Determination of Polar Solvents by Static Headspace Extraction – Gas Chromatography (SHE-GC)

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Determination of Polar Solvents by Static Headspace Extraction – Gas Chromatography (SHE-GC)

By

Michael Sithersingh

Dissertation submitted to the Department of Chemistry and Biochemistry of Seton Hall University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Chemistry

August, 2018
We certify that we have read this dissertation and that in our opinion it is sufficient in scientific scope and quality as a dissertation for the degree of Doctor of Philosophy.

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DEDICATION

This work is dedicated to:

My parents and grandmother:
Michael John Moses & Pushpam and Gnanammal

My wife:
Jesudha T. Sithersingh

My children:
Kevin Sithersingh and Rachel Sithersingh

Thank you for your unending inspiration, encouragement, and patience.
ACKNOWLEDGEMENTS

My heartfelt thanks to my Mentor, Dr. Nicholas H. Snow, Founding Endowed Professor of Chemistry and Biochemistry & Director of Research, for his guidance, inspiration, and support

Special thanks to Dr. Yuri Kazakevich, Professor of Chemistry,
Department of Chemistry and Biochemistry for his guidance

Thanks to Dr. Wyatt R. Murphy, Professor, Dr. Cicilia Marzabadi, Professor and Dr. Stephen P. Kelty, Professor & Department Chair, Department of Chemistry and Biochemistry,
for their support and inspiration

I would also like to thank Eyetech Pharmaceuticals Inc. / OSI Pharmaceuticals, Inc. for providing financial support for my research work and I also want to thank Sannova Analytical Inc., Somerset, NJ for allowing me to use their analytical laboratory for doing research
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ABSTRACT

The term “headspace” is defined as the vapor that forms above or around a liquid or solid sample in a closed container. Usually, the vapor is above the liquid or solid at the top of the container, hence the term headspace. Headspace sampling is a method for separating volatile materials that may be extracted from a more solid sample matrix and then analyzed using gas chromatography. Headspace extraction refers to the collection and analysis of the vapor phase in the container. Generally, the vapors are sampled from a system that is brought to equilibrium before sampling. Headspace sampling provides an excellent way for introducing a sample into a gas chromatograph. This sampling avoids introduction of non-volatile or high-boiling contaminants from the sample matrix, and it can often be used for trace or ultra-trace determination of volatile organics with little or no additional sample preparation.

The Static Headspace Extraction – Gas Chromatography (SHE-GC) technique has been used since the early days of Gas Chromatography (GC). In this procedure, the two phases in the sample vial are under static conditions, and sample transfer is conducted after they have reached equilibrium. The fundamentals of headspace analysis will be discussed along with modes and types of available headspace systems. The fundamental parameter that determines the sensitivity of an analyte in the sample is called the partition coefficient (K) and can be determined using the vapor phase calibration (VPC) and phase ratio variation (PRV) methods.

A breathalyzer is a device for estimating blood alcohol content (BAC) from a person’s breath sample. The blood breath partition ratio assumes that 2100 mL of breath contains the same amount of alcohol as 1 mL of blood. It was used for over forty years ago by the National Safety Council’s Committee for Tests on Intoxication. After reviewing blood-breath correlation data, the partition ratio was assigned a value of 2100, and that value has been used for calibration of breath testing instruments. VPC is used to determine the partition coefficient of ethanol in water at 37 °C.
A known amount of ethanol is mixed with a known volume of water and analyzed using static Headspace Sampler – Gas Chromatography (HS-GC) and quantified against an external standard. The partition coefficient of ethanol in the liquid phase (water) to the headspace of the vial can then be determined.

Using static HS-GC with a pressure balanced headspace sampling system, peak responses obtained by varying the solvents and headspace sampler parameters were compared and investigated. The following organic solvents were used as analytes: (1) methanol; (2) ethanol; (3) acetone; (4) acetonitrile; (5) methylene chloride; (6) tetrahydrofuran; and (7) pyridine. Peak responses obtained for the following solvents and solvent mixtures as diluents were compared: (1) water; (2) a 1:1 mixture of water and dimethyl sulfoxide (DMSO); and (3) DMSO. Also, peak responses from the above diluents with and without the electrolytes (salting out) were compared. Peak responses obtained by varying the following individual headspace sampler parameters were investigated: (1) oven thermostat time; (2) sample volume in the headspace vial; and (3) injection time. The resulting peak area responses for the varied headspace sampler parameters with different solvents and solvent mixtures were plotted for comparison and investigation.

