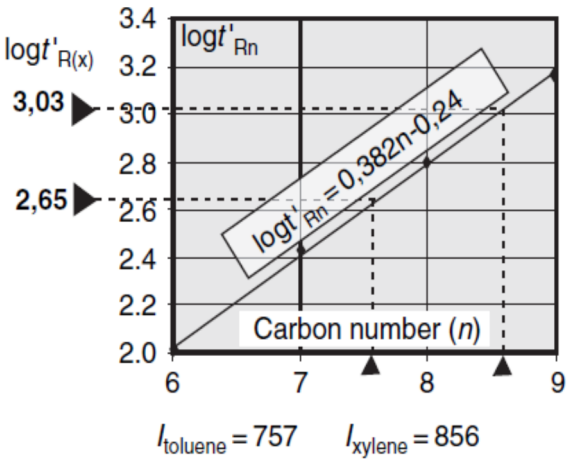


Figure 2.19 *Graphical measurement of Kovats' retention index* (I = 100 n_x) *on a column in the isothermal mode.*

The number of equivalent carbons n_x, is found from the logarithm of the adjusted retention time t'$_R$ of X. The chromatogram corresponds to the injection of a mixture of 4 n-alkanes and two aromatic hydrocarbons. By injecting periodically this mixture the modifications to the Kovats' indexes of these hydrocarbons permits the following of the column's performance. The calculations for retention indexes imply that the measurements were effected under isothermal conditions. With temperature programming they yield good results to the condition to adopt an adjusted formula, though this entails a reduction in precision.

There are tables of retention indexes of compounds currently in general used on the most common stationary phases. If several retention indexes of the same compound obtained on different stationary phases are available, then this unique collection of values could then characterize the compound more precisely. Identification by retention index is not as reliable as using more popular hyphenated techniques as GC/MS, but it requires a not inconsiderable material investment.

Retention time locking. The identification of compounds for which the retention times are very close and whose mass spectra are almost identical (certain forms of isomers) is obviously difficult. A current method consists of selecting an internal standard or a compound known to be present in all of the samples to be analyzed and through the use of computer software the value of its retention time is locked (unchangeable) for different analyses, even if undertaken on a different apparatus (but on the same stationary phase). The effect of this is to conserve equally the retention times of the other compounds of the mixture, facilitating their

identification. This approach, which avoids referring to the retention index, is possible with modern GC instruments and is known as retention time locking (RTL) [11].

PART III

EXPERIMENTAL

It requires a very unusual mind to undertake the analysis of the obvious

—Alfred North Whitehead (1861–1947)
English philosopher and mathematician

3.1 LOGIC OF QUALITATIVE ANALYSIS

The most important factor in qualitative gas chromatographic analysis is the collection of as much information as possible about the sample before beginning any laboratory work. This information is first gathered by the people involved in the collection of the sample. The sampling location, the person taking the sample, the method of sampling, and sample handling should be known. The sample matrix (solvent, etc.) should be investigated to determine the source of chromatographic peaks. A pure sample should be utilized to compare with the unknown sample. The technique of running blanks on solvents should certainly not be overlooked since the solvent used to work up a sample may be the contributing factor to unknown peaks. Furthermore, the chemist should always be alert to unknown peaks originating from simple decomposition in storage or isomerization under chromatographic conditions. All of the above items are important considerations, especially in the area of trace analysis. Many times impurities can be in excess of the amount of trace components being analyzed. One should keep in mind that the identification of an unknown by GC can easily turn into a major research project.

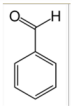
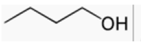
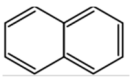
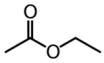
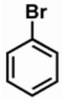
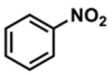
With this introduction to qualitative analysis via gas chromatography I started off my handling mission of the instrument by first figuring out a method in witch to operate the instrument under our specific conditions (column, carrier gas… etc.) thus providing a reliable method to be used in conducting the standardization to create a useful database for future reference.

3.2 Sample Collection, Preservation and Storage

Samples are collected, preserved and stored according to standard procedures for semi-volatile compounds, from the VTRS laboratory in our university at El Oued and courtesy of the National School of Teachers in Kouba, Algeries, and of the University of Ferhat Abbas, Setif.

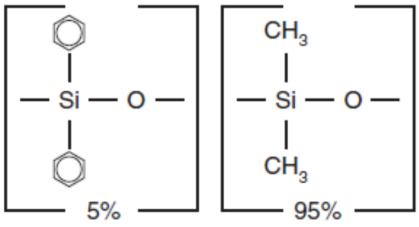
3.3 Equipment and Supplies

1. Chemicals

	Name	Boiling Temp (^oC)	Structure
1.	n-Hexane	69	
2.	Diethyl ether	35	
3.	Chloroform	61	
4.	Benzaldehyde	178	
5.	n-Heptane	98	
6.	Toluene	110	
7.	Benzene	80	
8.	n-Butanol	118	
9.	Naphthalene	218	
10.	Acetone	56	
11.	Ethyl Acetate	77	
12.	Ethanol	78	
13.	Methanol	65	CH_3OH
14.	BromoBenzene	156	
15.	NitroBenzene	210	
16.	ChloroBenzene	132	
17.	Cyclohexane	81	
18.	n-Alkanes standard		C_{14}-C_{17}

2. **Lab supplies**

   a. **J&W 30 meter DB-5 capillary column** (0.25IDmm, 0.25 µm film thickness)
   This column has small-diameter tubing in which the inner walls are used to support the stationary phase.
   Phase: 5% phenyl + 95% methyl Polysiloxane,



   b. SGE 5uL micro syringe

   c. **N_2 Carrier Gas tank** with two stroke pressure regulator.
   This is the phase that transports the sample through the column.

   d. **Shimadzu OPGU-2200S Hydrogen Generator**.
   To enable the flame thrower in the FID detector.

   e. **Air Compressor** with Humidity Filter.
   Also to enable the flame thrower in the FID detector

3. **Lab equipment**

   a. **Shimadzu Gas Chromatograph GC-2014** (specifications attached in Appendix B)

   b. **Dell Central Processing Unit** – equipped with a Dual Core Micro Processor
   Used to Process data from the GC and displaying the result chromatogram using GCsolution software version 2.3.

3.4 Operational Procedure

1. Open the nitrogen, compressed air and Hydrogen tanks and ensure that the line pressure for each is set to the marked level and that all valves in the gas line between the tanks and the chromatograph are open.
2. Turn on the gas chromatograph and the GCsolution computer.
3. Use the GCsolution software in the computer. Ensure that the “seif12" method is loaded. Take careful note of the oven temperature, flow rate and split ratio used by this method.

4. A flame ionization detector will be used to monitor the separated components. To establish the hydrogen flame, open the air and hydrogen needle valves for the Detector to a full counterclockwise position. Press the "ON" button until the flame lights. You can detect the flame by the signal.
5. Allow the system to warm up until the signal stabilizes, and then adjust the acquisition of the chromatogram to zero.
6. Rinse a 5-µL syringe several times with the solvent and then with the sample and inject a 1.0 µL sample and start data collection.
7. After completion of the run (about 60 mins), associate the major peaks in the chromatograms with the major components of the sample and note the retention time (t_R) for each of these peaks.

3.5 Definitions

1. n-Alkanes mixture – saturated aliphatic hydrocarbons.

2. Internal standard – a compound similar to the analyte(s) added to the sample at the beginning of the analyses to compensate for any variability in the analyses.

3. Adjusted Retention Time t'_R - The solute total elution time minus the retention time for an unretained peak (holdup time):

$$t'_R = t_R - t_M \qquad (3.1)$$

4. Holdup Time t_M. The time necessary for the carrier gas to travel from the point of injection to the detector. This is characteristic of the instrument, the mobile phase flow rate, and the column in use.
5. Capacity Factor k. This is a measure of the ability of the column to retain a sample component:

$$k = (t_R - t_M) / t_M \qquad (3.2)$$

6. *Holdup Volume* V_M. The volume of mobile phase from the point of injection to the point of detection. In GC it is a measure of the volume of carrier gas required to elute an unretained component (including injector and detector volumes):

$$V_M = t_M F_c \qquad (3.3)$$

3.6 THE EXPERIMENT: Effect of Linear Velocity

In order to find and provide a useful and reliable method for our analysis and because the instrument was never put to work, i conducted a series of experiments on 17 volatile compounds in two different linear velocities to figure out which was best to use with the carrier gas (N_2) and column (DB-5). Each of the samples was injected separately the results are listed in Table 3.1.

3.6.1 Operating Conditions:

The constant operating conditions for this experiment are as follows:
Phase: 5% phenyl + 95% methylPolysiloxane,
30 meter DB-5 capillary column (Int. Diam. 0.25 mm, 0.25 μm film thickness)
Carrier gas: N_2, Purge flow rate 3 ml/min
Temperature programming from 60 up to 240°C at a rate of 3°C/min
Injection temperature 220°C, split ratio 1:100
FID detector temperature 250°C

Linear Velocity:		**15 cm/s**	**12 cm/s**	**15 cm/s**	**12 cm/s**
Num.	Sample	Retention time (min)		Retention time (sec)	
1	Methanol	3,669	4,603	220	276
2	Acetone	3,798	4,856	228	291
3	Ethanol	3,837	4,769	230	286
4	Diethyl ether	3,894	4,876	234	293
5	n-Hexane (C6)	4,326	5,476	260	329
6	Chloroform	4,516	5,581	271	335
7	Ethyl Acetate	4,535	5,599	272	336
8	n-Butanol	4,935	6,056	296	363
9	Benzene	4,923	6,144	295	369
10	cyclohexane	5,044	6,22	303	373
11	n-Heptane (C7)	5,489	6,749	329	405
12	Toluene	6,696	8,401	402	504
13	chlorobenzene	8,590	10,316	515	619
14	bromobenzene	11,801	13,691	708	821
15	Benzaldhyde	12,486	15,025	749	902
16	Nitrobenzene	18,075	20,313	1085	1219
17	Naphtalene	19,921	25,416	1195	1525

Table 3.1 *Comparison of 17 samples in two different linear velocities 15 cm/sec and 12 cm/sec respectively*

As you observe the results you notice that as the linear velocity increases the retention time decreases which explain why the separated compound exits the column into the detector first when the linear velocity of the carrier gas transports them faster.

When you observe more deeply you notice that when the linear velocity increases the retention times move closer together, and the separation is harder to obtain when two molecules have nearly the same retention time.

To better explain this factor I injected a mixture of the 9 samples that are closest to each other in retention times, and the chromatograms are shown in figures 3.1 and 3.2.

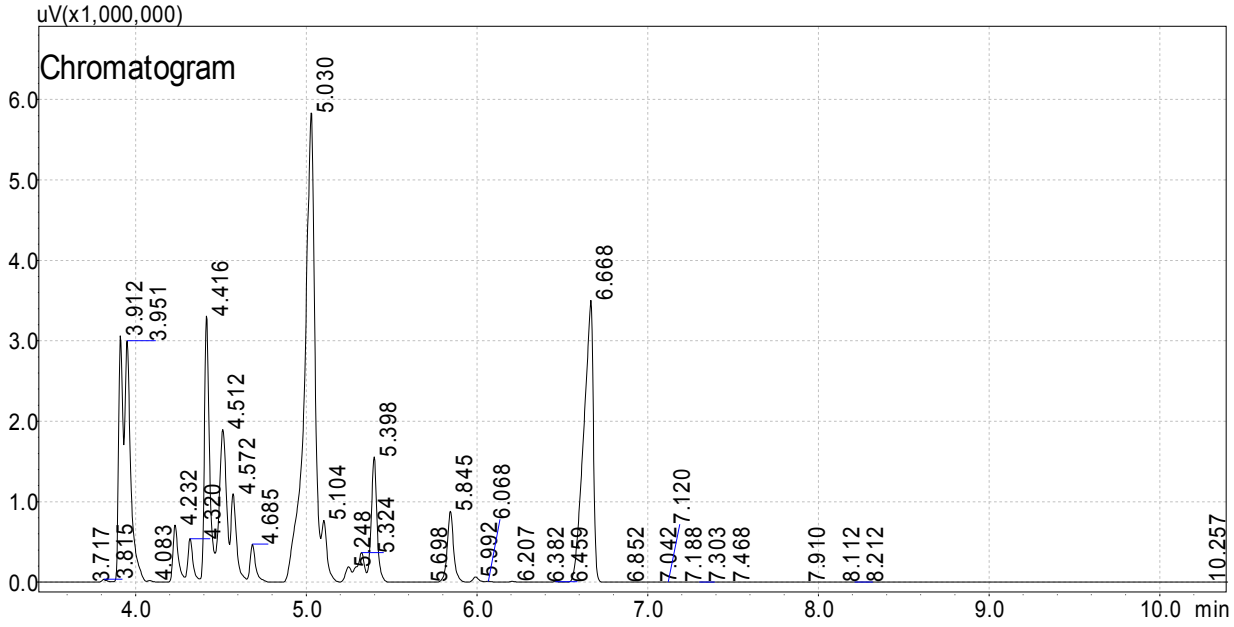


Figure 3.1 *Chromatogram for a 1 µl injection of a mixture of 9 components at carrier gas (N_2) linear velocity 15 cm/sec*

The peaks shown by order of retention time are: Acetone, Diethyl ether, n-Hexane, Chloroform, Ethyl Acetate, Benzene, Cyclohexane, n-Butanol, and Toluene respectively.

A sample chromatogram (Figure 3.1) illustrates that increase in linear velocity decreased retention time. it is a zoomed version of the chromatogram and as you can see the distance between a peak is what but of a few seconds away from the other, in order to make the reading of chromatogram more clear we need to make the separation go slower, thus leading to an accurate analysis.

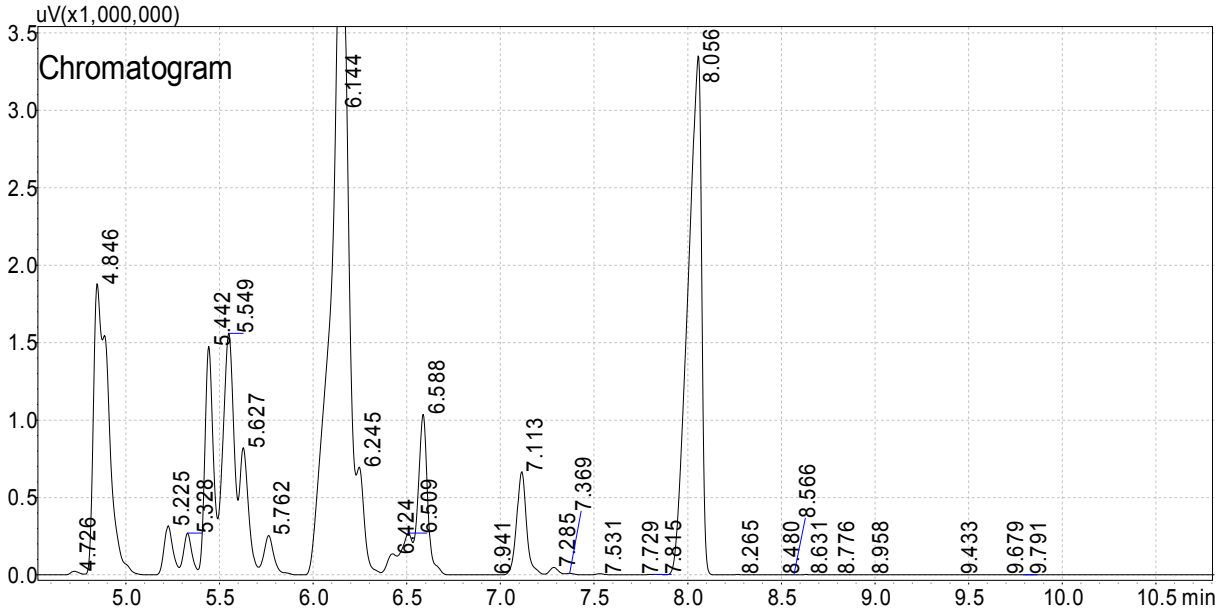


Figure 3.2 *Chromatogram for a 1 µl injection of a mixture of 9 components at carrier gas (N_2) linear velocity 12 cm/sec*

The peaks shown by order of retention time are: Acetone, Diethyl ether, n-Hexane, Chloroform, Ethyl Acetate, Benzene, Cyclohexane, n-Butanol, and Toluene respectively.

In this version of the chromatogram you notice that the peaks are farther away from each other in this linear velocity then they were before. Thus giving a better and more accurate analysis but taking a bit of a longer time, and to be even more persuasive a Chromatogram for a 1 µl injection of a mixture of 9 components at carrier gas (N_2) linear velocity 22.4 cm/sec is attached in APPENDIX A, **Figure A.42**

In conclusion; the linear velocity of 12 cm/sec shown to be of better and more accurate results than that of 15 and 22.4 cm/sec in our specific conditions of carrier gas N_2 and capillary column DB-5, and thus it is taken for a reliable method for our DATABASE.

3.7 THE EXPERIMENT: Effect of Temperature Programming

To understand the effect of temperature programming and difference between isothermal and gradient gas chromatography I have conducted a series of 6 experiments on 3 semi-volatile compounds with different temperature programs and the results are shown in Table 3.2

3.7.1 Operating Conditions:

The constant operating conditions for this experiment are as follows:

Phase: 5% phenyl + 95% methyl Polysiloxane,

30 meter DB-5 capillary column (Int. Diam. 0.25 mm, 0.25 µm film thickness)

Carrier gas: N_2, Purge flow rate 3 ml/min

Injection temperature 220°C, split ratio 1:100

FID detector temperature 250°C

Temperature programming for gradient; from 60 up to 240°C at a rate of 3°C/min

Temperature programming for isothermal; from 60 up to 80°C at a rate of 3°C/min

From 80 up to 150°C at a rate of 15°C/min

Remains 150°C for 10 minutes.

Temp. Programing:		Gradient	Isothermal	Gradient	Isothermal
Num.	Sample	Retention Time (min)		Retention Time (sec)	
1	Benzaldehyde 15 cm/s	12,486	10,928	749	656
2	Bromobenzene 15 cm/s	11,801	10,218	708	613
3	Nitrobenzene 15 cm/s	18,075	13,253	1085	795
4	Benzaldehyde 12 cm/s	15,025	12,506	902	750
5	Bromobenzene 12 cm/s	13,691	11,657	821	699
6	Nitrobenzene 12 cm/s	20,313	14,71	1219	883

Table 3.2 *Comparison of 3 samples in two different Linear Velocities and different temperature programs (Isothermal and gradient)*

As you observe the results, you clearly notice that retention times in Gradient temperature programs are considerably shorter than those of Isothermal temperature programs.

And the chromatograms in Figures 3.3 – 3.4 will show an example of the results and the rest are attached in APPENDIX A, Figures A.36 – A.41

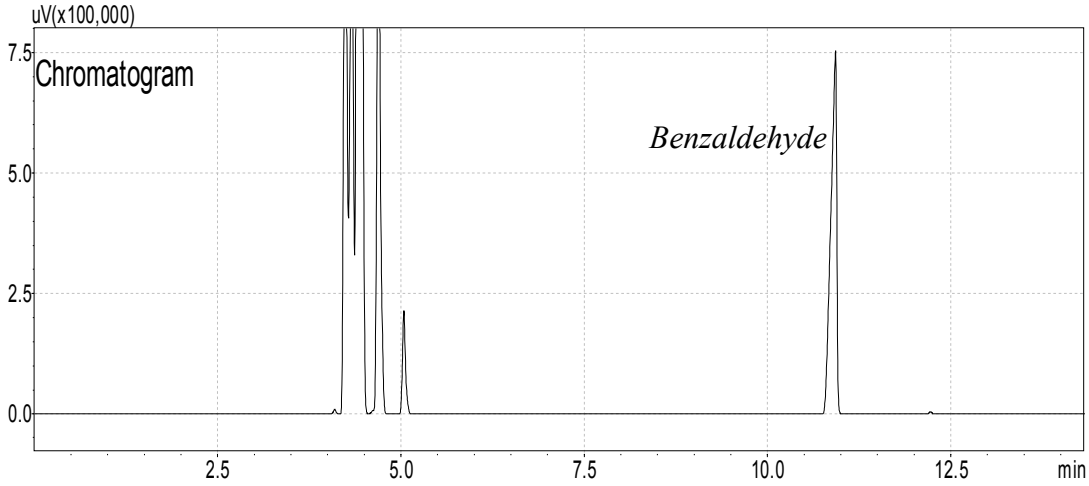


Figure 3.3 *Chromatogram of Benzaldehyde dissolved in n-Hexane at linear velocity of 15 cm/s and isothermal temperature program of 150 °C, (t_R =656).*

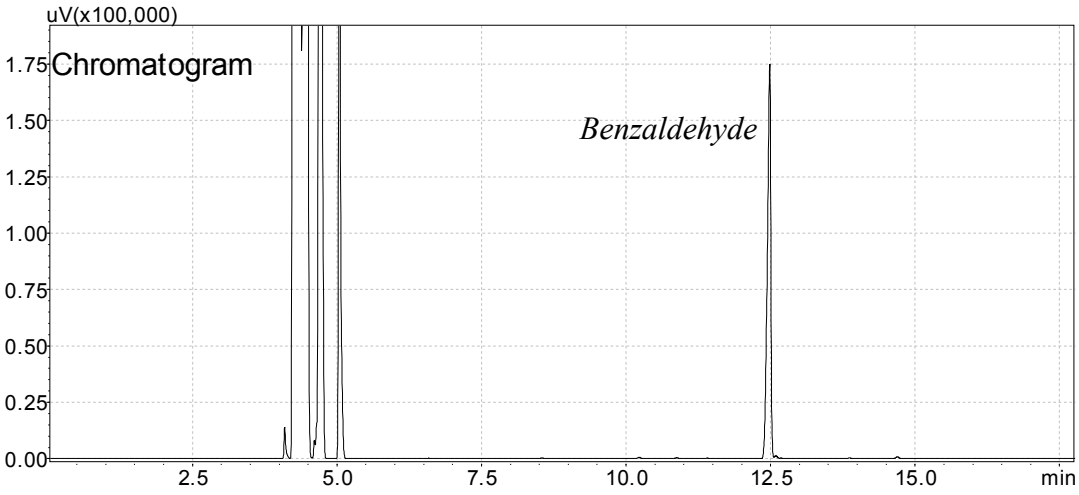


Figure 3.4 *Chromatogram of Benzaldehyde dissolved in n-Hexane at Linear Velocity of 15 cm/s and gradient temperature program from 60 to 240°C at a rate of 3°C/min, (t_R =749).*

3.7.2 Discussion

After discussing the results in table 3.2 we know that in gradient temperature programed gas chromatography the retention time is quite longer, and now from the chromatograms of Benzaldehyde dissolved in n-Hexane (Figures 3.3 and 3.4) at the same linear velocity.