Ionic liquids (IL) are salts in which the ions are poorly coordinated, resulting in liquid state forms at <100 °C or at room temperature (RTILs). At least one of the ions has a delocalized charge, and one component is organic, thus preventing a crystal lattice formation. ILs are largely composed of ions and short-lived ion pairs. Due to ILs’ unique properties (such as higher boiling points with high thermal stability), these compounds can be used as the stationary phase for capillary GC columns.

Currently, solvents including DMSO, dimethylformamide (DMF), dimethylacetamide (DMA), benzyl alcohol (BA), and water are used as diluents in the static HS-GC analysis of pharmaceutical residual solvents. As a drawback, these diluents preclude the analysis of high-
boiling solvents. Because of this drawback, static HS-GC cannot be employed for the residual solvents with very low vapor pressures, including 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, formamide, N-methylpyrrolidone, and sulfolane. These solvents have been designated as Class 2 residual solvents according to the United States Pharmacopeia National Formulary (USP–NF) General Chapter <467> on Residual Solvents, and the limited static HS-GC applicability for very high boiling residual solvents is specifically stated in the U.S. Pharmacopoeia [3]. A rapid and highly sensitive method has been developed for the determination of these high boiling class 2 solvents using static HS-GC with an IL:1-butyl 3-methylimidazolium tetrafluoroborate (Bmim BF4) as diluent. The method was successfully used to analyze a sample of caffeine.
1.0 Chapter 1 – Gas Chromatography

1.1 General Introduction

In 1952, Martin and Synge were both awarded Nobel Prizes for their work in the field of liquid/solid chromatography. In his award speech, Martin suggested that it might be possible to use a vapor as the mobile phase. Some years later, James and Martin used ethyl acetate vapor to desorb a mixture of fatty acids that had been affixed to an adsorbent and then placed in a tube. The vapor stream that was eluted from that tube was directed to an automated titration apparatus, resulting in a graph showing a series of steps that reflected sequential base additions as each eluted acid was neutralized by automated titration [1]. This process is considered the starting point of gas chromatography (GC). The theoretical basis for gas chromatography was first conceived by Erika Cremer, an Austrian scientist at the University of Innsbruck, Austria, in 1940s during the period of the Second World War. She and her students (with significant credit to Fritz Prior) constructed the first prototype of a gas chromatograph [2,3,4].

Perkin Elmer introduced its first commercial gas chromatograph, the Model 154 Vapor Fractometer in the year 1955. The Model 154 was the first gas chromatograph to use an oven (in order to adjust the column temperature), a flash vaporizer, and a syringe injector [5]. In 1961, PerkinElmer developed the Model 222, the first gas chromatograph with a resistance-heated packed column that removed the difference between set and actual column temperatures. The affiliate of PerkinElmer in Germany built the first modular gas chromatograph in the world, the Model F-6 in 1962, and this system allowed an operator to choose from multiple detector combinations and isothermal or programmed temperature operations. In 1967, PerkinElmer released its first flagship gas chromatograph, the Model 900, which had several improvements over the older models. In 1973, Hewlett-Packard (HP), introduced its first gas chromatograph, the HP 5830, which was also the first microprocessor-controlled analytical instrument ever made and
in 1980, Shimadzu launched the GC-8A, which changed GC system design by offering a smaller, compact size and a solid, die-cast frame.

1.2 Principles of separation

As per IUPAC (International Union of Pure and Applied Chemistry), chromatography is defined as “A physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction” and gas chromatography is defined as “A separation technique in which the mobile phase is a gas. Gas chromatography is always carried out in a column” [126]. In GC, separation is brought about by partitioning of a sample between a mobile gas phase and a thin nonvolatile liquid layer that has been coated on some inert solid particles. When a mixture of volatile material transported by a carrier gas progresses through a column containing a liquid absorbing phase coated over a solid material, each volatile component partitions between the carrier gas and the solid or the liquid. Depending upon the retention time of the compound in the column, the volatile compounds are eluted from the column at different times at which point a suitable detector identifies them.