Temperature programming refers to the temperature of the column oven increasing during the chromatographic analysis. As the column temperature increases the analytes vapor pressure increase and thus spend less time in the stationary phase. These results in a reduced retention time than if run at a lower temperature. Using faster ramping rates will cause

analytes to reach higher vapor pressures faster and thus elute peaks at even earlier retention times. All data confirmed that retention time decreased with an increasing temperature programming rate.

In conclusion; the gradient temperature programming method shown to be of better and more accurate results and thus it is taken for a reliable method for our DATABASE.

3.8 STANDARDIZATION

There is always the question of standards (known amounts of material generally in a matrix) regarding their preparation in the laboratory versus the purchase of readymade standards. In general, standards should be as close to the unknown samples as possible not only in the amounts of the materials to be analyzed, but also in the matrix of the sample itself. In all cases this requirement would dictate the preparation of standards in the laboratory. There is also the question of stability of the standards. With elapsed time, loss of either the matrix (e.g., hexane evaporation from a solution of pesticides in hexane) or the components of interest (e.g., adsorption of xylene on container walls of 50-ppm standard of xylene in air) cause the standard to be unreliable. In general, in the absence of prior knowledge, this dictates that standards be prepared, used, and then discarded all within a short period of time.

With this introduction to standardization, i conducted a series of experiments using the GC instrument provided in our laboratory at the University of El Oued, to provide a database for future reference with the use of our GC instrument (cf. Specifications of the GC-2014 in APPENDIX B) Using Kovats method that utilizes a series of normal n-alkanes as a reference base instead of one compound as in the relative retention method. Identification can be assisted with the use of the retention index *I*:

$$I_x = 100n + 100\left(\frac{\log t'_{R(X)} - \log t'_{R(n)}}{\log t'_{R(n+1)} - \log t'_{R(n)}}\right)$$ Applied in isothermal temperature programs.

$$I_x = 100n + 100\left(\frac{t_{R(X)} - t_{R(n)}}{t_{R(n+1)} - t_{R(n)}}\right)$$ Applied in gradient temperature programs.

The retention index is simply derived from a plot of the logarithm of the adjusted retention time versus carbon number times 100. To obtain a retention index, the compound of interest and at least three hydrocarbon standards are injected onto the column. At least one of the hydrocarbons must elute before the compound of interest and at least one must elute after it. A plot of the logarithm of the adjusted retention time versus the Kovats index is

constructed from the hydrocarbon data. The logarithm of the adjusted retention time of the unknown is calculated, and the Kovats index is determined from the curve.

This Method generally varies less than relative retention with temperature, flow, and column variation. And lets us profit from the qualitative analysis of the GC without coupling with a mass spectrometer (GC/MS) so in turn saving the university from inconsiderable material investment.

My method will provide a database that will not only enable the users (students of the university, teachers, or outside chemists from the market) for high resolution qualitative analysis but also provide technique for petrochemical analysis and water contamination with hydrocarbons impurities, the analysis of fatty acids in organic foods, pesticides and a whole new world of possibilities from identification by qualitative and quantitative analysis that will take both the chemical and chemical engineering departments and also the biochemistry department to a whole new level.

3.8.1 THE EXPERIMENT

One sample of a mixture of n-Alkanes Standard solution was dissolved in n-Hexane and 1 µl was injected at temperature of 220°C with five linear velocities of 12, 15, 18, 22.4 and 31.9cm/sec respectively, with an oven temperature program starting from of 60 up to 240°C rising at a rate of 3 °C/min. each of the analyses lasted exactly 60 minutes and the separated compounds passed through an FID detector at 250 °C. The results are shown in Table 3.3 and the Chromatogram in figure 3.3.

Standard C14-C17			C14	C15	C16	C17
31,9 cm/s	Retention Time	(min)	25,404	29,479	33,425	37,165
22,4 cm/s			28,461	32,571	36,572	40,346
18 cm/s			30,434	34,597	38,616	42,412
15 cm/s			32,245	36,407	40,455	44,276
12 cm/s			34,573	38,766	42,842	46,682
31,9 cm/s		(sec)	1524	1769	2006	2230
22,4 cm/s			1708	1954	2194	2421
18 cm/s			1826	2076	2317	2545
15 cm/s			1935	2184	2427	2657
12 cm/s			2074	2326	2571	2801

Table 3.3 *Retention times of four n-Alkanes in series (C_{14} to C_{17}).*

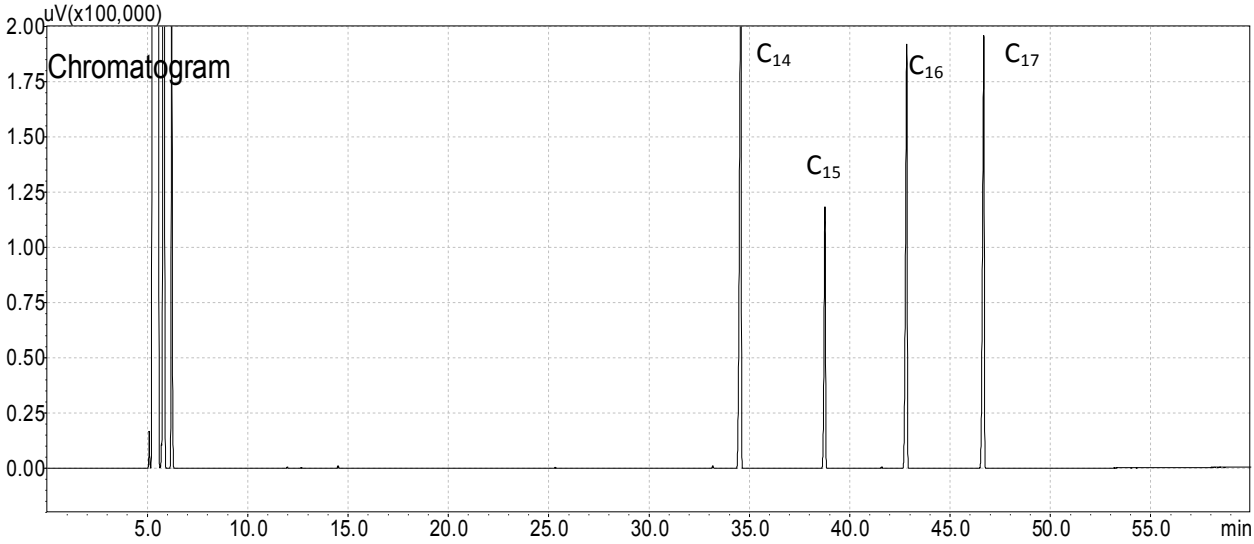


Figure 3.5 *Chromatogram of n-Alkanes Standard solution (C_{14} to C_{17}) at carrier gas linear velocity of 12 cm/sec*

Note: The peaks shown by order of retention time are: *n*-Hexane C_6, *n*-Tetradecane C_{14}, *n*-Pentadecane C_{15}, *n*-Hexadecane C_{16}, and *n*-Heptadecane C_{17} respectively.

The rest of the chromatograms associated with linear velocities of 15, 18, 22.4, and 31.9 cm/s are attached in appendix A. (Figure A.43 to A.47)

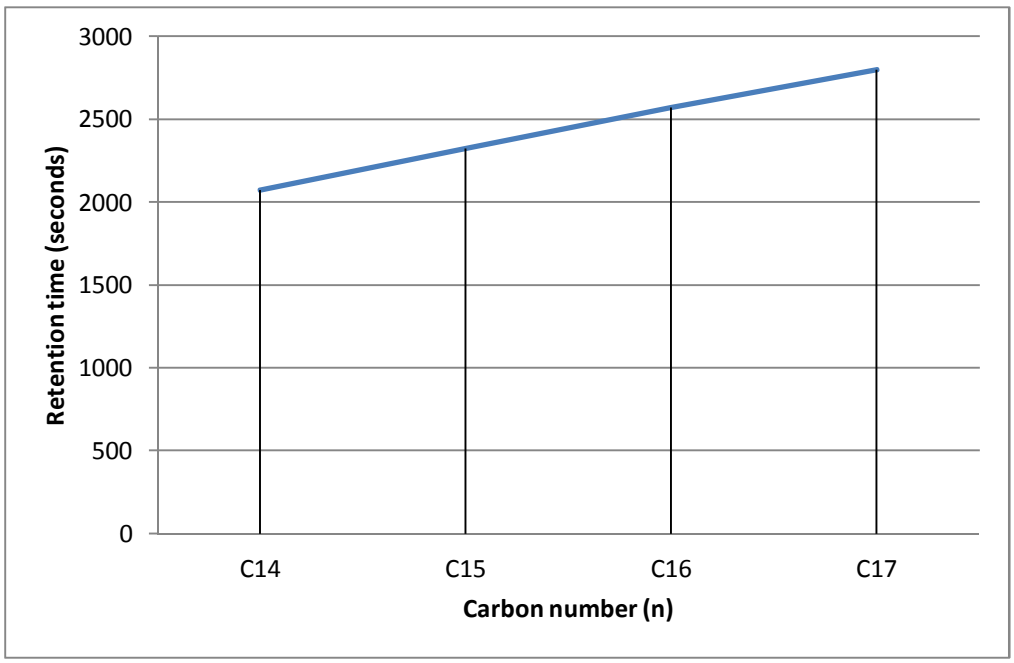


Figure 3.6*Graphical measurement of Kovats' retention index* (I = 100 n_x) *on DB-5 column in the gradient mode.*

The graphical representation yields a series of well lined up points according to the following semi-empirical mathematical relationship:

$$t_{R(n)} = a\,n + b$$

$a = \frac{2801-2074}{17-14} = 242.33$ ***and*** $b = 2074$

$t_{R(n)} = 242.33\,n + 2074\ (sec)$

3.8.2 RESULTS AND DISCUSSION:

To identify a compound (X), you will make an injection onto the column without changing the tuning of the instrument (my Method). The resulting chromatogram will enable I_x, the Kovats' retention index, to be calculated for X and the specific column employed:

This is equal to 100 times the equivalent number of carbon atoms n_x of the 'theoretical alkane' having the same adjusted retention time than X. Two methods can be used to find n_x:

- The first is based on the Kovats' relationship obtained from Figure 3.6

$$t_R = 242.33\,n + 2074$$

  This leads to a calculation of n_x (therefore I_x).
- The second gives a good estimation of I_x from the adjusted retention time of the two n-alkanes (n and n + 1) that bracket compound X on the chromatogram by using the Non-isothermal Kovats retention indices (from temperature-programming, using definition of Van den Dool and Kratz)

$$I_x = 100n + 100\,\frac{t_{R(X)} - t_{R(n)}}{t_{R(n+1)} - t_{R(n)}}$$

The final step is to use the index I_x and cross-reference it with the specific compound attached to it in Appendix B.

Remarks

1. For better accuracy it is advised to use at least 4 n-alkanes and calculate the Kovats index graphically.
2. When you find an index of 1040 this will not state the compound has 10,4 C-atoms. It merely tells you it has this retention index in the Kovats-scale. As an example: 3,3-dimethyloctane will have a different index from 2,7-dimethyloctane, although the number of carbon atoms and molecular-mass are equal.
3. The Kovats index is not absolute. It depends on flow rate and column stationary phase.

3.9 DETERMINING THE PARAMETERS:

Holdup time t_M:

$$t_M = {}^{L}/_{\mu}$$

L: length of the column (cm)

μ: linear velocity of the carrier gas. (cm/sec)

At μ = 12 cm/sec: $t_M = {}^{3000}/_{12} = 250\ sec$

Holdup Volume V_M;

$$V_M = t_M F_c$$

Fc: Flow rate (0.39 ml/min)

NA: $V_M = 250\ (0.39/60) = 1.625\ ml$

Equilibrium Constant K:

$$\boldsymbol{K = k\,\beta}$$

k: capacity Factor

β: phase ratio

By substitution of k and β: $K = \frac{(t_R - t_M)}{t_M} * \frac{d_c}{4d_f}$

NA: $K = \left(\frac{t_R - 250}{250}\right) * \frac{0.25 * 10^3}{4 * 0.25}$

Numerical Application for C_{14} , t_R = 2074 sec

$$K = \left(\frac{2074 - 250}{250}\right) * \frac{0.25 * 10^3}{4 * 0.25} = 1824$$

By calculating the equilibrium constant *K*, which specifically represents each peak in the chromatogram *(t_R)*, you can determine thermodynamic variation of free energy ΔG.

Efficiency of Column:

This is usually measured by column theoretical plate number and the height equivalent to a plate. It relates to peak sharpness or column performance.

$$N = 16\left(\frac{t'_R}{w_b}\right)^2$$

w_b: width of peak at baseline

Numerical Application for C_{14} *t'_R = 2074 – 250 = 1824 sec*

w_b = 18 sec

$$N = 16\left(\frac{1824}{18}\right)^2 = 164\,295$$

Which is a very high effective plate number due to the high performance of the new column applied in this experiment at the VTRS lab.

Next is to calculate the Height of the Plate (HETP):

$$HETP = \frac{L}{N}$$

L: Length of the column (30 meters)

$$HETP = 18.25\ \mu m$$

3.10 Demonstration of Quantitative analysis by GC

Quantitative analysis is to convert the size of the peak into some measure of the quantity of the particular material of interest. In some fashion this involves chromatographing known amounts of the materials to be analyzed and measuring their peak sizes. Then, depending on the technique to be used, the composition of the unknown is determined by relating the unknown peaks to be known amounts through peak size.

To demonstrate how qualitative analysis is done by GC, I have a conducted a series of experiments on three different compounds and four different concentrations for each, to plot a calibration curve.

3.10.1 Procedure

Prepare standard solutions containing graded amount of the standard object component, and inject a constant volume of each standard solution, exactly measured. With the chromatograms obtained, prepare a calibration curve by plotting the peak areas of the standard object component on the ordinate and the amounts of the standard object component on the abscissa. The calibration curve is a straight line through the origin. And the results obtained are recorded on table 3.4, demonstrated in figure 3.7, and plotted in figure 3.8.

3.10.2 Operating Conditions

The constant operating conditions for this experiment are as follows:
Phase: 5% phenyl + 95% methylPolysiloxane;
30 meter DB-5 capillary column (Int. Diam. 0.25 mm, 0.25 µm film thickness);
Carrier gas: N_2, Linear Velocity 12 cm/sec and Purge flow rate 3 ml/min;
Temperature programming from 60 up to 240°C at a rate of 3°C/min;
Injection temperature 220°C, split ratio 1:100;
FID detector temperature 250°C.

	Concentration Ratios	1:100	2:100	3:100	4:100
Compound Area (µV)	Cyclohexane	2969560	6306659	14173899	21864999
	Toluene	4477489	11815609	19731823	31775236
	Ethanol	1433825	2638168	4752852	6938407

Table 3.4 *Calibration of 4 different concentrations on 3 three compounds (area µV / concentration ratio)*

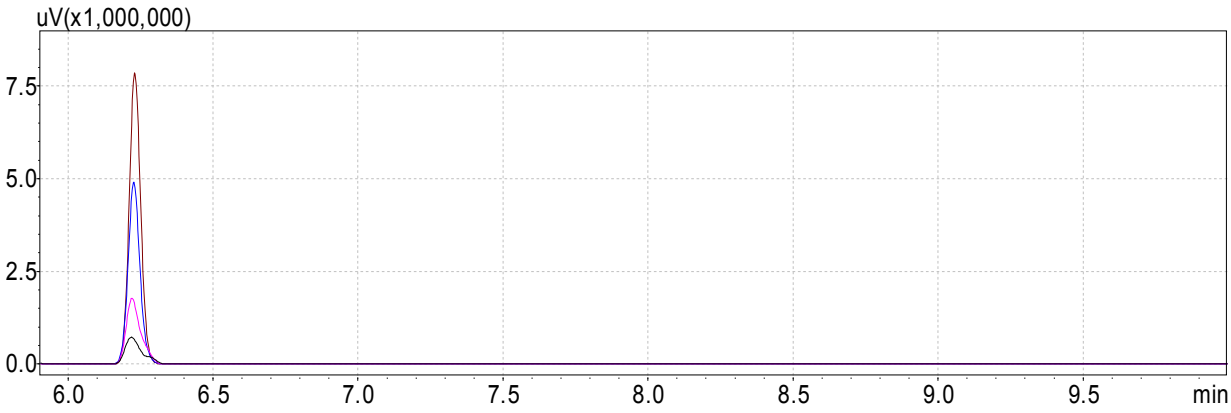


Figure 3.7 *Overlayed Chromatograms of Cyclohexane in 4 four concentrations.*

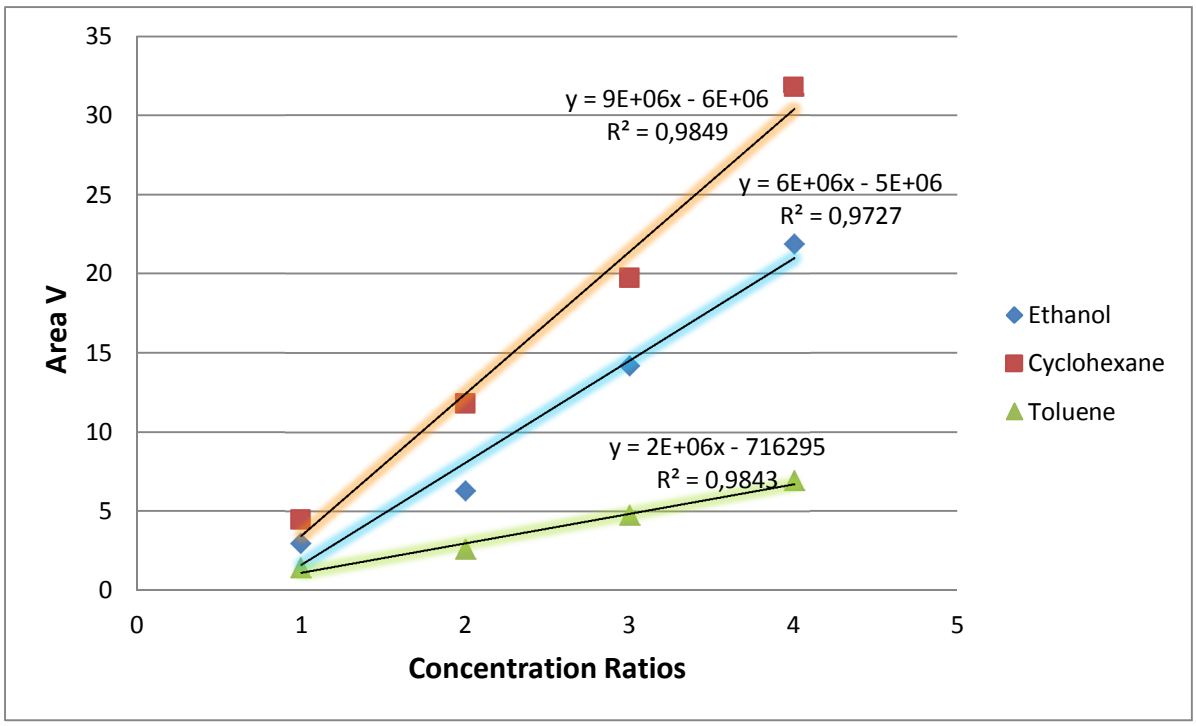


Figure 3.8 *Calibration Curve of three components in 4 different concentrations.*

This curve demonstrates the calibration on different concentrations that can be done by gas chromatography to determine an unknown concentration of a compound mixture, record a chromatogram under the same conditions as for the preparation of the calibration curve, measure the peak area of the object component, and perform the determination, using the calibration curve.

[redacted] SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The purpose of this research was to create a database for the GC-2014 in the VTRS laboratory, and to investigate the proper method;

For that a mixture of available volatile compounds was used to compare linear velocity of 12 cm/sec versus 15 cm/sec and 22.5 cm/s for gas chromatography with flame ionization detector (GC/FID). Linear velocity of 12 cm/sec was shown to give better and more consistent response than 15 cm/sec and 22.5 cm/sec resulting in potentially more accurate analyses.

Also I have conducted a series of 6 experiments on 3 semi-volatile compounds with different temperature programs, isothermal and gradient; all data confirmed that gradient temperature programing increased retention time and resolution.

The linear velocity of 12 cm/sec in gradient temperature programming was then used to evaluate a hydrocarbon mixture of n-alkanes standard C_{14} to C_{17} along with velocity of 15, 18, 22.4, and 31.9 cm/sec and shown, in general, to be able to be used as a reference for future analysis and give adequate qualitative results.

Also a demonstration of quantitative analysis by GC, through three compounds with four different concentrations in a calibration curve.

In summary, GC is an excellent analytical tool for qualitative analysis. However, common sense must be used in handling problems, and the entire system should be understood. The best technique should be used to standardize and for sample handling. The “weakest link” concept is no more pronounced that it is in qualitative GC.

REFERENCES

[1] H. M. McNair and J. M. Miller, *Basic Gas Chromatography*, Wiley, New York, 1998.

[2] R. L. Grob and E. F. Barry,*Modern Practice of Gas Chromatography,* New Jersey, 2004.

[3] R. P. W. Scott, *Gas Chromatogrphy*, D. H. Desty, London, 1958.

[4] G. Gambhir, *Chromatography (A Refresher Course CPDHE),* Acharya Narendra Dev College, USA

[5] Y. Guan, J. Kiraly and J. A. Rijks, *Journal of Chromatography,* 472 (1989) 129-143, Elsevier Science Publishers B.V., Amsterdam.

[6] http://www.chemistrydaily.com.chemistry/chromatography.html, 18 March 2012

[7] http://www.rpi.edu.html, Rensselaer Polytechnic Institute, 18 March 2012

[8] http://www.justchromatography.com.html, 18 March 2012

[9] http://www.library4science.com/eula.html. 08 April 2012

[10] http://www.chromatography-online.org/3/contents.html. 08 April 2012

[11] W. G. Jennings, M. Mittlefehdt, and P. Stremple, *Analytical Gas Chromatography, 2nd ed.,* Academic Press, New York, 1997.

[12] H. Rotzsche, *Stationary Phases in Gas Chromatography,* Elsevier Science Publishers, Germany, 1991.

[13] Adams, R.P. 1995. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream, IL.

[14] Van Den Dool, H., Kratz, P.D., *J. Chromatography*, 1963, 11, 463-471.

APPENDIXES

The following Chromatograms were run at carrier gas Linear Velocity of 12cm/sec

The constant operating conditions for the following experiment are:

Carrier gas: N_2, Purge flow rate 3 ml/min;

30 meter DB-5 capillary column (Int. Diam. 0.25 mm, 0.25 µm film thickness);

Temperature programming from 60 up to 240^{o}C at a rate of 3^{o}C/min;

Injection temperature 220^{o}C, split ratio 1:100;

FID detector temperature 250^{o}C;

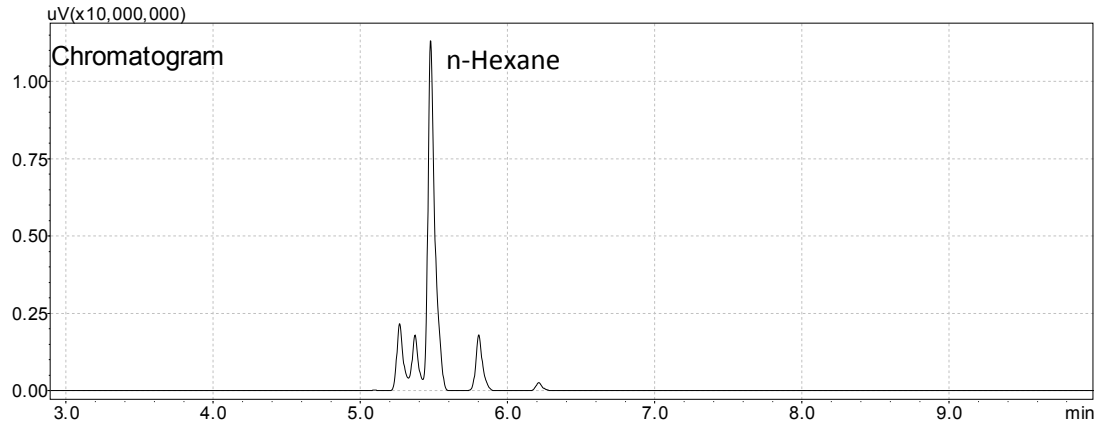


Figure A.1 *Chromatogram of n-Hexane (t_R = 329 sec).*

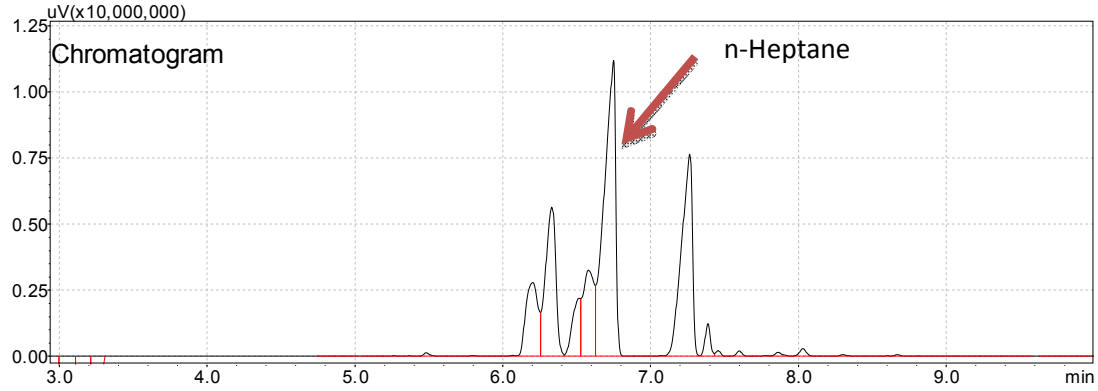


Figure A.2 *Chromatogram of n-Heptane (t_R= 405 sec).*

Note; the major peak represents the n-Heptane and the rest are impurities that came with the product since it was transported from the University of Farhat Abbas, Setif.

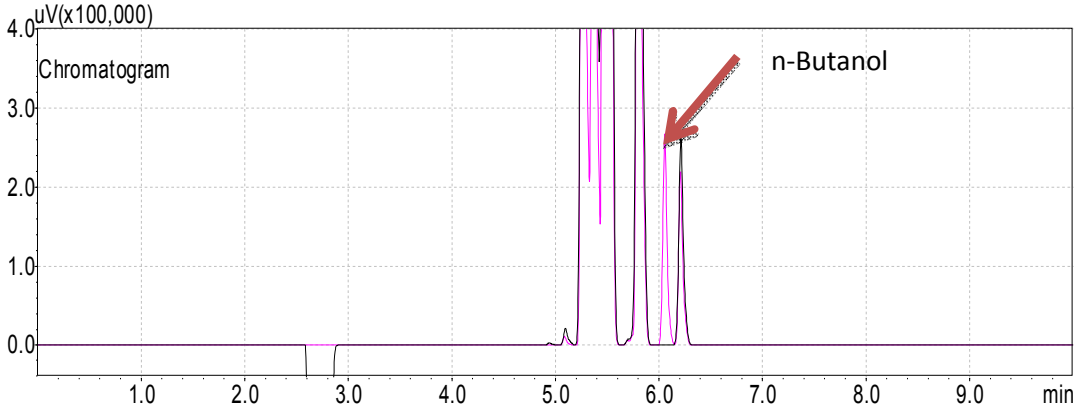


Figure A.3 *Chromatogram of n-Butanol (t_R = 363 sec).*

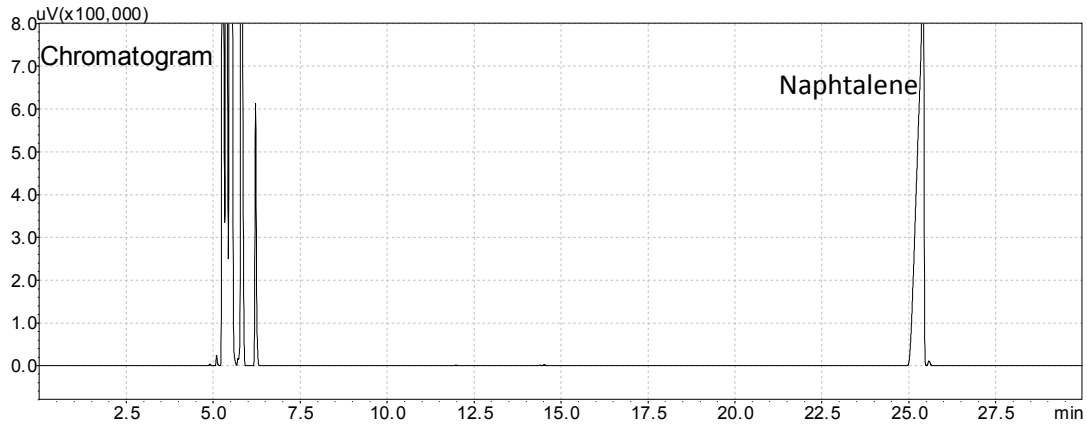


Figure A.4 *Chromatogram of Naphthalene dissolved in n-Hexane (t_R = 1525 sec).*

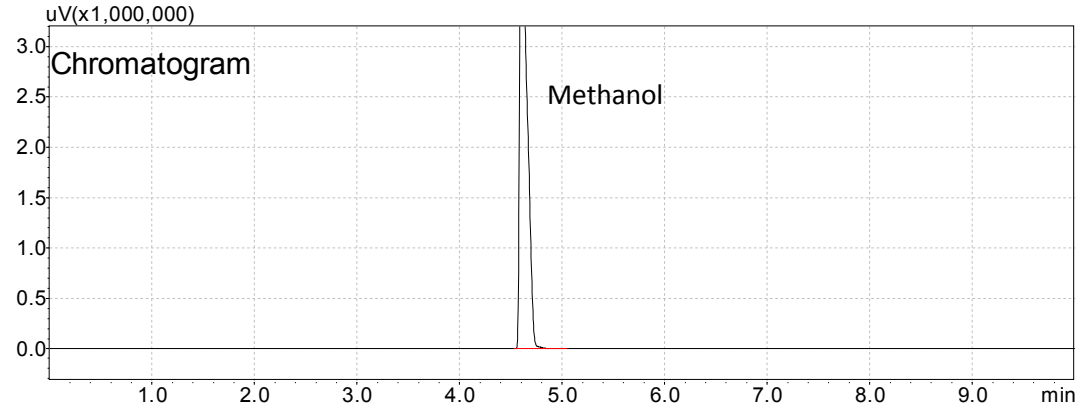


Figure A.5 *Chromatogram of Methanol (t_R = 276 sec).*

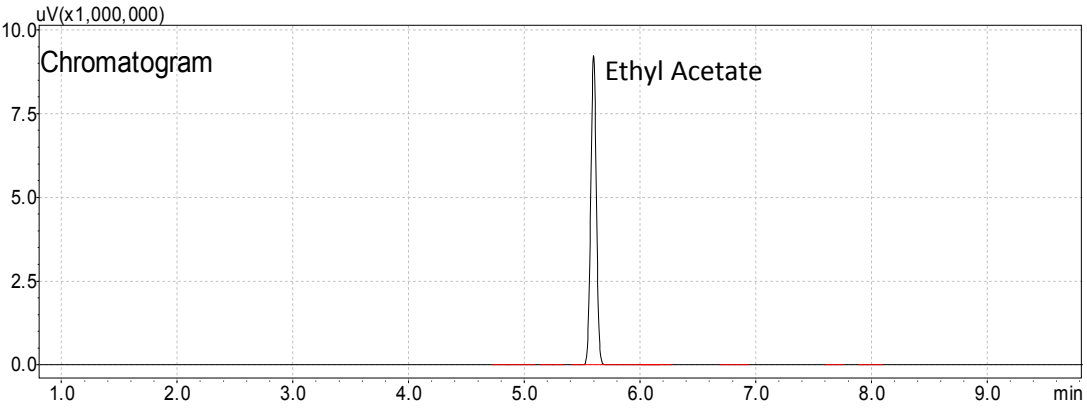


Figure A.6 *Chromatogram of Ethyl Acetate (t_R = 336 sec).*

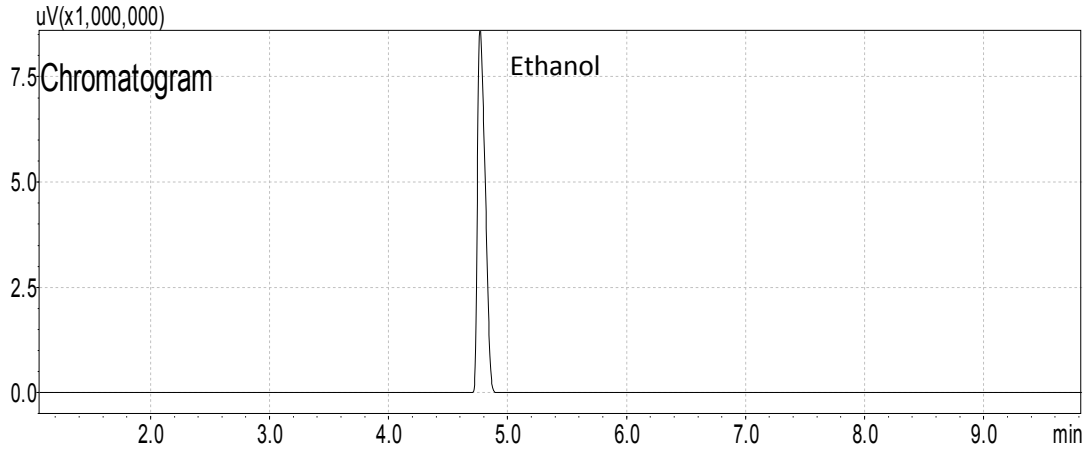


Figure A.7 *Chromatogram of Ethanol (t_R = 286 sec).*

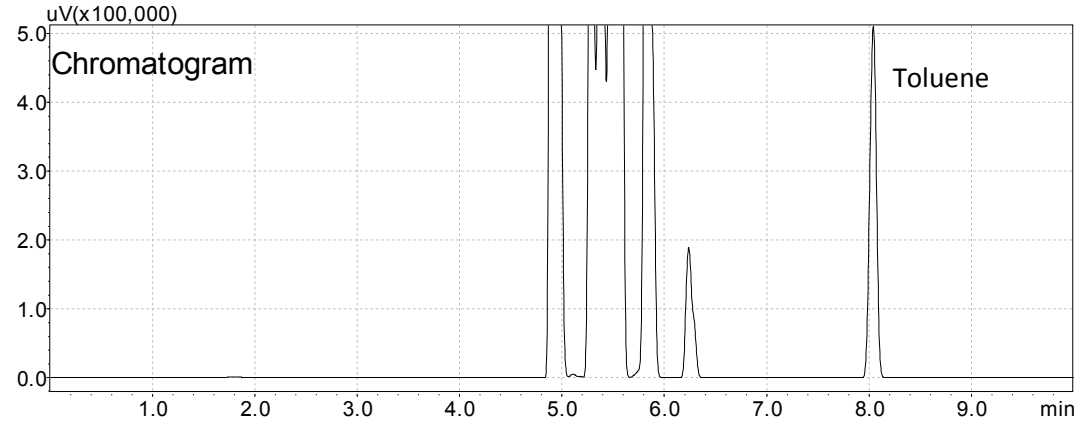


Figure A.8 *Chromatogram of Toluene with n- Hexane (t_R = 504 sec).*

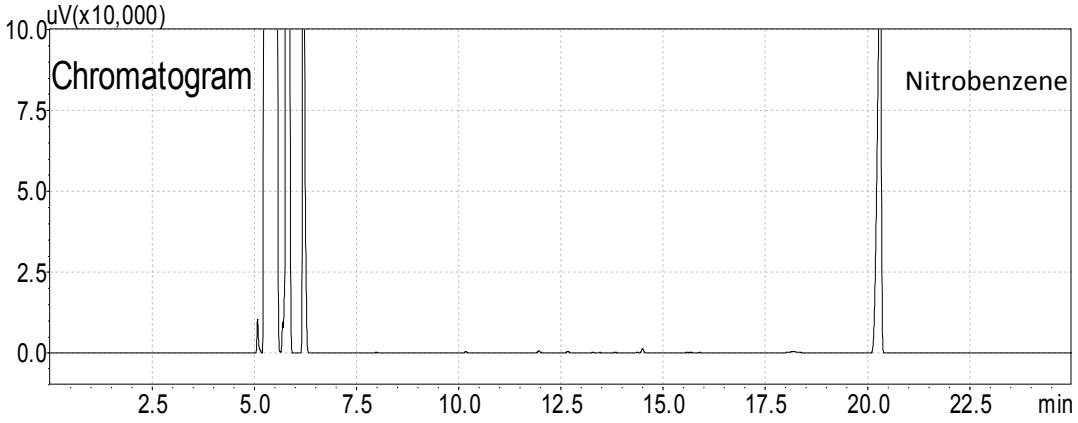


Figure A.9 *Chromatogram of Nitrobenzene dissolved in n-Hexane (t_R = 1219 sec).*

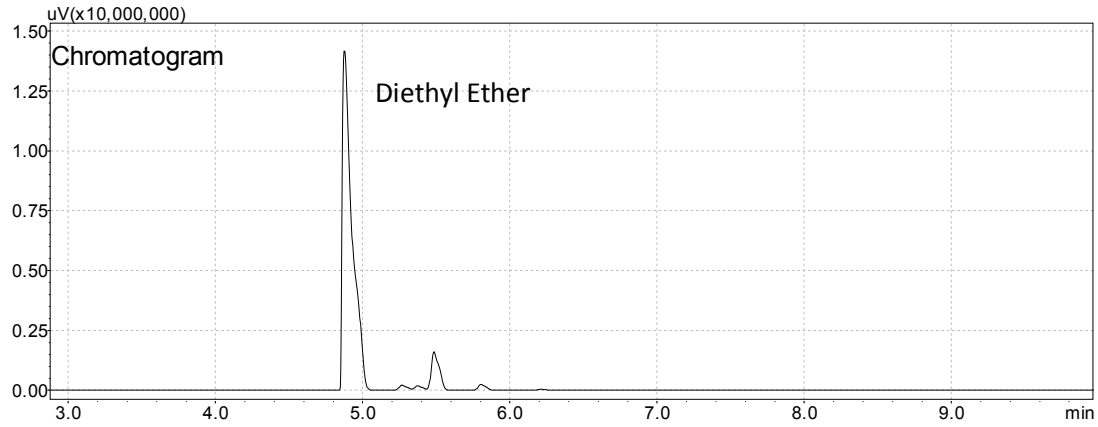


Figure A.10 *Chromatogram of Diethyl Ether (t_R = 293 sec).*

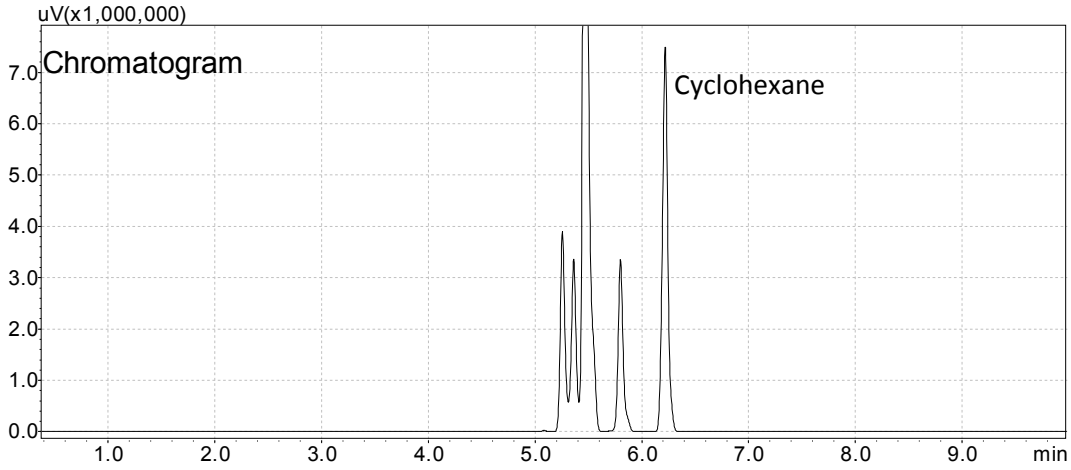


Figure A.11 *Chromatogram of Cyclohexane dissolved in n-Hexane (t_R = 373 sec).*

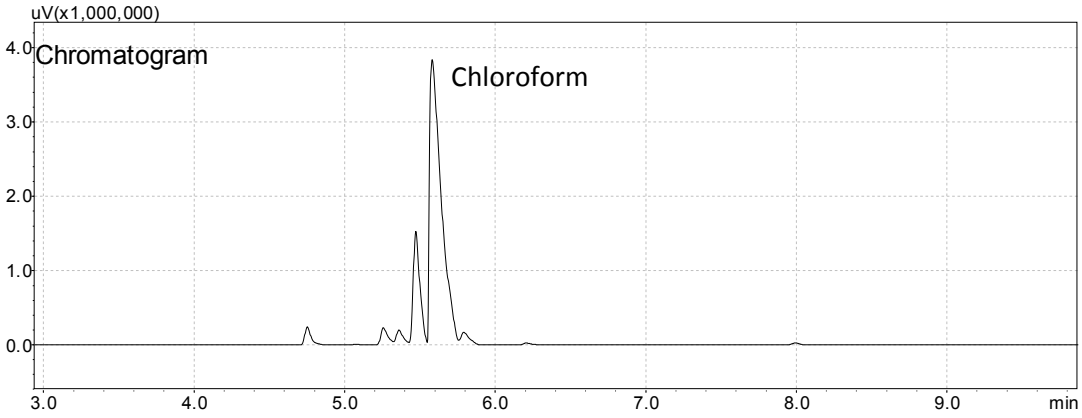


Figure A.12 *Chromatogram of Chloroform (t_R = 335 sec).*

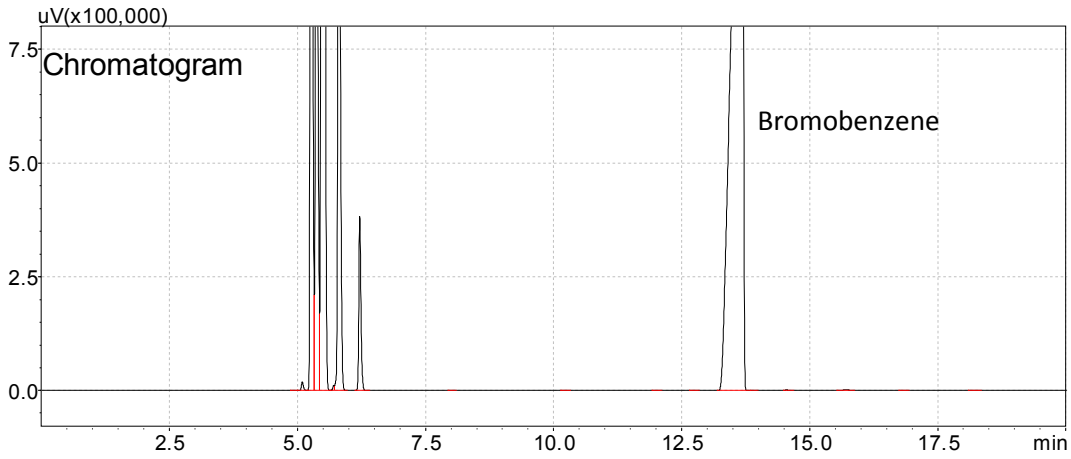


Figure A.13 *Chromatogram of Bromobenzene dissolved in n-Hexane (t_R = 699 sec).*