Usually, an inert gas such as helium or an unreactive gas such as nitrogen is used as a mobile phase; this gas is called the carrier gas. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support. GC is also similar to fractional distillation since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure) differences. First, the process of separating the compounds in a mixture is carried out between liquid stationary and gas mobile phases. (Hence, the full name of the procedure is Gas–liquid chromatography (GLC), referring to the mobile and stationary phases, respectively). Second, the column, through which the gas phase passes, is located in an oven in which the gas temperature
can be controlled. Finally, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas.

GC operates by introducing a sample via an injection port into the inlet. Solid samples have to be dissolved in some solvent, whereas gaseous samples require special sample introduction valves. The detectors, placed at the exit of the separation chamber, detect and measure the amounts of separated components present in the stream of carrier gas leaving the column.

1.3 Theory

1.3.1 Thermodynamics of Analytical Separation

Applying thermodynamic principles to a chromatographic separation may explain the way in which the distribution coefficient (which itself determines the magnitude of retention) is controlled by the standard distribution-related energy and the absolute temperature. The dynamic process in chromatographic separation involves the repeated analyte transfer between flowing mobile and fixed stationary phases [6,8]. The physicochemical properties of the stationary / mobile phases and analytes control the degree to which partitioning between the flowing and fixed phases occur. Analytes that strongly interact with the stationary phase will be mostly retained, whereas those that do not will be rapidly pass through the chromatographic system along with the mobile phase. The thermodynamic equilibrium concepts can be applied in order to better characterize or understand the molecular-scale energetics (such as free energy, enthalpy, and entropy).

The distribution of an analyte, A, between the mobile phase (mp) and the stationary phase (sp) at equilibrium can be represented by the following Equation 1.1).

\[ A_{(mp)} \leftrightarrow A_{(sp)} \]  

(Equation 1.1)
The distribution of the analyte between two phases, stationary phase and a mobile (gas) phase can be described by the distribution constant or partition coefficient. The relationship between the retention factor \( k \) and equilibrium constant \( K \) of an analyte is shown in equation 1.2, using the phase ratio \( \beta \) expression, which is the ratio of the mobile phase volume to the stationary phase volume in the chromatographic system:

\[
K = k \times \beta
\]  
(Equation 1.2)

The equilibrium constant for an analyte that is partitioning between the mobile and stationary phases consists of the ratio of the equilibrium concentration of the analyte in the stationary phase to that of the analyte in the mobile phase. The retention factor is taken as the ratio of the adjusted retention time \( t'_{R} = t_{R} - t_{0} \) to the dead time; it can also be considered the amount of time the analyte spends in the stationary phase \( t'_{R} \) divided by the amount of time it spends in the mobile phase \( t_{0} \); all analytes spend \( t_{0} \) amount of time in the mobile phase).

\[
K = \frac{C_{\text{Stationary Phase}}}{C_{\text{Mobile Phase}}}
\]  
(Equation 1.3)

The retention factor value should represent the ratio of the amount of analyte in each phase at any given time point. It should then be multiplied by the phase ratio to obtain the equilibrium constant. The equilibrium constant can be related to the Gibbs free energy change \( \Delta G \) via the equation:

\[
\Delta G = -RT \ln K
\]  
(Equation 1.4)

\( R \) is the gas constant and \( T \) is the temperature (in Kelvin). The Gibbs free energy change is related to the change in enthalpy \( \Delta H \) and change in entropy \( \Delta S \) for a system as indicated by the Gibbs-Helmholtz equation:

\[
\Delta G = \Delta H - T\Delta S
\]  
(Equation 1.5)
A spontaneous reaction (in this case, the analyte favoring interaction with the stationary phase) occurs when $\Delta G$ is negative. A negative $\Delta G$ indicates the processes that give up free energy are favored. A spontaneous process could be induced by a decrease in enthalpy (that is, $\Delta H$ becomes more negative) or by a large increase in entropy which means even if $\Delta H$ is positive, a positive $\Delta S$, multiplied by the temperature, could also result in an overall negative $\Delta G$ and a favorable process. The mode, or the types of noncovalent forces, by which analytes interact with the stationary phase controls both enthalpy and entropy in the phase transfer/partitioning process.