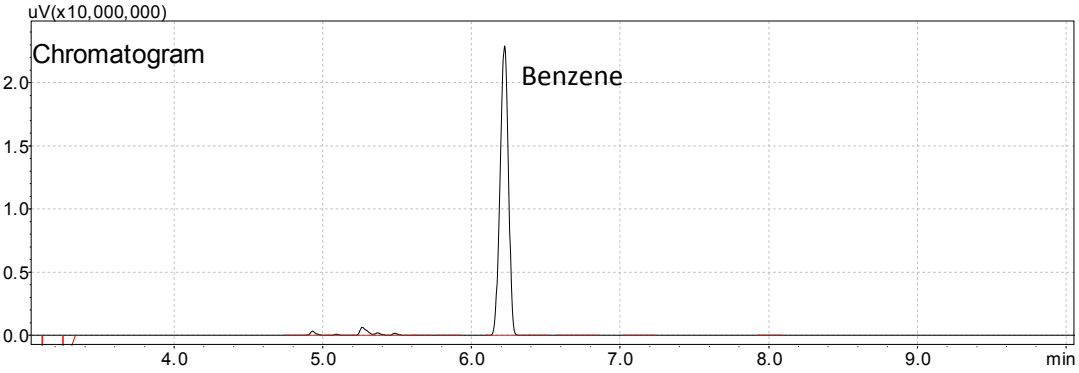


Figure A.14 *Chromatogram of Benzene (t_R = 373 sec).*

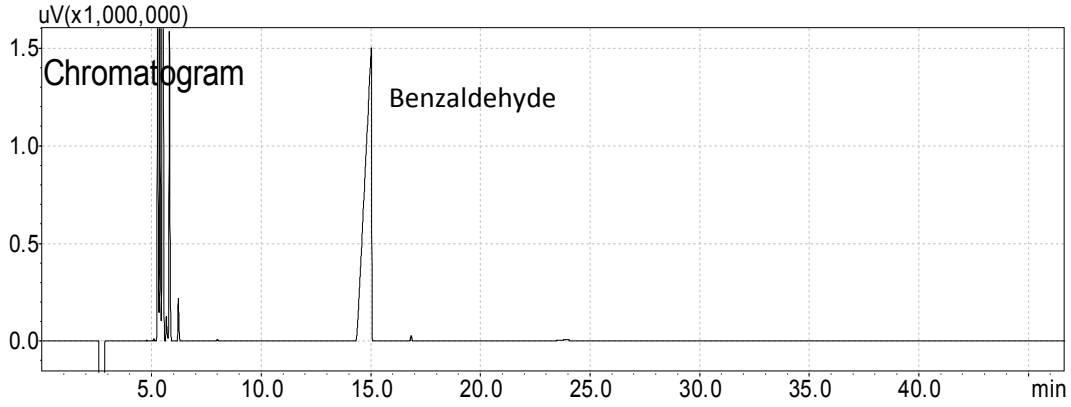


Figure A.15 *Chromatogram of Benzaldehyde dissolved in n-Hexane (t_R = 905 sec).*

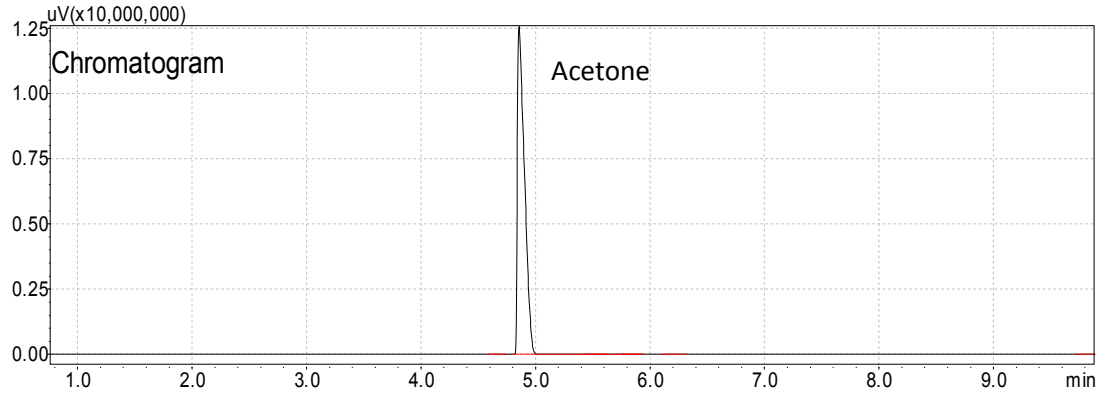


Figure A.16 *Chromatogram of Acetone (t_R = 291 sec).*

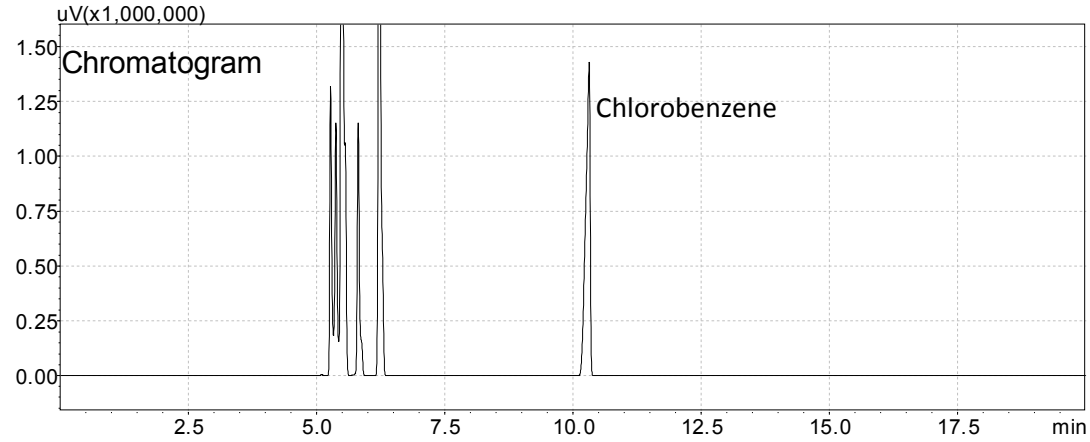


Figure A.17 *Chromatogram of Chlorobenzene dissolved in n-Hexane (t_R= 619 sec).*

The following Chromatograms were run at carrier gas Linear Velocity of 15cm/sec

The constant operating conditions for the following experiment are:

Carrier gas: N_2, Purge flow rate 3 ml/min;

30 meter DB-5 capillary column (Int. Diam. 0.25 mm, 0.25 µm film thickness);

Temperature programming from 60 up to 240^{o}C at a rate of 3^{o}C/min;

Injection temperature 220^{o}C, split ratio 1:100;

FID detector temperature 250^{o}C;

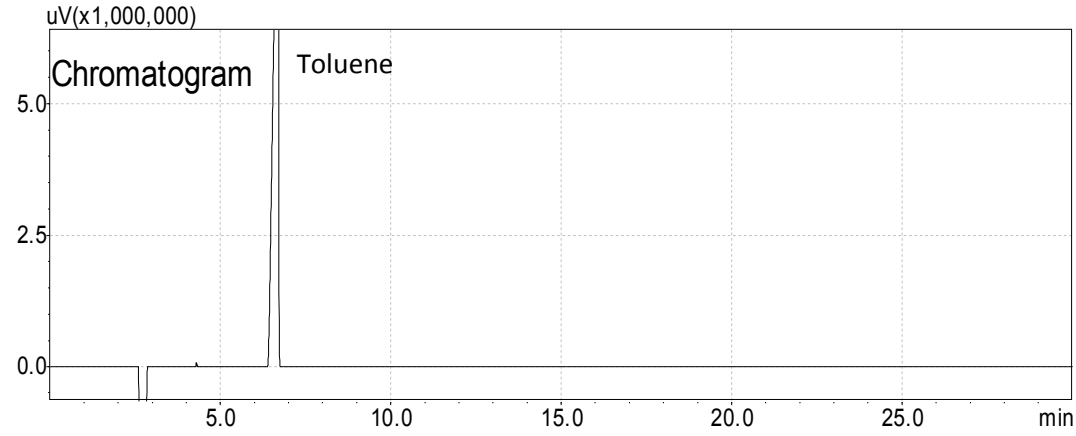


Figure A.18 *Chromatogram of Toluene (t_R =402 sec).*

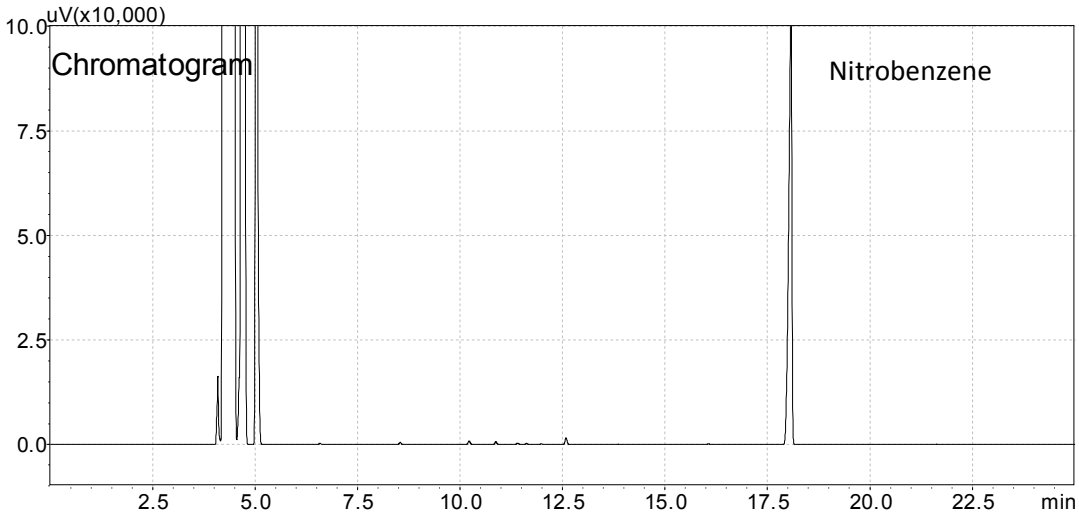


Figure A.19 *Chromatogram of Nitrobenzene dissolved in n-Hexane (t_R = 1084 sec).*

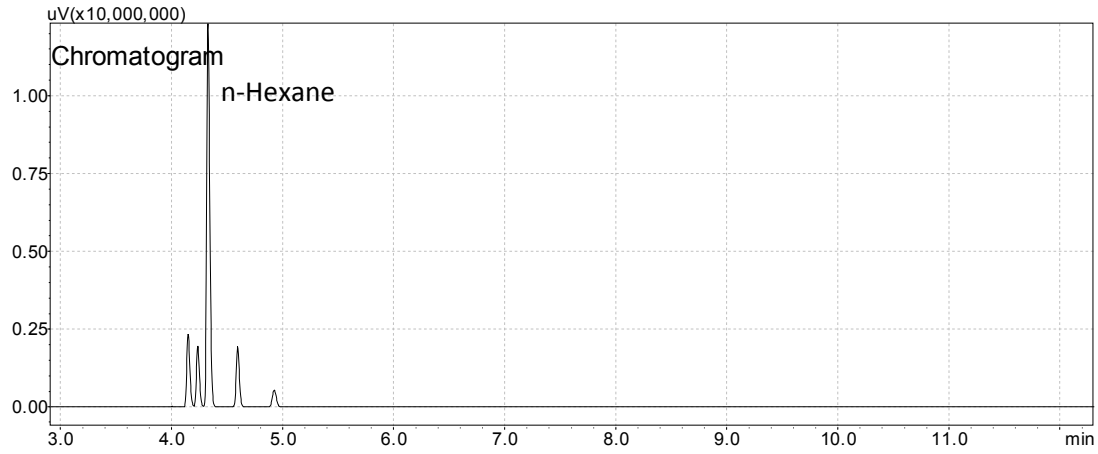


Figure A.20 *Chromatogram of n- Hexane (t_R =260 sec).*

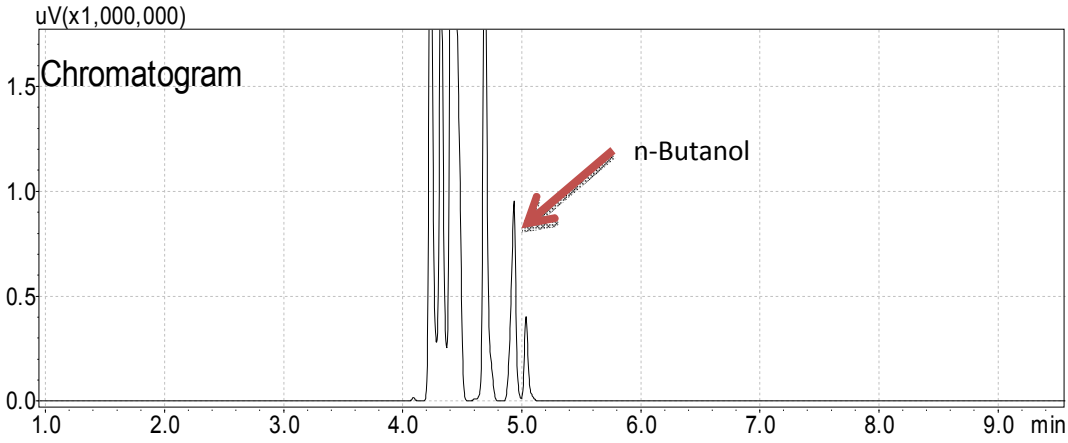


Figure A.21 *Chromatogram of n-Butanoldissolved in n-Hexane (t_R =296sec).*

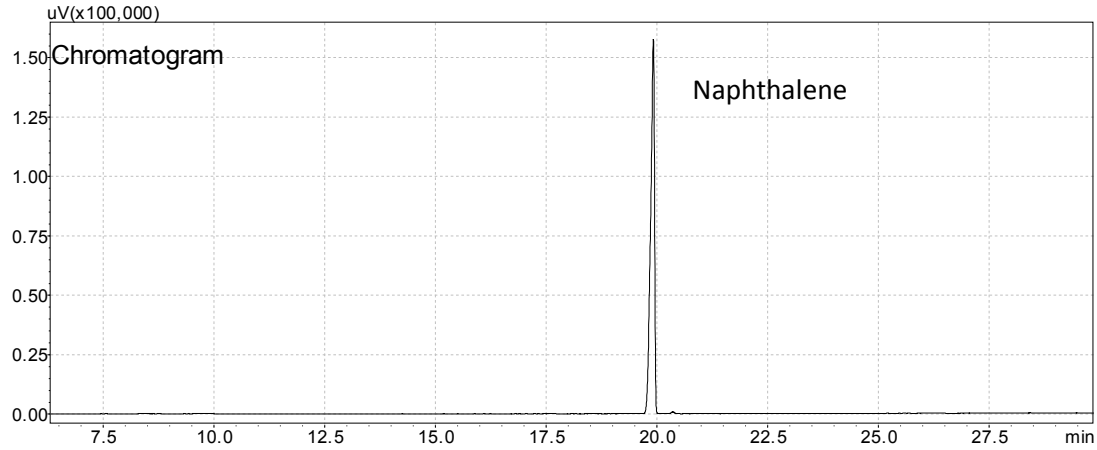


Figure A.22 *Chromatogram of Naphthalene (t_R = 1195sec).*

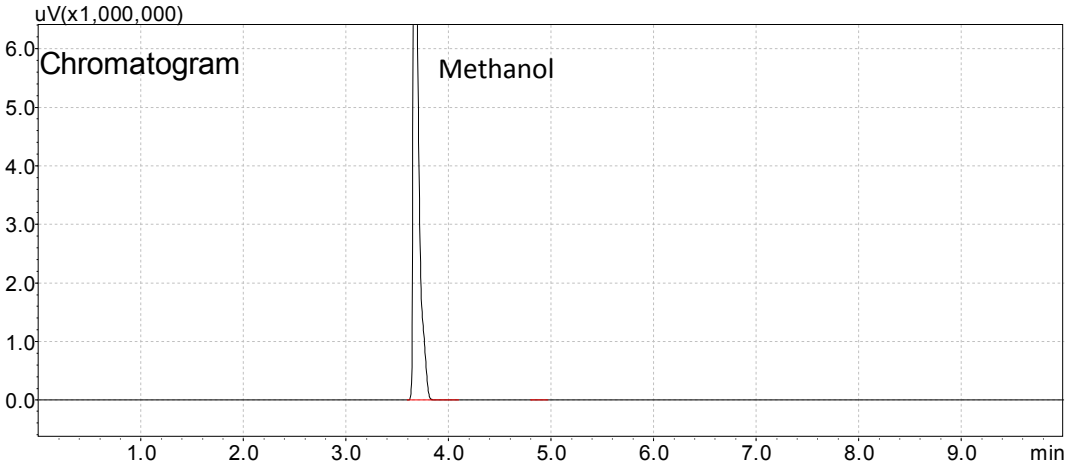


Figure A.23 *Chromatogram of Methanol (t_R = 220sec).*

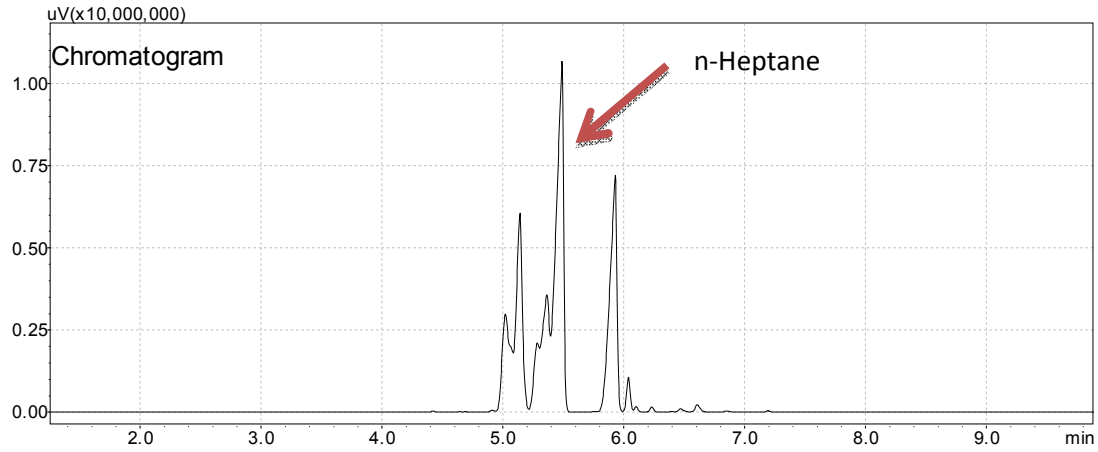


Figure A.24 *Chromatogram of n-Heptane (t_R = 329sec).*

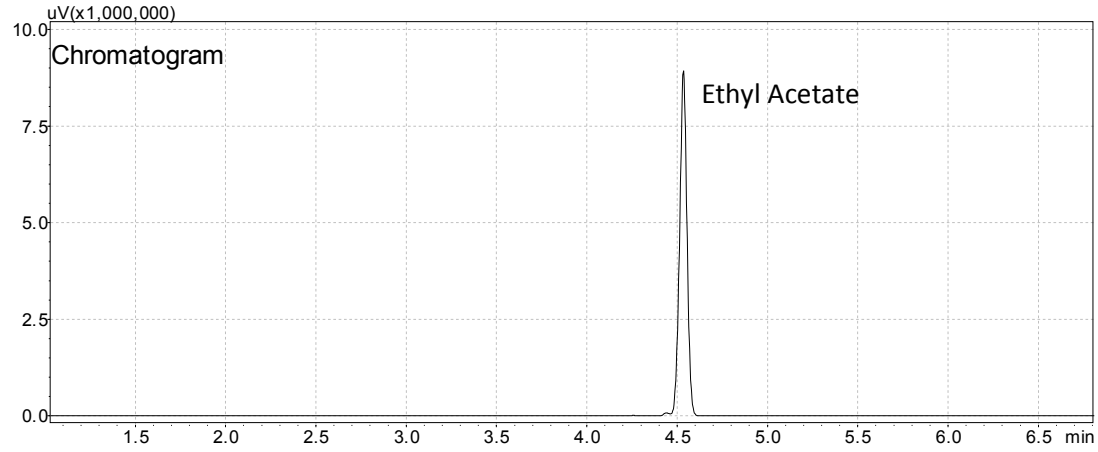


Figure A.25*Chromatogram of Ethyl Acetate (t_R = 272sec).*

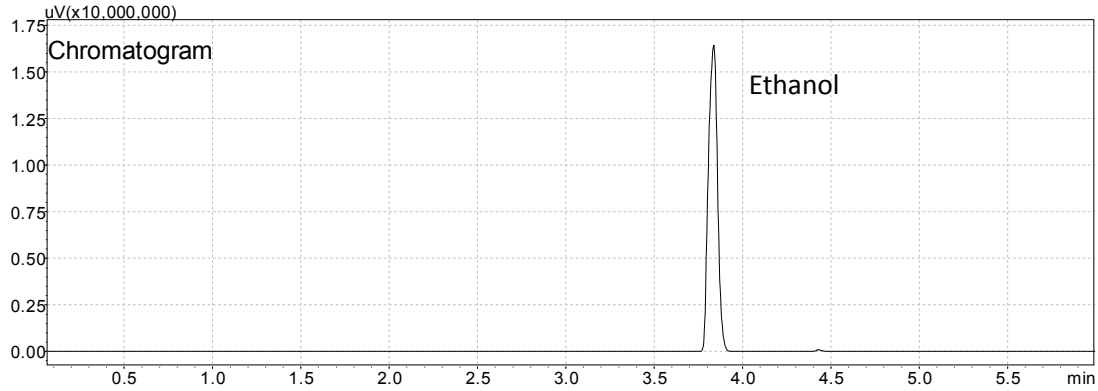


Figure A.26 *Chromatogram of Ethanol (t_R = 230sec).*

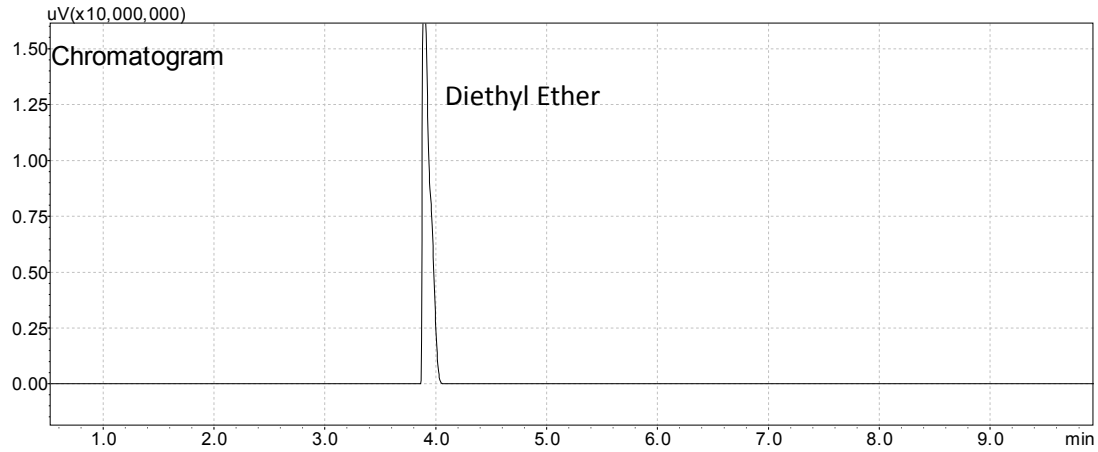


Figure A.27 *Chromatogram of Diethyl Ether (t_R = 234sec).*

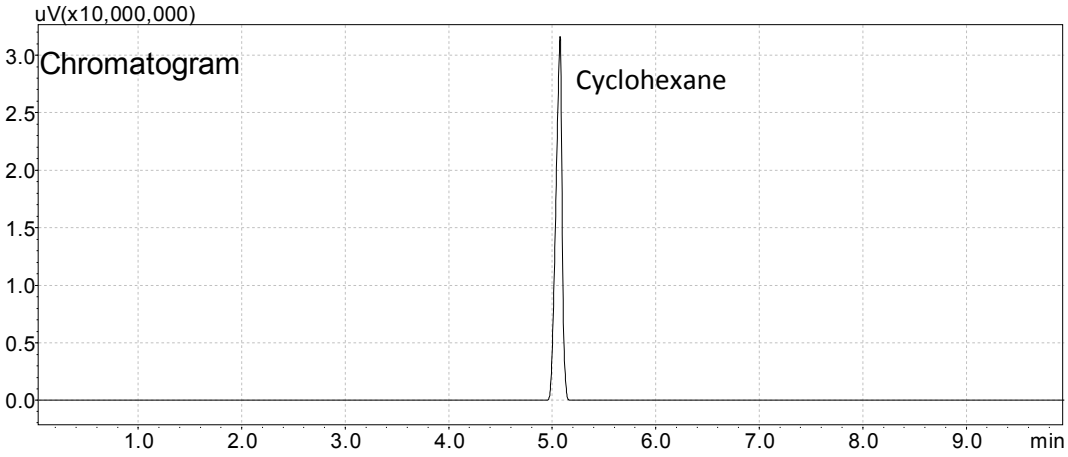


Figure A.28 *Chromatogram of Cyclohexane (t_R = 303sec).*

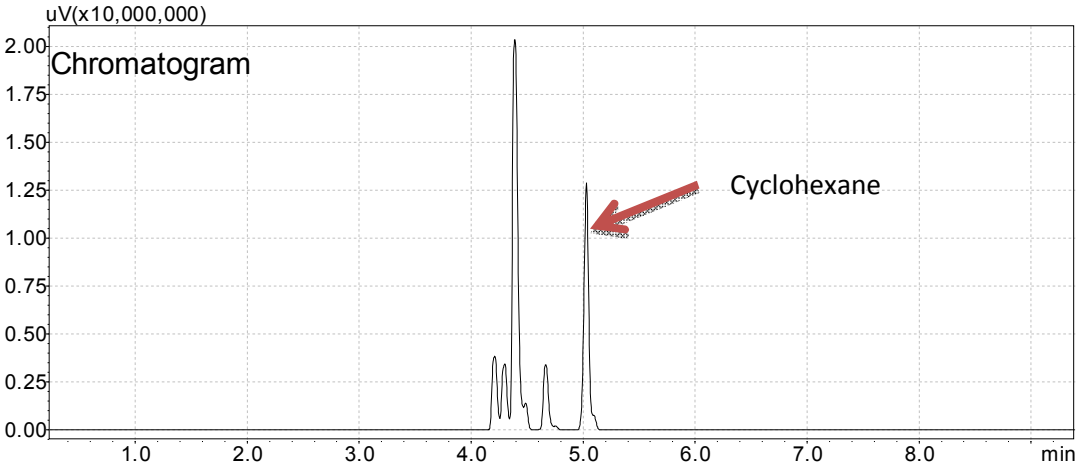


Figure A.29 *Chromatogram of Cyclohexane dissolved in n-Hexane (t_R = 303sec).*

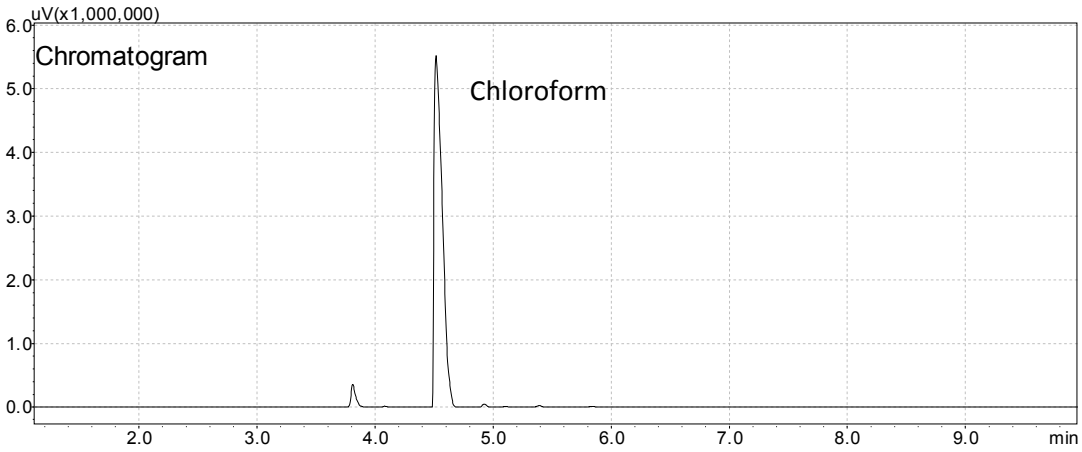


Figure A.30 *Chromatogram of Chloroform (t_R = 271sec).*

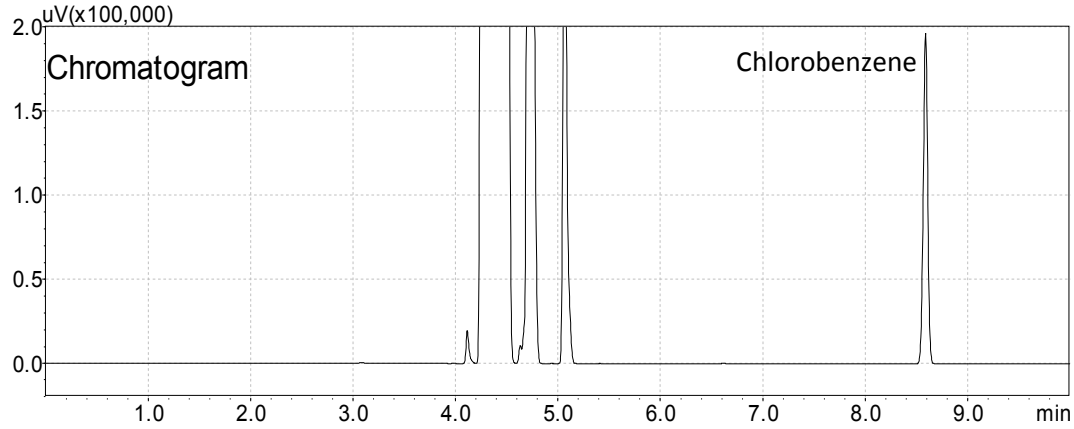


Figure A.31 *Chromatogram of Chlorobenzene dissolved in n-Hexane (t_R = 515 sec).*

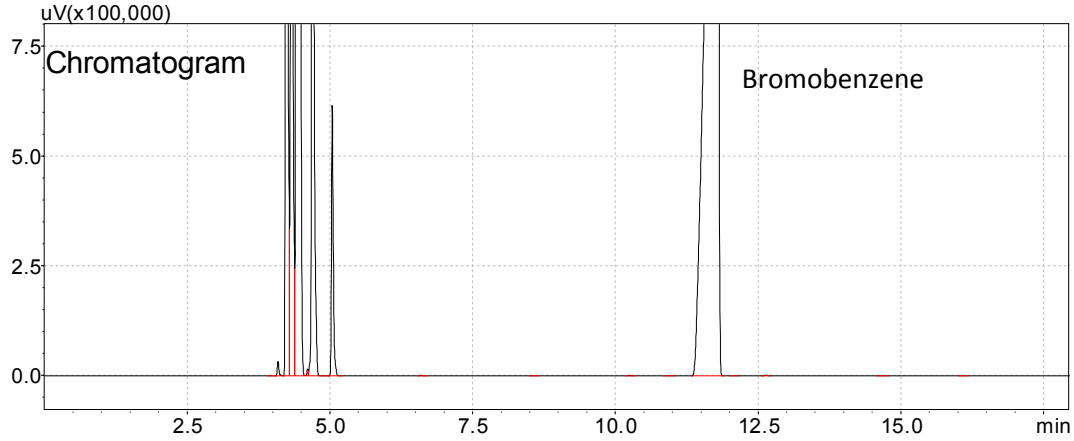


Figure A.32 *Chromatogram of Bromobenzene dissolved in n-Hexane (t_R = 708 sec).*

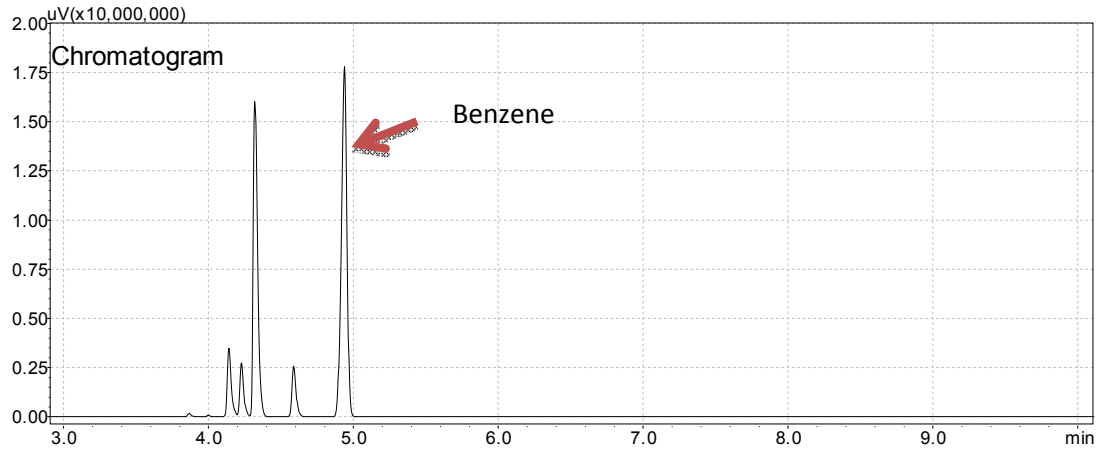


Figure A.33 *Chromatogram of Benzene dissolved in n-Hexane (t_R = 295 sec).*

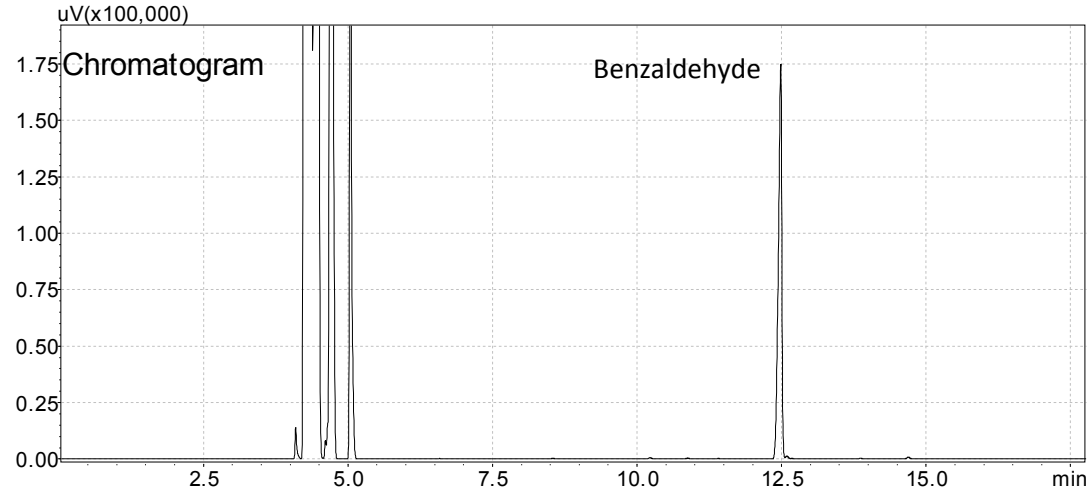


Figure A.34 *Chromatogram of Benzaldehyde dissolved in n-Hexane (t_R = 749 sec).*

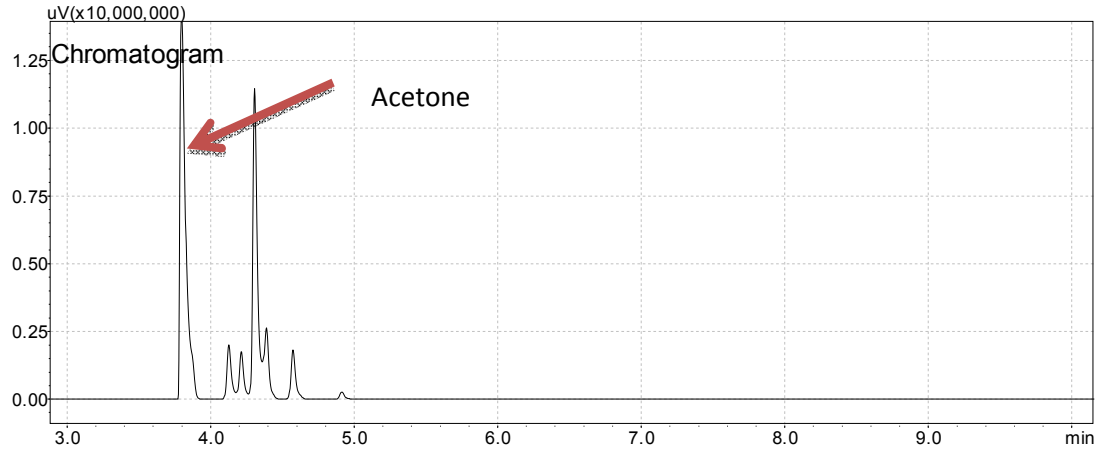


Figure A.35 *Chromatogram of Acetone dissolved n-Hexane (t_R = 228 sec).*

The following chromatograms were run at Isothermal Temperature Programing

The constant operating conditions for this experiment are as follows:

Carrier gas: N_2, Purge flow rate 3 ml/min;

30 meter DB-5 capillary column (Int. Diam. 0.25 mm, 0.25 μm film thickness);

Injection temperature 220°C, split ratio 1:100;

FID detector temperature 250°C;

Temperature programming for isothermal; from 60 up to 80°C at a rate of 3°C/min.
From 80 up to 150°C at a rate of 15°C/min.
Remains 150°C for 10 minutes.