1.3.2 The Plate Theory of Chromatography

There are two basic theories applicable to chromatography: (1) the Plate Theory and (2) the Rate Theory. The Plate Theory was developed by Martin and Synge in 1941 to describe the mechanism of retention [9]. It offers an equation that allows the calculation of the retention volume of a solute and the column efficiency. This theory assumes that the solute, during its passage through the column, is always in equilibrium with the mobile and stationary phases. However, equilibrium between the phases never actually occurs. In order to compensate for this non-equilibrium condition, the column is considered to be divided into a number of cells or plates, called “theoretical plates”. Each plate is allotted a specific length, and thus, the solute will spend a finite time in each plate. The size of the cell is chosen to provide sufficient residence time for the solute in order to establish equilibrium with the two phases. The smaller the plate, the faster equilibrium will be achieved, and more plates will exist in the column. The number of theoretical plates contained in a column will be directly related to the equilibrium rate and for this reason, has been termed column efficiency. Separate equilibrations of the sample between the stationary and mobile phase occur within these "plates". The analyte moves down the column via transfer of equilibrated mobile phase from one plate to the next. Plates also serve as a way of measuring
column efficiency, either by reporting the number of theoretical plates in a column, $N$ (the more plates the better) or by indicating the plate height; as the height equivalent to a theoretical plate (HETP). The smaller the height, the better it is for separation. If the length of the column is $L$, then the HETP is

$$HETP = \frac{L}{N}$$  \hspace{1cm} (Equation 1.6)

The number of theoretical plates that a real column possesses can be found by examining a chromatographic peak after elution:

$$N = 16 \left( \frac{t_r}{w_b} \right)^2 = 5.55 \left( \frac{t_r}{w_{1/2}} \right)^2$$  \hspace{1cm} (Equation 1.7)

In which $t_r$ is the peak retention time, $w_{1/2}$ is the peak width at half-height, and $w_b$ is the peak width.

As can be seen from this equation, columns behave as if they have different numbers of plates for different solutes in a mixture. A schematic diagram of the column separated into plates is shown in Figure 1.1.

The Plate Theory is old and does not acknowledge mass transfer kinetics; therefore it reveals little about the factors influencing HETP values. Also, it does not account for the presence and influence of other solutes; thus, the behavior of the column plate is calculated based on the assumption that the distribution coefficient remains unaffected by the presence of other solutes and that the distribution isotherm is linear. Furthermore, this theory also neglects the concept of solute diffusion and flow path.
Figure 1.1 – Schematic representation of diagram of the column separated into plates
1.3.3 The Rate Theory of Chromatography

The Rate Theory was introduced by Van Deemter and describes the process of peak dispersion (band spreading) [10]. This theory provides an equation that allows the calculation of the variance per unit length of a column (HETP) in terms of the mobile phase velocity and other physiochemical properties of the solute and distribution system to be performed. The rate theory describes the processes at work inside a column while taking into account the time it takes for the solute to equilibrate between the stationary and mobile phases. The Rate Theory has been interpreted differently by a number of well-established scientists in the field. These interpretations have resulted in a number of different equations; such as the Van Deemter, the Giddings, the Huber, the Horvath, and Knox Equations. The equations differ slightly from each other and were developed from first principles except the Knox equation, which was developed from experimentally observed relationships and subsequently rationalized on a first principle basis. All of the equations give a type of hyperbolic function that predicts a minimum plate height at an optimum velocity and thus, yields a maximum efficiency. At normal operating velocities, it has been demonstrated that the Van Deemter equation offers the best fit for the experimental data. The Van Deemter equation considers various mechanisms that contribute to band broadening. The plate height is written as follows:

\[ HETP = A + \frac{B}{u} + C_u \]  

(Equation 1.8)

Where, \( u \) is the average velocity of the mobile phase and the terms \( A, B, \) and \( C \) are factors that contribute to band broadening. The band shape of a chromatographic peak is affected by the rate of elution. It is also affected by the different paths available to solute molecules as they travel among stationary phase particles. Figure 1.2 shows the schematic representation of the Van Deemter curve.
Figure 1.2 – Schematic representation of Van Deemter curve - plot of plate height vs. average linear velocity of mobile phase [11]. Reproduced with Permission, Courtesy of Restek Inc.
A – **Eddy diffusion**

The mobile phase moves through a column that is packed with stationary phase. Solute molecules will take different random paths through the stationary phase. This random movement will cause broadening of the solute band because different paths are of different lengths.