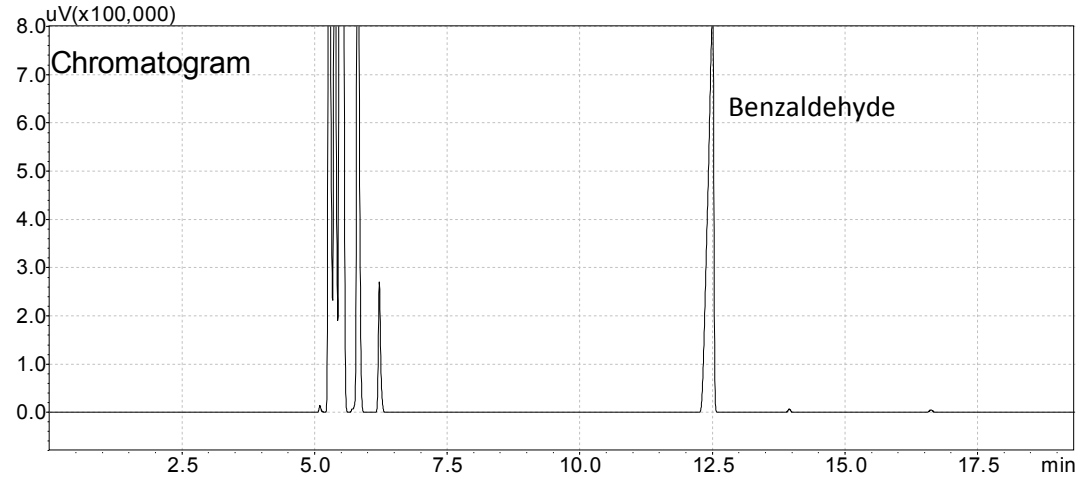


Figure A.36 *Chromatogram of Benzaldehyde in n-Hexane at LV= 12 cm/s (t_R = 750 sec).*

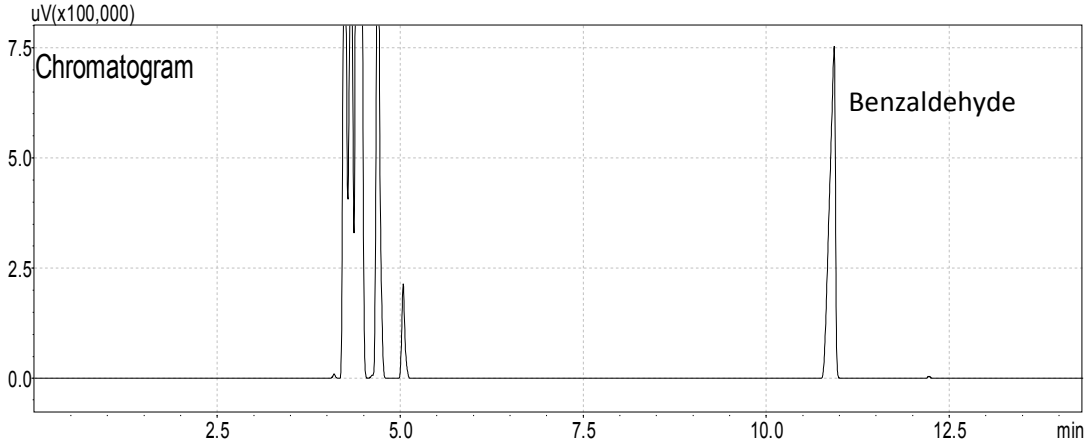


Figure A.37 *Chromatogram of Benzaldehyde in n-Hexane at LV = 15 cm/s (t_R =656 sec).*

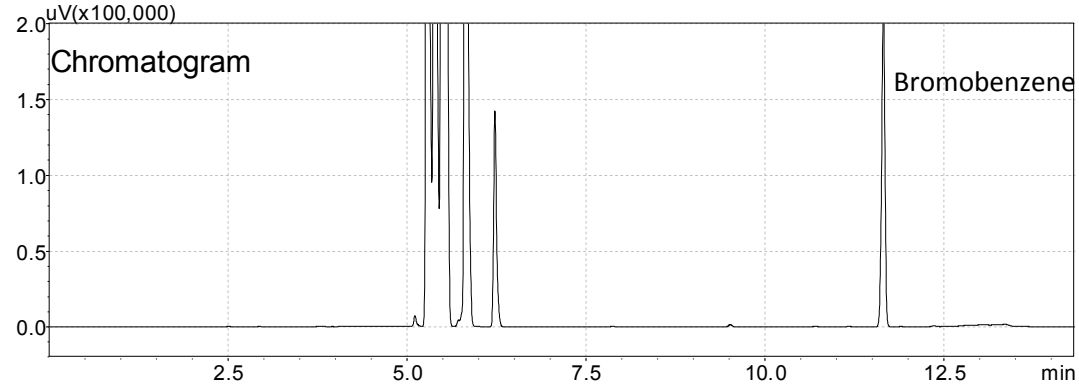


Figure A.38 *Chromatogram of Bromobenzene in n-Hexane at LV = 12 cm/s (t_R =699 sec).*

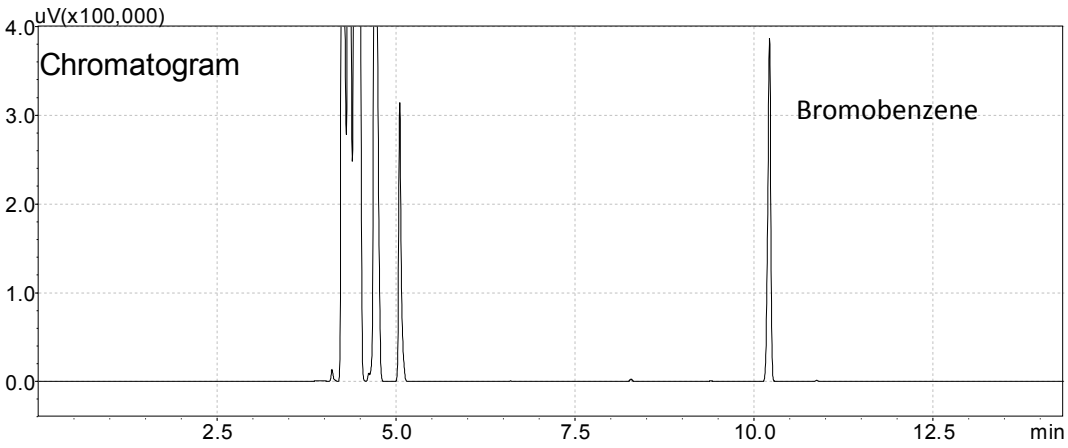


Figure A.39 *Chromatogram of Bromobenzene in n-Hexane at LV = 15 cm/s (t_R = 613 sec).*

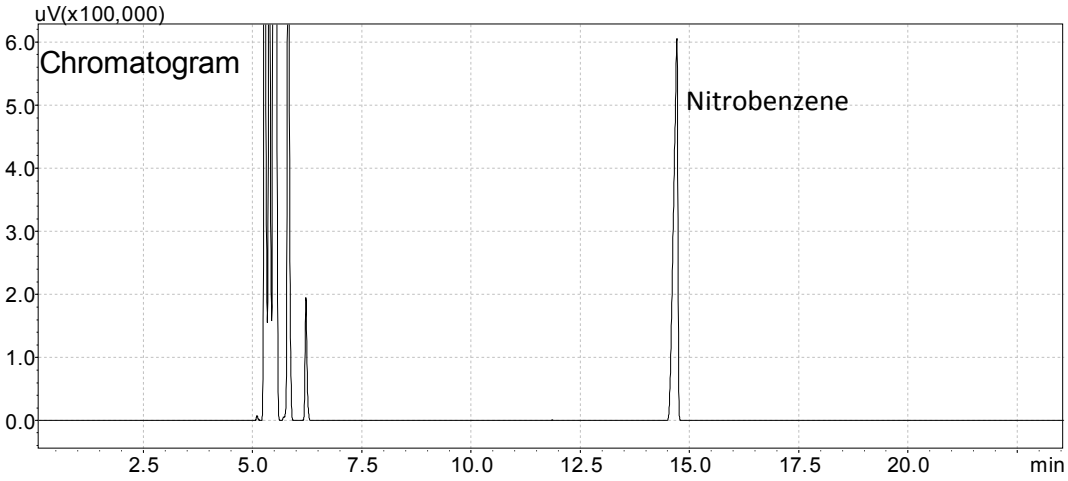


Figure A.40 *Chromatogram of Nitrobenzene in n-Hexane at LV = 12 cm/s (t_R = 883 sec).*

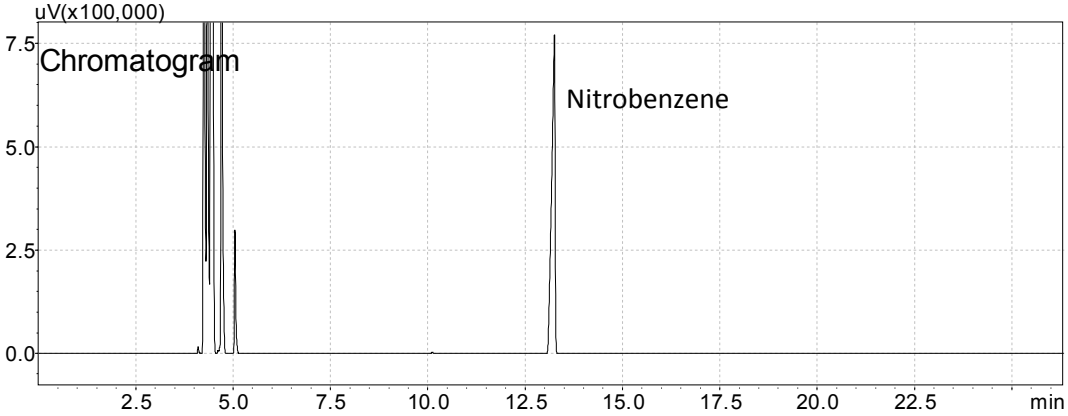


Figure A.41 *Chromatogram of Nitrobenzene in n-Hexane at LV = 15 cm/s (t_R = 795 sec).*

Chromatogram for a 1 µl injection of a mixture of 9 components at carrier gas (N_2) linear velocity of 22.4 cm/sec

(Standard conditions apply);

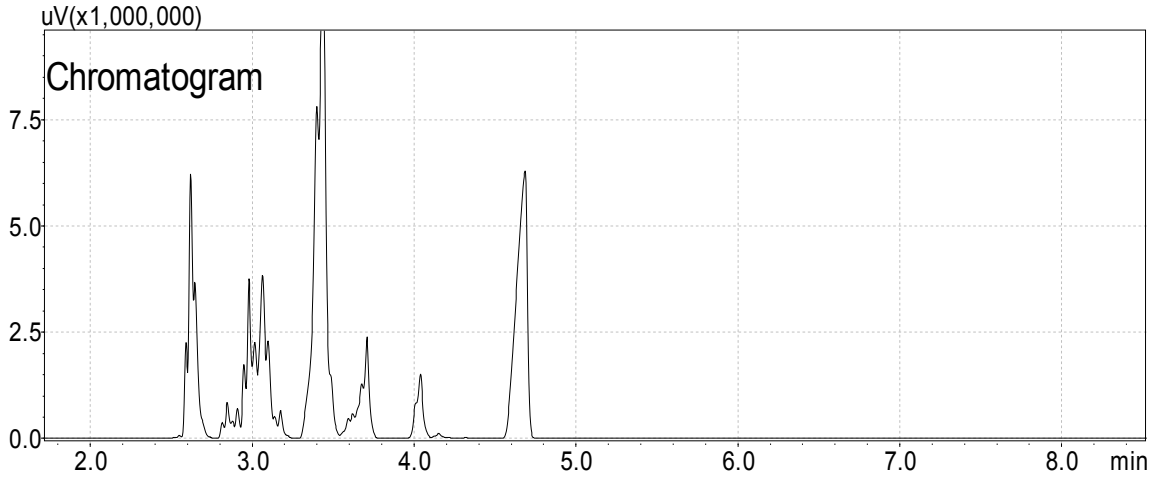


Figure A.42 *Chromatogram for a 1 µl injection of a mixture of 9 components at carrier gas (N_2) linear velocity of 22.4 cm/sec.*

The following are Chromatograms of a Standard n-Alkane Mixture C_{14} – C_{17} at 5 different Linear Velocities

The constant operating conditions for the following experiment are:

Carrier gas: N_2, Purge flow rate 3 ml/min;

30 meter DB-5 capillary column (Int. Diam. 0.25 mm, 0.25 µm film thickness);

Temperature programming from 60 up to 240^{o}C at a rate of 3^{o}C/min;

Injection temperature 220^{o}C, split ratio 1:100;

FID detector temperature 250^{o}C.

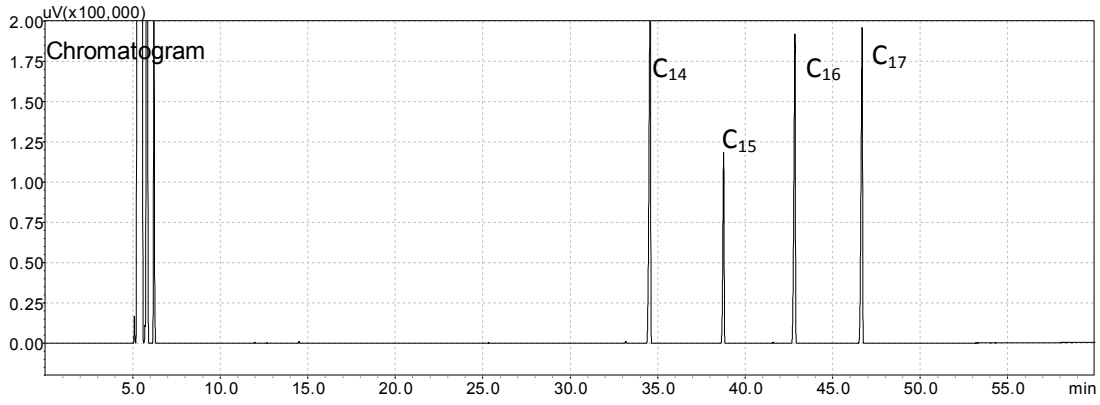


Figure A.43 *Chromatogram of n-Alkanes Standard solution (C_{14} to C_{17}) at carrier gas linear velocity of 12 cm/sec.*

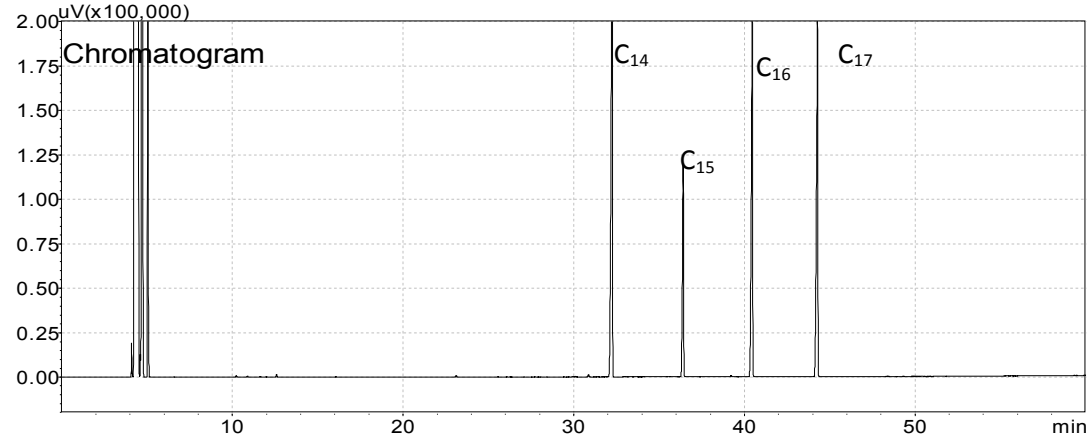


Figure A.44 *Chromatogram of n-Alkanes Standard solution (C_{14} to C_{17}) at carrier gas linear velocity of 15 cm/sec.*

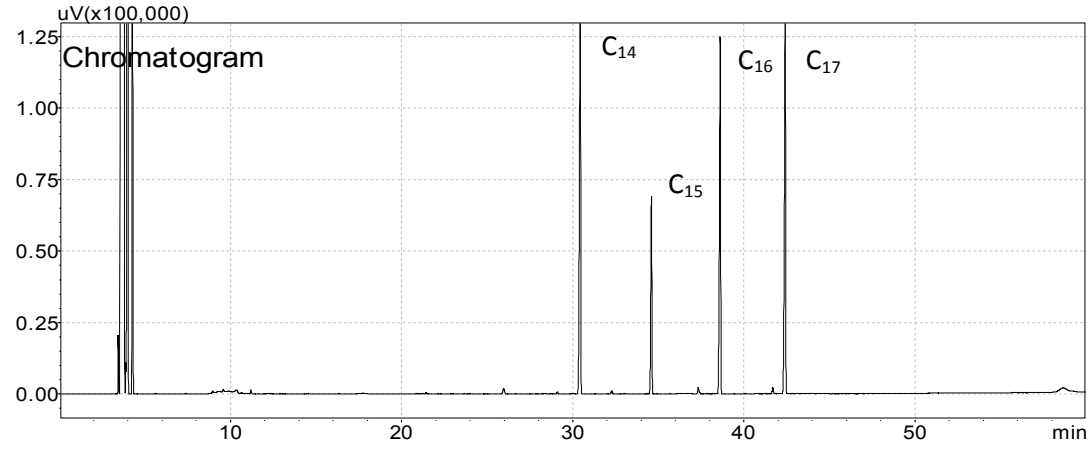


Figure A.45 *Chromatogram of n-Alkanes Standard solution (C_{14} to C_{17}) at carrier gas linear velocity of 18 cm/sec.*

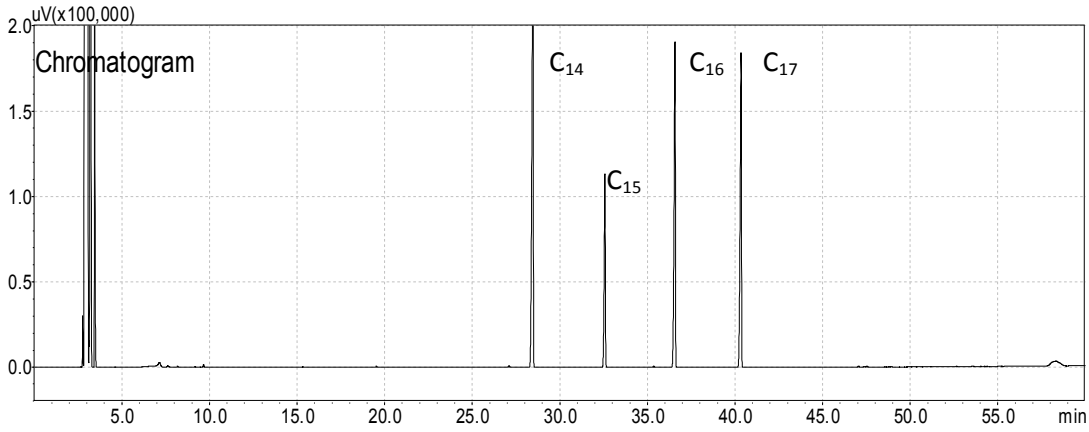


Figure A.46 *Chromatogram of n-Alkanes Standard solution (C_{14} to C_{17}) at carrier gas linear velocity of 22.4 cm/sec.*

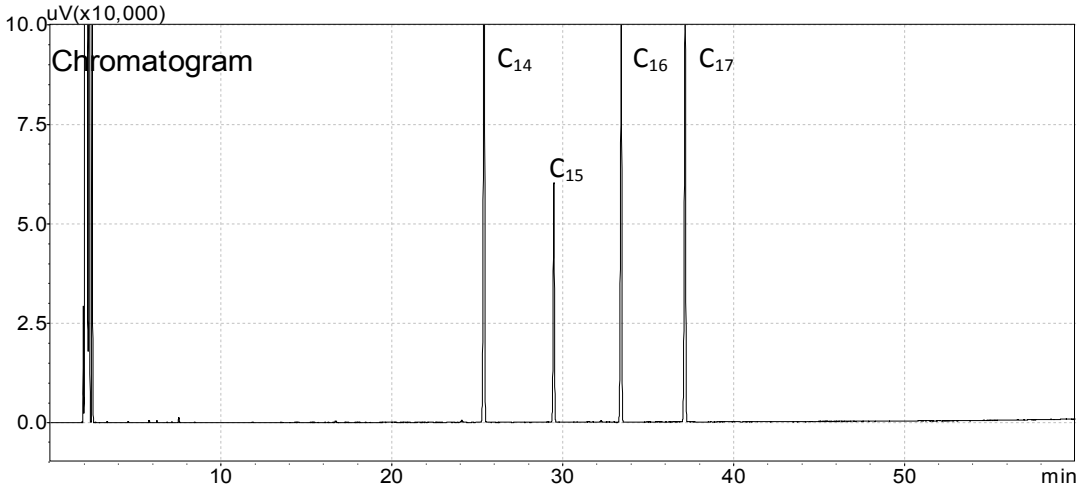


Figure A.47 *Chromatogram of n-Alkanes Standard solution (C_{14} to C_{17}) at carrier gas linear velocity of 31.9 cm/sec.*

Kovats	Compound Name	Formula
1401	1,2-Dimethoxy-4-(2-propenyl)-benzene	C11H14O2
1401	Dodecanal	C12H24O
1402	1,2-Dibutylcyclopentane	C13H26
1402	1,3,4,5,6,7-Hexahydro-1,1,5,5-tetramethyl-2H-2,4alpha-methanonaphthalene	C15H24
1402	2-Methoxy-4-((Z)-1-propenyl)-phenol	C10H12O2
1402	4,8,8-Trimethyl-9-methylenedecahydro-1,4-methanoazulene	C15H24
1402	Methyl 2-(methylamino)-benzoate	C9H11NO2
1403	(E)-9-Dodecenal	C12H22O
1404	(E)-9-Dodecenal	C12H22O
1404	(Z,1R,9S)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	C15H24
1406	(E)-2-Tetradecene	C14H28
1407	(Z)-9-Dodecenal	C12H22O
1407	(Z,1S,9R)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	C15H24
1407	Dodecanal	C12H24O
1408	4-Pyridinecarboxamide	C6H6N2O
1408	Decyl acetate	C12H24O2
1409	(5-Isopropyl-2-methyl-2-cyclohexen-1-yl)-propan-1-one	C13H22O
1409	(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	C13H20O
1409	2-Nitrobenzenamine	C6H6N2O2
1409	3R-(3à,3aá,7á,8aà)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3alpha,7-methanoazulene	C15H24
1409	alpha-Gurjunene	C15H24
1409	Dodecanal	C12H24O
1410	(Z)-9-Dodecenal	C12H22O
1410	3R-(3à,3aá,7á,8aà)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3alpha,7-methanoazulene	C15H24
1410	4-Hydroxy-3-methoxybenzaldehyde	C8H8O3
1410	Ethyl 2-aminobenzoate	C9H11NO2
1411	2-ethyl-1,4-dimethylbenzene	C10H14
1411	N-Cyclohexylcyclohexanamine	C12H23N
1412	(E)-10-Dodecenal	C12H22O
1413	(Z,1S,9R)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	C15H24
1413	Dodecanal	C12H24O
1414	(4à,4aà,7à,7aà)-Hexahydro-4,7-dimethylcyclopenta[c]pyranone	C10H16O2
1414	(Z)-2-Tetradecene	C14H28
1416	4,8,8-Trimethyl-9-methylenedecahydro-1,4-methanoazulene	C15H24
1418	(3R-(3à,3aá,7á,8aà))-Octahydro-3,8,8-trimethyl-6-methylene-1H-3a,7-methanoazulene	C15H24
1418	1R-(1R*,4E,9S*)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	C15H24
1419	4-(1-Methylethyl)-benzenemethanol acetate	C12H16O2
1420	1,7-Dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0(2,6)]heptane	C15H24
1420	4-Hydroxy-3-methoxybenzaldehyde	C8H8O3
1420	Allyl 3-cyclohexylpropionate	C12H20O2
1421	1-Phenylhexan-3-one	C12H16O
1422	3,7-Dimethyl-1,6-octadien-3-yl butyrate	C14H24O2
1422	Ionone	C13H20O
1422	Isobutyl 2-hydroxybenzoate	C11H14O3
1423	beta-Gurjunene	C15H24
1424	(Z)-10-Dodecenal	C12H22O
1424	2-(4-Methylcyclohex-3-enyl)-propyl acetate	C12H20O2

1424	2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)-ethyl acetate	C13H20O2
1425	Methyl undecanoate	C12H24O2
1426	(E)-4-(2,6,6-Trimethylcyclohex-2-enyl)-3-buten-2-one	C13H20O
1426	Ethyl 4-methoxybenzoate	C10H12O3
1427	2-Ethyl-1,4-hydroquinone	C8H10O2
1428	1,2,3,4,5,5alpha,6,8alpha-Octahydro-2,2,5alpha,8-tetramethylcyclopropa[j]naphthalene	C15H24
1428	1R-(1R*,4E,9S*)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	C15H24
1428	2H-Chromen-2-one	C9H6O2
1429	1,2,3,4,5,5alpha,6,8alpha-Octahydro-2,2,5alpha,8-tetramethylcyclopropa[j]naphthalene	C15H24
1429	2H-Chromen-2-one	C9H6O2
1430	1-Methyl-2,4-bis(1-methylethylidene)-1-vinylcyclohexane	C15H24
1432	beta-Gurjunene	C15H24
1433	1-Methyl-2,4-bis(1-methylethylidene)-1-vinylcyclohexane	C15H24
1433	4-(2,6,6-Trimethyl-1-cyclohexenyl)-butan-2-one	C13H22O
1434	(Z)-6,10-Dimethyl-5,9-undecadien-2-one	C13H22O
1434	trans-2,6-Dimethyl-6-(4-methylpent-3-enyl)-bicyclo[3.1.1]hept-2-ene	C15H24
1435	3,7-Dimethyl-6-octen-1-ol	C10H20O
1436	(E)-6,10-Dimethyl-5,9-undecadien-2-one	C13H22O
1436	(Z,E)-5,7-Dodecadienal	C12H20O
1436	trans-2,6-Dimethyl-6-(4-methylpent-3-enyl)-bicyclo[3.1.1]hept-2-ene	C15H24
1438	cis-Muurola-3,5-diene	C15H24
1439	1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene	C15H24
1439	2-Phenylethyl butyrate	C12H16O2
1439	2-Phenylpropyl isobutyrate	C13H18O2
1439	7-Isopropenyl-1,4-dimethyl-1,2,3,4,5,6,7,8-octahydroazulene	C15H24
1440	(E,E)-1,4,4-Trimethyl-8-methylene-1,5-cycloundecadiene	C15H24
1441	1-Methoxynaphthalene	C11H10O
1441	2,3,4,5,6,7,8,8alpha-Octahydro-1,9,9-trimethyl-4-methylene-1H-3alpha,7-methanoazulene	C15H24
1441	3R-(3à,3aá,6à,7á,8aà)-Octahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulene	C15H26
1442	(E)-9,11-Dodecadienal	C12H20O
1442	(E,Z)-5,7-Dodecadienal	C12H20O
1442	7-Isopropenyl-1,4-dimethyl-1,2,3,4,5,6,7,8-octahydroazulene	C15H24
1443	(E)-3-Phenyl-2-propenyl acetate	C11H12O2
1443	(Z)-7,11-Dimethyl-3-methylene-1,6,10-dodecatriene	C15H24
1443	2,6-Dimethoxyphenylacetate	C10H12O4
1443	2,6-Di-tert-butylphenol	C14H22O
1444	(E,E,E)-2,6,6,9-Tetramethyl-1,4,8-cycloundecatriene	C15H24
1444	2-(1-Methylethyl)-naphthalene	C13H14
1444	2-Methoxy-4-((E)-1-propenyl)-phenol	C10H12O2
1444	3-(4-Isopropylphenyl)-2-methylpropanal	C13H18O
1444	3,7-Dimethyl-6-octenyl propionate	C13H24O2
1444	Isopentyl octanoate	C13H26O2
1446	2-Methylphenol	C7H8O
1447	1,2,4alpha,5,6,7,8,9,9alpha-Nonahydro-3,5,5-trimethyl-9-methylene-2H-benzo[7]annulene	C15H24
1447	2-Methoxy-4-((E)-1-propenyl)-phenol	C10H12O2
1447	5,6,7,8-Tetrahydro-1-naphthalenol	C10H12O
1448	(E)-6,10-Dimethyl-5,9-undecadien-2-one	C13H22O

1449	(1S-endo)-2-Methyl-3-methylene-2-(4-methyl-3-pentenyl)-bicyclo[2.2.1]heptane	C15H24
1450	cis-Muurola-4(14),5-diene	C15H24
1450	Isopentyl octanoate	C13H26O2
1451	2-(2-(2-(Isobutoxy)-ethoxy)-ethoxy)-ethyl trifluoroacetate	C12H21F3O5
1452	(E)-3-Dodecen-1-ol	C12H24O
1452	(Z,Z)-5,7-Dodecadienal	C12H20O
1452	3-Ethoxy-4-hydroxybenzaldehyde	C9H10O3
1453	(E)-6,10-Dimethyl-5,9-undecadien-2-one	C13H22O
1454	(E,E,E)-2,6,6,9-Tetramethyl-1,4,8-cycloundecatriene	C15H24
1454	(Z)-3,7-Dimethyl-2,6-octadienyl propionate	C13H22O2
1454	5-Methyltetradecane	C15H32
1454	Neoclovene	C15H24
1456	2,3,6,7,8,8alpha-Hexahydro-1,4,9,9-tetramethyl-1H-3alpha,7-methanoazulene	C15H24
1457	(Z)-3-Dodecen-1-ol	C12H24O
1458	(E)-7,11-Dimethyl-3-methylene-1,6,10-dodecatriene	C15H24
1458	2-Vinyl-4(H)-1,3-dithiin	C6H8S2
1458	Thujopsadiene	C15H22
1459	(Z)-4-Dodecen-1-ol	C12H24O
1459	3-(4-Isopropylphenyl)-2-methylpropanal	C13H18O
1460	2-Methyl-1,3-dinitro-benzene	C7H6N2O4
1460	4-Methyltetradecane	C15H32
1460	Decahydro-1,4,8alpha-trimethyl-9-methylene-1,6-methanonaphthalene	C15H24
1460	Ethyl (E)-3-phenyl-2-propionate	C11H12O2
1461	(1S-exo)-2-Methyl-3-methylene-2-(4-methyl-3-pentenyl)-bicyclo[2.2.1]heptane	C15H24
1461	(7S)-Decahydro-1,1,7-trimethyl-4-methylene-1H-cyclopropa[e]azulene	C15H24
1461	(Z)-5-Dodecen-1-ol	C12H24O
1461	3,7-Dimethyl-1,6-octadien-3-yl 2-methylbutanoate	C15H26O2
1462	(1S-exo)-2-Methyl-3-methylene-2-(4-methyl-3-pentenyl)-bicyclo[2.2.1]heptane	C15H24
1462	(E)-2-Dodecenal	C12H22O
1462	(E)-4-Dodecen-1-ol	C12H24O
1462	(E)-6-Dodecen-1-ol	C12H24O
1462	(Z)-6-Dodecen-1-ol	C12H24O
1462	2,6,10-Trimethyltridecane	C16H34
1462	3-Methyltetradecane	C15H32
1462	Ethyl (E)-3-phenyl-2-propenoate	C11H12O2
1463	(Z)-7-Dodecen-1-ol	C12H24O
1463	1,8-Dimethyl-4-isopropenylspiro[4.5]dec-7-ene	C15H24
1463	5-Hexyl-dihydrofuran-2(3H)-one	C10H18O2
1464	(E)-5-Dodecen-1-ol	C12H24O
1464	1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene	C15H24
1464	2-(2-Isopentoxyethoxy)-ethyl acetate	C11H22O4
1465	(E)-7-Dodecen-1-ol	C12H24O
1465	1,8-Dimethyl-4-isopropenylspiro[4.5]dec-7-ene	C15H24
1465	4-(Hydroxymethyl)-benzoate	C8H7O3-
1465	Retronecine	C8H13NO2
1466	(E)-6-Dodecenal	C12H22O
1466	(E)-8-Dodecen-1-ol	C12H24O
1466	11-Dodecen-1-ol	C12H24O
1466	Dehydroaromadendrene	C15H22
1467	(E,E)-4,10-Dodecadienyl acetate	C14H24O2

1467	(Z)-2-Dodecenal	C12H22O
1467	1R-(1R*,4E,9S*)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	C15H24
1467	2-Furylmethyl heptanoate	C12H18O3
1467	2-Methyltetradecane	C15H32
1468	(Z)-8-Dodecen-1-ol	C12H24O
1468	1,5,8-Trimethyl-1,2,3,4-tetrahydronaphthalene	C13H18
1468	Butyl 2-hydroxybenzoate	C11H14O3
1469	(E)-9-Dodecen-1-ol	C12H24O
1469	p-Menth-1-en-7,8-diol	C10H18O2
1470	(E)-9-Dodecen-1-ol	C12H24O
1471	2-(4-Methyl-3-cyclohexenyl)-2-propyl isobutyrate	C14H24O2
1471	2,5,8-Trimethyltetralin	C13H18
1472	1,2,4alpha,5,8,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1472	2-Hydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one	C10H12O2
1472	3-Methyltetradecane	C15H32
1472	5-Hexyl-dihydrofuran-2(3H)-one	C10H18O2
1473	(Z)-9-Dodecen-1-ol	C12H24O
1473	1,2,4alpha,5,8,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1473	Dodecan-1-ol	C12H26O
1473	gamma-Gurjunene	C15H24
1473	Pentyl benzoate	C12H16O2
1475	(E)-3,7-Dimethyl-2,6-octadienyl propionate	C13H22O2
1475	(E,E)-5,7-Dodecadienal	C12H20O
1475	(R)-3,7,7-Trimethyl-11-methylenespiro[5.5]undec-2-ene	C15H24
1476	(R)-3,7,7-Trimethyl-11-methylenespiro[5.5]undec-2-ene	C15H24
1476	14-Methyl-(Z)-8-hexadecen-1-ol	C17H34O
1476	2-(2-(2-(Butoxy)-ethoxy)-ethoxy)-ethyl trifluoroacetate	C12H21F3O5
1476	4-Pentylbenzaldehyde	C12H16O
1477	(1à,4aà,8aà)-1,2,3,4,4a,5,6,8alpha-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	C15H24
1477	Allyl decanoate	C13H24O2
1478	(E)-10-Dodecen-1-ol	C12H24O
1478	1,2-Diallyldisulfane	C6H10S2
1479	2-Hydroxy-5-isopropyl-2,4,6-cycloheptatrien-1-one	C10H12O2
1480	(E,E)-1-Methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene	C15H24
1480	1,2,4alpha,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1481	1-Methyl-4-(6-methylhept-5-en-2-yl)-benzene	C15H22
1481	Tridecan-2-one	C13H26O
1482	3,7-Dimethyl-6-octenyl isobutyrate	C14H26O2
1483	1-Methyl-4-((S)-6-methylhept-5-en-2-yl)-benzene	C15H22
1483	trans-4,5-Epoxy-(E)-2-undecenal	C11H18O2
1484	2-Phenylpropyl butyrate	C13H18O2
1484	4alpha-Methyl-1-methylene-7-(1-methylethylidene)-decahydronaphthalene	C15H24
1485	(E)-4-(2,6,6-Trimethylcyclohex-1-enyl)-3-buten-2-one	C13H20O
1485	1,2,4alpha,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1485	7-Isopropenyl-4alpha-methyl-1-methylenedecahydronaphthalene	C15H24
1486	Dodecanenitrile	C12H23N
1489	2-Methyl-1-tetradecene	C15H30
1489	4-Methoxybenzoic acid	C8H8O3
1489	Phenylethyl 3-methylbutanoate	C13H18O2

1490	(Z)-10-Dodecen-1-ol	C12H24O
1490	1,2,3,5,6,7,8,8alpha-Octahydro-1,8alpha-dimethyl-7-(prop-1-en-2-yl)-naphthalene	C15H24
1490	1,4-Dimethyl-7-(1-methylethylidene)-1,2,3,4,5,6,7,8-octahydroazulene	C15H24
1490	Undecanoic acid	C11H22O2
1491	(E)-1,2-Dimethoxy-4-(1-propenyl)-benzene	C11H14O2
1491	(Z)-3,7-Dimethyl-2,6-octadienyl isobutyrate	C14H24O2
1491	1,2,3,5,6,7,8,8alpha-Octahydro-1,8alpha-dimethyl-7-(prop-1-en-2-yl)-naphthalene	C15H24
1493	(Z)-1,2,3,5,6,7,8,8alpha-Octahydro-1,4-dimethyl-7-(prop-1-en-2-yl)azulene	C15H24
1493	1,1,4,7-Tetramethyl-1alpha,2,3,5,6,7,7alpha,7b-octahydro-1H-cyclopropa[e]azulene	C15H24
1493	3-Methyl-2H-chromen-2-one	C10H8O2
1494	(E)-4-(2,6,6-Trimethylcyclohex-1-enyl)-3-buten-2-one	C13H20O
1494	1,1,4,7-Tetramethyl-1alpha,2,3,5,6,7,7alpha,7b-octahydro-1H-cyclopropa[e]azulene	C15H24
1494	1,2,3,4,4alpha,5,6,8alpha-Octahydro-4a,8-dimethyl-2-(prop-1-en-2-yl)-naphthalene	C15H24
1494	3,7,11,11-Tetramethylbicyclo[8.1.0]2,6-undecadiene	C15H24
1494	Ethyl undecanoate	C13H26O2
1495	(R)-2-Methyl-5-((S)-6-methylhept-5-en-2-yl)-cyclohexa-1,3-diene	C15H24
1495	6-Isopropyl-4,8alpha-dimethyl-1,2,3,7,8,8alpha-hexahydronaphthalene	C15H24
1495	Heliotridine	C8H13NO2
1496	5-Isopropenyl-3,6-dimethyl-6-vinyl-4,5,6,7-tetrahydro-1-benzofuran	C15H20O
1496	Benzyl (E)-2-methyl-2-butenoate	C12H14O2
1497	Epizonarene	C15H24
1499	(E,E)-1-Methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene	C15H24
1499	(Z)-1,2,4alpha,5,6,7,8-Heptahydro-3,5,5,9-tetramethyl-2H-benzo[7]annulene	C15H24
1499	1,2,4alpha,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1500	(E)-1,2-Dimethoxy-4-(1-propenyl)-benzene	C11H14O2
1500	(R)-1,5,5,9-Tetramethylspiro[5.5]undeca-1,8-diene	C15H24
1500	Pentadecane	C15H32
1500	trans-2,3-Epoxydodecanal	C12H22O2
1501	(E)-9,11-Dodecadien-1-ol	C12H22O
1502	(Z,E)-1,5-Dimethyl-8-(prop-1-en-2-yl)-1,5-cyclodecadiene	C15H24
1502	1-Methyl-4-(1,2,2-trimethylcyclopentyl)-benzene	C15H22
1503	(Z)-1,2,3,5,6,7,8,8alpha-Octahydro-1,4-dimethyl-7-(prop-1-en-2-yl)azulene	C15H24
1503	(Z,E)-1,5-Dimethyl-8-(prop-1-en-2-yl)-1,5-cyclodecadiene	C15H24
1503	1-Methyl-4-(1,2,2-trimethylcyclopentyl)-benzene	C15H22
1503	Tetrahydro-6-pentylpyran-2-one	C10H18O2
1504	(S)-1-Methyl-4-((Z)-6-methylhepta-2,5-dien-2-yl)-cyclohex-1-ene	C15H24
1504	2,6-Dimethylphenol	C8H10O
1504	2,6-di-t-Butyl-4-methylphenol	C15H24O
1504	4-Methyl-9H-fluorene	C14H12
1504	alpha-Alaskene	C15H24
1504	beta-Dihydroagarofuran	C15H26O
1505	(Z)-1,2,3,5,6,7,8,8alpha-Octahydro-1,4-dimethyl-7-(prop-1-en-2-yl)azulene	C15H24
1505	(Z,E)-1,5-Dimethyl-8-(prop-1-en-2-yl)-1,5-cyclodecadiene	C15H24
1506	1,2,4alpha,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1506	5-Isopropenyl-3,6-dimethyl-6-vinyl-4,5,6,7-tetrahydro-1-benzofuran	C15H20O
1507	(E)-2-Pentadecene	C15H30
1507	2-(2-Pentoxyethoxy)-ethyl acetate	C11H22O4
1507	2,6,10-Trimethyl-9-undecenal	C14H26O
1507	6-(1-Methylethyl)-quinoline	C12H13N
1508	(E,E)-3,7,11-Trimethyl-1,3,6,10-dodecatetraene	C15H24

1509	(5R,7R,10S)-Selina-4(14),11-diene	C15H24
1509	1-Methyl-4-(6-methylhepta-1,5-dien-2-yl)-cyclohex-1-ene	C15H24
1510	4-Methoxybenzyl propionate	C11H14O3
1511	(Z,E)-5,7-Dodecadien-1-ol	C12H22O
1511	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	C15H26O
1512	1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4alpha,5,6,8alpha-octahydro-naphthalene	C15H24
1512	2,6-di-t-Butyl-4-methylphenol	C15H24O
1512	2-Ethylphenol	C8H10O
1512	beta-Dehydro-ar-himachalene	C15H20
1512	Tridecan-1-ol	C13H28O
1513	1-(4-Hydroxy-3-methoxyphenyl)-propan-2-one	C10H12O3
1513	1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4alpha,5,6,8alpha-octahydro-naphthalene	C15H24
1513	3-Methyl-2-phenylpyridine	C12H11N
1513	Pentadecanal	C15H30O
1514	(E)-3,7-Dimethyl-2,6-octadienyl isobutyrate	C14H24O2
1514	(E,Z)-5,7-Dodecadien-1-ol	C12H22O
1514	7-epi-alpha-Selinene	C15H24
1515	(Z,E)-4,4-(1,5-Dimethyl-4-heptenylidene)-1-methylcyclohexene	C16H26
1515	2-(2-(2-(Isobutoxy)-ethoxy)-ethoxy)-ethanol	C10H22O4
1515	2-Isopropyl-5-methylcyclohexyl 3-methylbutanoate	C15H28O2
1516	4-(4-Methyl-3-pentenyl)-3-cyclohexene-1-carbaldehyde	C13H20O
1517	(Z)-2-Pentadecene	C15H30
1517	1,2,3,4,4alpha,5,6,8alpha-Octahydro-4a,8-dimethyl-2-(prop-1-en-2-yl)-naphthalene	C15H24
1517	1-Methyl-4-((R)-6-methylhept-5-en-2-yl)-cyclohexa-1,4-diene	C15H24
1518	(E)-4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one	C14H22O
1518	7-Isopropyl-4,10-dimethyltricyclo[4.4.0.0(1,5)]decan-4-ol	C15H26O
1520	1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl 3-methylbutanoate	C15H26O2
1520	6-Allyl-4-methoxy-1,3-benzodioxole	C11H12O3
1521	Bibenzyl	C14H14
1522	3-(4-tert-Butylphenyl)-2-methylpropanal	C14H20O
1524	1,2,3,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1524	1,3,7-Trimethylnaphthalene	C13H14
1524	2-Methoxy-4-(2-propenyl)-phenol acetate	C12H14O3
1524	4-(Acetyloxy)-3-methoxy-benzaldehyde	C10H10O4
1524	8-Methoxyquinoline	C10H9NO
1525	2-Naphthol	C10H8O
1525	Methyl 4-hydroxy-3-methoxybenzoate	C9H10O4
1525	Methyl dodecanoate	C13H26O2
1526	4-Hydroxy-3-(methoxymethyl)-benzoate	C9H9O4-
1526	8-Isopropyl-2,5-dimethyl-1,2,3,4-tetrahydronaphthalene	C15H22
1527	2,6-Di-tert-butyl-4-methoxyphenol	C15H24O2
1527	gamma-Dehydro-ar-himachalene	C15H20
1528	1-Methyl-2,4-dinitrobenzene	C7H6N2O4
1529	2-Chlorophenol	C6H5ClO
1529	3,7-Dimethyl-6-octenyl butyrate	C14H26O2
1530	1,2,3,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1530	1,3,6-Trimethylnaphthalene	C13H14
1530	2-(4,8-Dimethyl-3,7-cyclodecadien-1-yl)-propan-2-ol	C15H26O
1531	1,2,3,4,4alpha,7-Hexahydro-4-isopropyl-1,6-dimethylnaphthalene	C15H24
1531	Isopentyl 2-hydroxybenzoate	C12H16O3