**B - Longitudinal diffusion**

The analyte’s concentration is less at the band edges than at the center. The analyte diffuses out from the center toward the edges. This diffusion causes band broadening. If the velocity of the mobile phase is high, the analyte will spend less time on the column, which decreases longitudinal diffusion effects.

**C - Resistance to mass transfer**

The analyte requires a certain amount of time to equilibrate between the stationary and mobile phases. The analyte in the mobile phase will move ahead of the analyte in the stationary phase if the velocity of the mobile is high and the analyte has a strong affinity for the stationary phase. The band of analyte will be broad. A mobile phase with high velocity favors more band broadening.

### 1.3.4 Factors influencing component separation

**Vapor pressure**

The boiling point of a compound is often related to its polarity. Lower boiling points, higher compound vapor pressures, and shorter retention times occur when the compound spends more time in the gas phase. A lower boiling point is one of the main reasons why low boiling solvents (such as diethyl ether, dichloromethane) are used as solvents for sample dissolution. The temperature of the column does not need to be above the boiling point because every compound, even a solid, has a non-zero vapor pressure at any given temperature. However, the vapor pressures of solids are low compared to liquids.
Polarity of stationary phase of column versus polarity of components

If the polarity of the stationary phase and compound are similar, the retention time increases because the compound interacts more extensively with the stationary phase. As a result, polar compounds have longer retention times on polar stationary phases and shorter retention times on non-polar columns using the same temperature. Chiral stationary phases that are based on amino acid derivatives, cyclodextrins, and chiral silanes are capable of separating enantiomers because one enantiomer interacts to a stronger degree with the stationary phase than does the other enantiomer; this type of interaction is often due to steric effects or other very specific interactions.

Column temperature

Excessively high column temperatures result in very short retention time but also in a very poor separation because all compounds stay mainly in the gas phase. However, in order for the separation to occur, the components need to be able to interact with the stationary phase. If the compound does not interact with the stationary phase, its retention time will decrease. At the same time, the separation’s quality deteriorates because the differences in retention times are no longer as pronounced.

Carrier gas flow rate

A high flow rate causes a reduction in retention times, but poor separation would also be observed. As described above, the compounds have very little time to interact with the stationary phase and are just passing through the column.

Column length

A longer column generally improves compound separation. The trade-off is a retention time that increases proportionally with the column length. Significant peak broadening will also be observed because of increased longitudinal diffusion inside the column. It should be noted that the
gas molecules are not only traveling in one direction but also side-to-side and backward. Peak broadening is inversely proportional to the flow rate. Broadening is also observed because there is a finite rate of mass transfer between the phases and because the molecules move along different paths through the column.

**Amount of material injected**

Ideally, the peaks in the chromatogram display symmetric shapes (Gaussian curve). If too much of the sample is injected, the peaks show significant fronting which causes poor separation. Under standard conditions, only 1% – 2% of the compound injected into the injection port passes through the column because most GC instruments are operated in split-mode to prevent column and detector overloading. The split-less mode will only be used if the concentration of the analyte in the sample is difficult to detect with the split-mode.

**1.4 Instrumentation**

As described above in the Introduction, Perkin Elmer introduced their first gas chromatograph, the Model 154 Vapor Fractometer, in the year 1955. The essential elements of these types of instruments had been developed by the early 1960s [12] with further developments occurring during short periods of innovation and technological advances followed by longer evolutionary periods of changes and consolidation. Many advances were facilitated by advances in column technology or electronics. For example, use of the microprocessor brought about a radical change in instrument design and use. From this point onward, circuit networks, via communication with each other through a central controller, monitored and controlled instrument functions. This paved the way for the emergence of software-controlled keyboard instrumentation on a personal computer, which were the dominant instruments for laboratory use by the early 1990s. Achieving full instrument operation automation was a significant milestone and paved the
way for the introduction of the electronic pressure control in the early 1980s. This allowed carrier and support gas pressures and flow rates to be set and monitored by a central processor connected to an electromechanical device. Modern-day gas chromatograph essentially consists of several components: (1) Inlet; (2) Oven; (3) Detector; and (4) Data handling device. A schematic diagram of a gas chromatograph containing injector, column, oven, and detector is shown in Figure 1.3, and an example of the gas chromatograph model, Clarus 690 from Perkin Elmer, is shown in Figure 1.4.
Figure 1.3 - Schematic diagram of a gas chromatograph.
Figure 1.4 - Example of a Gas Chromatograph, Perkin Elmer Model Clarus 690 [14].