1532	1,2,3,4,4alpha,5,6,8alpha-Octahydro-4alpha,8-dimethyl-2-(propan-2-ylidene)-naphthalene	C15H24
1532	1,2,3,4,4alpha,7-Hexahydro-4-isopropyl-1,6-dimethylnaphthalene	C15H24
1532	Epiglobulol	C15H26O
1534	(5R,7R,10S)-Selina-3,11-diene	C15H24
1534	(Z)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	C15H26O
1534	1,2,4alpha,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1534	1,4,6-Trimethylnaphthalene	C13H14
1537	(E,E)-5,7-Dodecadien-1-ol	C12H22O
1537	1,2,3-Trimethylnaphthalene	C13H14
1537	1,2,4alpha,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1538	1,2,4alpha,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	C15H24
1539	(E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	C15H26O
1540	5-Allyl-1,2,3-trimethoxybenzene	C12H16O3
1540	8,14-Cedranoxide	C15H24O
1541	cis-Muurol-5-en-4alpha-ol	C15H26O
1542	1,2,3,4,4alpha,5,6,8alpha-Octahydro-4alpha,8-dimethyl-2-(propan-2-ylidene)-naphthalene	C15H24
1543	1,3,5-Trimethylnaphthalene	C13H14
1544	Cedranoxide	C14H22O
1545	p-Hydroxyphenylacetic acid	C8H8O3
1546	2,5-Dimethylphenol	C8H10O
1546	3-Chlorophenol	C6H5ClO
1547	2-(3-Isopropenyl-4-methyl-4-vinylcyclohexyl)-propan-2-ol	C15H26O
1548	1,10-Decanediol	C10H22O2
1548	1,2-Dihydro-1-isopropyl-4,7-dimethylnaphthalene	C15H20
1548	2-(2-(2-(Isopentyloxy)-ethoxy)-ethoxy)-ethyl trifluoroacetate	C13H23F3O5
1549	6-Methyl-2H-chromen-2-one	C10H8O2
1552	1,2,6-Trimethylnaphthalene	C13H14
1554	5-Allyl-1,2,3-trimethoxybenzene	C12H16O3
1555	4-Chlorophenol	C6H5ClO
1556	5-Allyl-1,2,3-trimethoxybenzene	C12H16O3
1557	4-Methylpentadecane	C16H34
1560	(E,E)-1,5-Dimethyl-8-(1-methylethylidene)-1,5-cyclodecadiene	C15H24
1561	1,2,7-Trimethylnaphthalene	C13H14
1561	3-(4-Methoxyphenyl)-2-propenal	C10H10O2
1561	Undecanoic acid	C11H22O2
1562	(E)-3,7-Dimethyl-2,6-octadienyl butyrate	C14H24O2
1564	(E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	C15H26O
1564	2-Methylpentadecane	C16H34
1565	(Z)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	C15H26O
1565	1,5-di-tert-Butyl-3,3-dimethylbicyclo[3.1.0]hexan-2-one	C16H28O
1565	4,4-Bipyridine	C10H8N2
1565	Diethyl phthalate	C12H14O4
1565	Ledol	C15H26O
1566	1,2,4-Trimethylnaphthalene	C13H14
1567	2-Furylmethyl octanoate	C13H20O3
1568	Decahydro-2,2,4,8-tetramethyl-4,8-methanoazulen-9-ol	C15H26O
1568	Dodecanoic Acid	C12H24O2
1570	(Z)-3-Hexenyl benzoate	C13H16O2

1572	Isobutyl-(E)-7,9-decadienoate	C15H26O2
1572	Pentyl 2-hydroxybenzoate	C12H16O3
1573	1,4-Dipropyltetrasulfane	C6H14S4
1573	2,6-Dimethyl-3-(methoxymethyl)-benzoquinone	C10H12O3
1573	Caryophyllene oxide	C15H24O
1574	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	C15H26O
1574	3-(4,8-Dimethylnona-3,7-dienyl)-furan	C15H22O
1575	[1ar-(1aà,4aà,7á,7aá,7bà)]-Decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol	C15H24O
1575	2-(2-(2-(Pentyloxy)-ethoxy)-ethoxy)-ethyl trifluoroacetate	C13H23F3O5
1575	Tridecan-1-ol	C13H28O
1576	[1ar-(1aà,4aà,7á,7aá,7bà)]-Decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol	C15H24O
1576	2-(Pyridin-3-yl)-pyridine	C10H8N2
1576	Globulol	C15H26O
1576	Hexyl benzoate	C13H18O2
1578	7-Isopropyl-1-methylnaphthalene	C14H16
1578	9H-Fluorene	C13H10
1580	1,2,5-Trimethylnaphthalene	C13H14
1581	(E,E)-6,10-Dimethyl-3,5,9-undecatrien-2-one	C13H20O
1581	Caryophyllene oxide	C15H24O
1582	2,2-Dimethyl-6-vinylchroman-4-one	C13H14O2
1583	(E)-2-Hexenyl benzoate	C13H16O2
1583	(Z)-4-Dodecenyl acetate	C14H26O2
1584	2,3-Dimethylphenol	C8H10O
1584	2-Phenylethyl (E)-2-methyl-2-butenoate	C13H16O2
1585	beta-Oplopenone	C15H24O
1585	Diethyl phthalate	C12H14O4
1586	6-Methyl-2-(5-methyl-5-vinyltetrahydro-2-furanyl)-5-hepten-3-one	C15H24O2
1586	Diethyl octanedioate	C12H22O4
1587	2-Methyl-1-pentadecene	C16H32
1588	6-Methyl-2-(5-methyl-5-vinyltetrahydro-2-furanyl)-5-hepten-3-one	C15H24O2
1588	Epiglobulol	C15H26O
1589	(Z)-3-Tridecen-2-yl acetate	C15H28O2
1589	(Z)-6-Dodecenyl acetate	C14H26O2
1590	trans-4,5-Epoxy-(E)-2-dodecenal	C12H20O2
1590	Viridiflorol	C15H26O
1591	(Z)-3-Dodecenyl acetate	C14H26O2
1591	2-Propylphenol	C9H12O
1592	(Z)-5-Dodecenyl acetate	C14H26O2
1592	4-Hydroxy-3-methoxybenzoic acid	C8H8O4
1592	Longiborneol	C15H26O
1592	Tridecanenitrile	C13H25N
1593	(E)-3-Dodecenyl acetate	C14H26O2
1593	(E)-6-Dodecenyl acetate	C14H26O2
1593	1-Hexadecene	C16H32
1593	4-Isopropenyl-5-methyl-2-(1-methylethylidene)-5-vinylcyclohexanone	C15H22O
1593	9-Methyl-9H-fluorene	C14H12
1593	Ethyl dodecanoate	C14H28O2
1594	(Z)-1,2,3,3alpha,4,5,8,8alpha-Octahydro-3-isopropyl-6,8alpha-dimethylazulen-3a-ol	C15H26O

1594	(Z)-5-Tetradecenal	C14H26O
1594	cis-4-Isopropenyl-5-methyl-2-(1-methylethylidene)-5-vinylcyclohexanone	C15H22O
1594	trans-Sesquilavandulol	C15H26O
1595	2-(1,2,3,4,5,6,7,8-Octahydro-1,4-dimethylazulen-7-yl)-propan-2-ol	C15H26O
1595	Ethyl dodecanoate	C14H28O2
1596	(E)-4-Dodecenyl acetate	C14H26O2
1596	Cedrol	C15H26O
1597	(E)-5-Dodecenyl acetate	C14H26O2
1597	(Z)-2,3,4,4alpha,5,6,7,8-octahydro-1,1,4alpha,7-tetramethyl-1H-benzo[7]annulen-7-ol	C15H26O
1597	(Z)-7-Tetradecenal	C14H26O
1598	(E)-6-Dodecenyl acetate	C14H26O2
1598	(Z)-7-Dodecenyl acetate	C14H26O2
1599	(E)-5-Tetradecenal	C14H26O
1599	(Z)-3-Tetradecenal	C14H26O
1599	(Z)-4-Tetradecenal	C14H26O
1600	(E)-7-Dodecenyl acetate	C14H26O2
1600	Hexadecane	C16H34
1600	trans-4-Isopropenyl-5-methyl-2-(1-methylethylidene)-5-vinylcyclohexanone	C15H22O
1601	(E)-3-Tetradecenal	C14H26O
1602	(E)-4-Tetradecenal	C14H26O
1602	(E)-8-Dodecenyl acetate	C14H26O2
1602	(Z)-7-Tetradecenal	C14H26O
1602	10-epi-gamma-Eudesmol	C15H26O
1603	(Z)-9-Tetradecenal	C14H26O
1604	(Z)-5-Tridecen-2-yl acetate	C15H28O2
1604	11-Dodecenyl acetate	C14H26O2
1604	Benzophenone	C13H10O
1604	Cedrenol	C15H24O
1604	Cedrol	C15H26O
1605	(Z)-8-Dodecenyl acetate	C14H26O2
1605	(Z,Z)-4,7-Tridecadien-(2S)-2-yl acetate	C15H26O2
1605	N-Nitrosodiphenylamine	C12H10N2O
1606	(E)-10-Tetradecenal	C14H26O
1606	1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	C15H24O
1606	Caryophyllene oxide	C15H24O
1606	cis-1,3,4,6,7,8alpha-Hexahydro-1,1,5,5-tetramethyl-2H-2,4alpha-methanonaphthalen-8(5H)-one	C15H24O
1607	(E)-2-Hexadecene	C16H32
1607	(E)-9-Dodecenyl acetate	C14H26O2
1607	1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	C15H24O
1609	(Z)-10-Tetradecenal	C14H26O
1609	(Z)-6-Tridecen-2-yl acetate	C15H28O2
1609	10-epi-gamma-Eudesmol	C15H26O
1609	Dodecyl acetate	C14H28O2
1610	(E)-11-Tetradecenal	C14H26O
1610	(Z)-7-Tridecen-2-yl acetate	C15H28O2
1611	(Z)-9-Dodecenyl acetate	C14H26O2
1611	2-(2-(2-(Isopentyloxy)-ethoxy)-ethoxy)-ethanol	C11H24O4
1611	2-Methoxy-4-[(E)-1-propenyl]phenyl acetate	C12H14O3

1611	beta-Himachalene oxide	C15H24O
1611	Tetradecanal	C14H28O
1613	1-epi-Cubenol	C15H26O
1614	(E)-5-Tridecen-2-yl acetate	C15H28O2
1614	(E)-6-Tridecen-2-yl acetate	C15H28O2
1615	(E)-10-Dodecenyl acetate	C14H26O2
1615	(Z)-11-Tetradecenal	C14H26O
1615	1-(Methoxycarbonyl)-naphthalene	C12H10O2
1615	Tetradecanal	C14H28O
1616	(E)-4-Tridecen-2-yl acetate	C15H28O2
1616	1-epi-Cubenol	C15H26O
1617	(Z)-4-Tridecen-2-yl acetate	C15H28O2
1618	(E)-7-Tridecen-2-yl acetate	C15H28O2
1618	(Z)-2-Hexadecene	C16H32
1618	(Z)-8-Tridecen-2-yl acetate	C15H28O2
1618	2-Bromophenol	C6H5BrO
1618	trans-1,3,4,6,7,8alpha-Hexahydro-1,1,5,5-tetramethyl-2H-2,4alpha-methanonaphthalen-8(5H)-one	C15H24O
1619	(E)-8-Tridecen-2-yl acetate	C15H28O2
1620	(E)-3-Tridecen-2-yl acetate	C15H28O2
1620	cis-Arteannuic alcohol	C15H24O
1622	(Z)-1,2,4-Trimethoxy-5-(1-propenyl)-benzene	C12H16O3
1622	6-Allyl-4,5-dimethoxy-1,3-benzodioxole	C12H14O4
1623	1,1-Diphenylhydrazine	C12H12N2
1623	12-Tridecen-2-yl acetate	C15H28O2
1624	(Z)-9-Tridecen-2-yl acetate	C15H28O2
1626	2,4,6-Tribromophenol	C6H3Br3O
1626	Propyl dodecanoate	C15H30O2
1627	(E)-10-Tridecen-2-yl acetate	C15H28O2
1630	(Z)-10-Dodecenyl acetate	C14H26O2
1630	(Z)-10-Tridecen-2-yl acetate	C15H28O2
1630	2-((4aR)-1,2,3,4,4alpha,5,6,7-octahydro-4alpha,8-dimethylnaphthalen-2-yl)-propan-2-ol	C15H26O
1631	(Z)-3-Hexenyl phenylacetate	C14H18O2
1631	Phenethyl dodecanoate	C20H32O2
1631	Tridecan-2-yl acetate	C15H30O2
1632	(Z)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-yl acetate	C17H28O2
1635	(E)-11-Tridecen-2-yl acetate	C15H28O2
1635	1,3,5,7-Tetramethylnaphthalene	C14H16
1636	(Z,E)-5,7-Dodecadienyl acetate	C14H24O2
1636	1,2,3,4,4alpha,7,8,8alpha-Octahydro-4-isopropyl-1,6-dimethylnaphthalen-1-ol	C15H26O
1638	2-(6,10-Dimethylspiro[4.5]dec-6-en-2-yl)-propan-2-ol	C15H26O
1638	Acenaphthenone	C12H8O
1638	Daucol	C15H26O2
1639	2,3,6-Trimethylphenol	C9H12O
1639	4-Methyldibenzofuran	C13H10O
1640	(E,Z)-5,7-Dodecadienyl acetate	C14H24O2
1641	1,2,3,4,4alpha,5,6,8alpha-Octahydro-1-isopropyl-4,7-dimethylnaphthalen-4-ol	C15H26O
1642	1,2,3,4,4alpha,5,6,8alpha-Octahydro-1-isopropyl-4,7-dimethylnaphthalen-4-ol	C15H26O
1642	2-(6,10-Dimethylspiro[4.5]dec-6-en-2-yl)-propan-2-ol	C15H26O

1642	alpha-Selin-11-en-4-ol	C15H26O
1643	3-Bromophenol	C6H5BrO
1644	1,2,3-Trimethoxy-5-((E)-prop-1-enyl)-benzene	C12H16O3
1644	6-Allyl-4,5-dimethoxy-1,3-benzodioxole	C12H14O4
1645	Torreyol	C15H26O
1646	2-(2-(2-(Isobutoxy)-ethoxy)-ethoxy)-ethyl acetate	C12H24O5
1647	Himachalol	C15H26O
1647	Methyl 2-(3-oxo-2-((Z)-pent-2-enyl)-cyclopentyl)-acetate	C13H20O3
1648	(Z)-11-Tridecen-2-yl acetate	C15H28O2
1649	2-(Decahydro-4a-methyl-1-methylenenaphthalen-7-yl)-propan-2-ol	C15H26O
1651	2-((4aR)-1,2,3,4,4alpha,5,6,7-octahydro-4alpha,8-dimethylnaphthalen-2-yl)-propan-2-ol	C15H26O
1651	5-Methylhexadecane	C17H36
1651	Methyl 2-naphthoate	C12H10O2
1652	1,2,3,4,4alpha,7,8,8alpha-Octahydro-4-isopropyl-1,6-dimethylnaphthalen-1-ol	C15H26O
1652	2-(1,2,3,4,4alpha,5,6,8alpha-Octahydro-4alpha,8-dimethylnaphthalen-2-yl)-propan-2-ol	C15H26O
1652	4-Bromophenol	C6H5BrO
1653	(Z,Z)-5,7-Dodecadienyl acetate	C14H24O2
1653	1,2,3,4,4alpha,7,8,8alpha-Octahydro-4-isopropyl-1,6-dimethylnaphthalen-1-ol	C15H26O
1654	(Z)-9,13-Tetradecadien-11-ynal	C14H20O
1654	2-(2-(2-(Pentyloxy)-ethoxy)-ethoxy)-ethanol	C11H24O4
1654	2-(Decahydro-4a-methyl-1-methylenenaphthalen-7-yl)-propan-2-ol	C15H26O
1654	3,7-Dimethyl-1,6-octadien-3-yl 3-methylhexanoate	C17H30O2
1655	2-[5-Methyl-5-(4-methyl-3-cyclohexen-1-yl)-tetrahydro-2-furanyl]propan-2-ol	C15H26O2
1655	Selin-11-en-4alpha-ol	C15H26O
1656	(Z)-2-Methyl-2-butenoic acid	C5H8O2
1656	(Z)-6-Dodecen-4-olide	C12H20O2
1656	1,3,6,7-Tetramethylnaphthalene	C14H16
1656	4-Hydroxy-3,5-dimethoxybenzaldehyde	C9H10O4
1656	4-Isopropylphenol	C9H12O
1656	Decylcyclohexane	C16H32
1657	(Z)-6-Dodeceno-gamma-lactone	C12H20O2
1658	2-(4alpha,8-Dimethyldecahydro-2-naphthalenyl)-propan-2-ol	C15H28O
1658	2-Senecioyl-4-vinylphenol	C13H14O2
1658	7-epi-alpha-Eudesmol	C15H26O
1659	4-Methylhexadecane	C17H36
1659	Patchouli alcohol	C15H26O
1660	(Z)-4-Tetradecen-1-ol	C14H28O
1660	(Z)-5-Tetradecen-1-ol	C14H28O
1660	(Z)-7-Tetradecen-1-ol	C14H28O
1662	(Z)-3-Tetradecen-1-ol	C14H28O
1663	(E)-3-Tetradecen-1-ol	C14H28O
1663	(E)-7-Tetradecen-1-ol	C14H28O
1663	(Z)-8-Tetradecen-1-ol	C14H28O
1663	14-Hydroxy-9-epi-beta-caryophyllene	C15H24O
1664	(E)-4-Tetradecen-1-ol	C14H28O
1665	(E)-5-Tetradecen-1-ol	C14H28O
1665	4-Chloro-3-cresol	C7H7ClO
1666	(E,E)-5,7-Dodecadienyl acetate	C14H24O2

1666	(R)-6-((Z)-Hept-1-enyl)-5,6-dihydropyran-2-one	C12H18O2
1666	2-(3,8-Dimethyl-1,2,3,3alpha,4,5,6,7-octahydro-5-azulenyl)-propan-2-ol	C15H26O
1666	2-Methylhexadecane	C17H36
1667	(Z)-9-Tetradecen-1-ol	C14H28O
1667	3,7-Dimethyl-6-octenyl-2-methyl-2-butenoate	C15H26O2
1668	(E)-9-Tetradecen-1-ol	C14H28O
1668	Cedr-8-en-13-ol	C15H24O
1669	(E)-10-Tetradecen-1-ol	C14H28O
1669	1-Methyl-2-quinolone	C10H9NO
1670	3,4-Dimethoxybenzoic acid	C9H10O4
1671	1,2,4,7-Tetramethylnaphthalene	C14H16
1673	(E)-11-Tetradecen-1-ol	C14H28O
1673	(Z)-10-Tetradecen-1-ol	C14H28O
1673	3-Methylhexadecane	C17H36
1674	(E)-2-Tetradecenal	C14H26O
1674	(E,Z)-8,10-Tetradecadienal	C14H24O
1674	(Z)-2-Tetradecenal	C14H26O
1674	2,4-Dichlorophenol	C6H4Cl2O
1674	3,5-Dichlorophenol	C6H4Cl2O
1674	4-Isopropyl-1,6-dimethylnaphthalene	C15H18
1675	1,2,5,7-Tetramethylnaphthalene	C14H16
1675	Hexyl 2-hydroxybenzoate	C13H18O3
1676	(S)-2-Methylbutyl (E)-7,9-decadienoate	C17H30O2
1676	1,3,6,8-Tetramethylnaphthalene	C14H16
1676	2,3,5-Trimethylphenol	C9H12O
1676	Methyl 2-((1R,2S)-3-oxo-2-((Z)-pent-2-enyl)-cyclopentyl)-acetate	C13H20O3
1676	Tetradecan-1-ol	C14H30O
1677	1-(3-Methylbut-2-enyloxy)-4-((E)-prop-1-enyl)-benzene	C14H18O
1677	Tetradecan-1-ol	C14H30O
1678	(R,Z)-5-(2,3-Dimethyltricyclo[2.2.1.0(2,6)]hept-3-yl)-2-methyl-2-penten-1-ol	C15H24O
1678	(Z)-11-Tetradecen-1-ol	C14H28O
1678	cis-Guai-9-en-11-ol	C15H26O
1679	(E)-1,2,4-Trimethoxy-5-(1-propenyl)-benzene	C12H16O3
1679	2,4,6-Trifluorophenanthrene	C14H7F3
1679	5-Allyl-4,7-dimethoxy-1,3-benzodioxole	C12H14O4
1680	(E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-yl acetate	C17H28O2
1680	2,3,6,7-Tetramethylnaphthalene	C14H16
1680	5-Allyl-4,7-dimethoxy-1,3-benzodioxole	C12H14O4
1681	6-Heptyl-tetrahydropyran-2-one	C12H22O2
1683	6-Methyl-2-(3-methyl-3-cyclohexen-1-yl)-5-hepten-2-ol	C15H26O
1685	5-Allyl-4,7-dimethoxy-1,3-benzodioxole	C12H14O4
1685	Dihydro-5-octylfuran-2(3H)-one	C12H22O2
1686	1,3-Difluorophenanthrene	C14H8F2
1686	4-Propylphenol	C9H12O
1686	alpha-caryophyllene acetate	C17H28O2
1687	2,4-Difluorophenanthrene	C14H8F2
1687	Pentadecanal	C15H30O
1689	(Z,Z)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	C15H26O
1689	2-Methyl-1-hexadecene	C17H34
1690	1,2,6,7-Tetramethylnaphthalene	C14H16

1697	(Z,E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	C15H26O
1699	1,2,3,6-Tetramethylnaphthalene	C14H16

[13] Adams, R.P. 1995. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream, IL.

Now, my own suspicion is that the universe is not only queerer than we suppose, but queerer than we can suppose. I have read and heard many attempts at a systematic account of it, from materialism and theosophy to the Christian system or that of Kant, and I have always felt that they were much too simple. I suspect that there are more things in heaven and earth that are dreamed of, or can be dreamed of, in any philosophy. That is the reason why I have no philosophy myself, and must be my excuse for dreaming.

—John Burden Sanderson Haldane (1892-1964) English geneticist.
Possible Worlds (1927).