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1.4.1 GC Inlets

Modern-day gas chromatographs use many inlet types for introducing the sample into the column, which is separate from the headspace sampler, and among them, the following three inlets are popular:

- Split/Splitless
- Programmed Thermal Vaporizing (PTV)
- Cool-on-column (COC)

Split/Splitless Inlet

A combined split/splitless inlet is the most popular inlet for capillary column GC. It can be used in either a split or splitless mode and thus, it provides a very effective combination that can cover most analytical requirements [15,16]. A sample can be introduced into a heated small chamber using a syringe through a septum, and then the heat facilitates volatilization of the sample and sample matrix. The carrier gas then either sweeps the entire sample (split-less mode) or a portion of the sample (split mode) into the column.

In the split mode, part of the sample/carrier gas mixture in the injection chamber will eventually be exhausted through the split vent. Split injection is the preferred mode when working with samples containing high concentrations of analyte (>0.1%), whereas split-less injection is best suited for trace analysis of samples with low analyte concentrations (<0.01%). In the split-less mode, the split valve opens after a pre-set amount of time in order to purge heavier elements that would otherwise cause system contamination. This pre-set (split-less) time should be optimized. Shorter times (such as 0.2 min) ensure less tailing but a decrease in response, and longer times (such as 2 min) cause an increase in the signal but also in the tailing. A schematic diagram of a split/split-less inlet describing various components is shown in Figure 1.5.
Figure 1.5 - Schematic diagram of a Split / Splitless Inlet.

Reproduced with permission from reference [15].
1.4.2 GC Detectors

As solutes elute from the column, they interact with the detector. The detector converts this interaction into an electronic signal that is sent to the data system. The magnitude of the signal is plotted versus time (from the time of injection), and a chromatogram is then generated. Some detectors respond to any solute eluting from the column, while others respond only to solutes with specific structures, functional groups, and/or atoms. Most detectors require one or more gases to function correctly. There are different combinations of combustion, reagent, auxiliary, and makeup gases. In some cases, one gas may serve multiple purposes. The type of detector gas is dependent on the specific detector and is relatively universal among GC manufacturers.

Flame ionization detector (FID)

Flame ionization detectors (FID) are based on the electrical conductivity measurement of gases [15,16]. At normal temperatures and pressures, gases will act as bad conductors or insulators; however, when gases undergo ionization, they will act as good electrical current conductors. When gases and vapors emerge from the separation column, they are mixed with hydrogen, and then burned in the air in order to produce a flame that ionizes the molecules of compound in the carrier gas. The burning jet is the negative electrode, whereas the anode usually contains a small wire loop which a small voltage is applied and extends across into the tip of the flame. The resulting ions are collected at the electrodes, and a current is generated, which is proportional to the number of ionized molecules within the compound. The burning of the carrier gas with hydrogen produces a constant signal, but a higher current is observed in cases the ionized mixture of components that are undergoing the analysis, emerges. The detector is equipped with an automatic recording device, which records these fluctuations and then fed into an integrator. The final data is handled by a computer and software and a graph is displayed that has retention time on the x-axis and total ion on the y-axis. This detector is very sensitive toward organic
molecules, but is relatively insensitive for a few small molecules, including N₂, NOₓ, H₂S, CO, CO₂, and H₂O. If proper amounts of hydrogen/air are mixed, the resulting combustion produces none or very few ions, resulting in a low background signal. If other carbon-containing compounds are introduced to this stream, additional cations will appear in the effluent stream. More carbon atoms in the molecule lead to more fragmentation, and the more sensitive this detector is for this compound. Unfortunately, there is no direct relationship between the number of carbon atoms and the signal size. As a result, the individual response factors for each compound must be experimentally determined and standardized for each one of these instruments. FID was used for all of the studies performed as part of the research. Figure 1.6 shows a schematic diagram of FID.
Figure 1.6 - Schematic diagram of a Flame Ionization Detector (FID) used in the gas chromatograph (GC). Reproduced with permission from reference [15].
2.0 Chapter 2 – Headspace Gas Chromatography (HS-GC)

2.1 Introduction

Headspace in GC indicates the gas phase in a chromatography vial above the sample. The definition of headspace is the vapor above a liquid and/or solid phase sample (sometimes called the sample phase). Headspace analysis is the analysis of the vapor lying in equilibrium over a solid/liquid sample in a sealed vial.

In this technique, the sample in a sealed vial is heated to a pre-determined temperature in an oven with a thermostat. When equilibrium is reached between the sample and the vapor phases, the volatile material in the sample vial in the vapor phase will be at equilibrium with the sample phase. A defined amount of the vapor is taken and carried to the column in the gas chromatograph for the analysis. With this technique, only volatile substances reach the column and the nonvolatile substances remain in the sample vial. Headspace chromatography is the cleanest form of GC analysis since no nonvolatile residues are deposited in the injector of the gas chromatograph.

Complex sample matrices, which would otherwise require sample extraction or preparation or difficult to directly analyze, are ideal candidates for headspace analysis, since they can be placed directly in a vial with little or no preparation. Suitable fields of application are polymer, volatile components in drink and foodstuff, blood alcohol level, water, and environmental analyses. Other common applications include industrial analyses of fragrances in perfumes and cosmetics.

In the pharmaceutical industry, headspace GC is widely used to determine the residual solvents in active pharmaceutical ingredients (API). A few forms of headspace GC are listed below:

- Static headspace extraction (SHE)
- Dynamic headspace extraction (DHE)
- Multiple headspace extraction (MHE) and
- Solid phase micro extraction (SPME).

Static headspace extraction was exclusively used in this study.

2.2 Evolution of Headspace Sampler

Headspace analysis, which consists of analyzing a gas in contact with a liquid or solid sample and drawing conclusions from the results concerning the nature and/or composition of the original sample, has been carried out long before GC development and before the combination of the two techniques [20]. The first report about headspace analysis was found in the abstract of a paper presented by Harger, Bridwell, and Raney in the Department of Biochemistry and Pharmacology of Indiana University School of Medicine (Indianapolis, Indiana) published in the year 1939. It deals with an aerometric method for the rapid determination of alcohol in water and body fluids [20]. The paper describes a method representing a combination of static and dynamic sampling in which the headspace above the liquid sample was conducted through a sulfuric acid–permanganate reagent to rapidly determine alcohol in water and body fluids including blood and urine. In this paper and a later one in 1950 by the same author, air-water partition coefficients of alcohol were also determined and compared to values published by other authors over temperatures ranges from 0°C to 40°C [21–31]. They used the partition coefficients to calculate alcohol concentrations in the original sample based on the amount present in the gas phase.

Headspace analysis was also used in Hungary in the first part of the 1950s by Schulek and colleagues at the University of Budapest to conduct various physicochemical measurements of aqueous solutions. They could not publish their results due to political reasons. In 1955, the political situation in that country relaxed somewhat, and Schulek was permitted to present a paper
in Vienna on his group’s work. This paper was published in 1956 followed by seven additional publications on similar topics [20-32]. His group used a self-constructed, all-glass apparatus to investigate changes in the tension of alcohol and phenol aqueous solutions, in which various nonvolatile substances were dissolved. Classical analytical techniques were used to determine concentrations of the analytes in the headspace.

In the early days, scientists had no specific expression in English to characterize the technique. As mentioned, Harger spoke about an aerometric method, and in the English summaries of their paper Schulek and colleagues translated the German expression, “Dampfraumanalyse”, as “vapor space analytical procedure.” This term, along with “Dampfphase”, (vapor phase) has remained in use in German ever since [20, 32]. Bovijn used no specific term; he spoke only about the “gaseous phase in equilibrium with a liquid phase.” The terms headspace and headspace analysis were used first in 1960, in the paper of Stahl [34]. The expressions most likely were adapted from the food packaging industry in which the gas layer above the food in sealed containers was characterized as the headspace.

During 1958 and 1959, a chemist working in a food industry requested that Ettre investigate the nature of the rancid odor in potato chips as a chemist heard about the detector (FID) in GC functions similar to our nose, "smelling" the volatile components. The question asked was, "Would GC not be able to detect or measure rancidity?". This investigation led to the invention of what we call now "the headspace gas chromatography (HS-GC) technique". Ettre monitored differences between properly and improperly stored bags at room temperature and also at temperatures warmer than room temperature. This experiment was soon mimicked and published by Stahl. To perform this analysis, the bag was pierced with a hypodermic needle; 0.5 to 1 mL of the gas was withdrawn and then injected into the gas chromatograph with the intention of determining gases such as oxygen in the headspace using a polarographic oxygen
sensor coupled to a direct readout device [33, 34]. However, it was Stahl and his coworkers in 1960 used the headspace combination with GC for the first time. The first instrument to perform headspace analysis was developed by Perkin Elmer from 1966 to 1967 and was based on the fundamental work of Professor Macheta from 1964 to 1966. The popularity of this technique has grown over the past 30 years due to instrumentation automation.

The first use of headspace sampling in combination with GC was monitoring hydrogen content in the water from high-pressure power stations. The results were published in 1958, but the authors claimed to have used in an operational system for more than a year by the time of publication [20, 33]. By using a thermal-conductivity detector on a gas chromatograph, trace amounts of hydrogen (at the parts per billion level) could be analyzed from aliquots of the equilibrium gas.

In 1960, Beckman Corporation created a device that could be used in combination with Stahl's work. This device had a puncturing tool connected to a small closed volume that could be evacuated; in this way, gas was drawn into this sampling volume after piercing the container. The principal use of the device was the determination of the presence of oxygen in cans, and for those measurements, it could be connected directly to a polarographic oxygen sensor that was coupled with a direct readout. In addition, gas samples also could be withdrawn from the Beckman device with a syringe through a rubber septum located on the side of the device for subsequent GC analysis.

When a sample is ready to be extracted, an air-tight syringe (at room temperature) enters an air-tight valve at the top right. A sample is withdrawn and transferred to an analytical instrument such as a gas chromatograph. There are inherent problems associated with syringe-based manual sample transfer in the headspace sampling system. Even if the samples can be incrementally heated at elevated temperatures, the syringe is at room temperature, and thus
higher-boiling, but volatile compounds nonetheless, could condense in it. The second problem is related to the pressures. When the closed sample vial is incrementally heated at elevated temperatures, the pressure in its headspace will be higher than atmospheric pressure. This gas is sampled with the syringe. However, the internal volume of the syringe is open to the atmosphere through the needle; therefore, after withdrawal from the vial, the pressurized gas will expand to atmospheric pressure. In this way, part of the sample is lost, and its actual volume in the syringe will depend upon atmospheric pressure.

The use of headspace GC to investigate volatile organic compounds was accelerated by the introduction of the flame and argon ionization detectors toward the end of the 1950s. These detectors were more sensitive than the thermal-conductivity detectors used in previous years [20, 35]. With these detectors, chromatographers could detect trace quantities of odorous compounds. These detectors could detect trace quantities of volatile compounds and at the time were being used to investigate a range of raspberry, banana, pear, carrot, onion, peppermint oil, and coffee samples [36–42]. In all of these investigations, the headspace above the liquid or solid sample, confined to a closed container, was sampled with a gastight syringe and the withdrawn aliquot then was injected directly into a gas chromatograph.

2.3 Instrumentation

In the early 1960s, Machata developed a way to overcome the problem of non-volatiles in blood samples. He used a pre-column for blood alcohol analysis [42]. Machata also started to investigate the possibility headspace, as the mode of injection, could be used after placing the blood sample with the added internal standard into a serum vessel, heating it at 60 °C, and injecting 1–2 mL of the headspace into the gas chromatograph with a heated syringe. This method was in use in his laboratory for routine analysis starting in 1963. The use of a syringe to manually transfer samples led to some pressure, temperature, and volume related difficulties such as
sample loss or condensation. These difficulties hampered ability of the researchers to make quantitative measurements.

The first automated headspace GC instrument was introduced in 1967 as a result of a coordinated effort between Machata and Bodenseewerk from Perkin Elmer, Inc. [42, 43]. The instrument was designed to contain the vial carousel located on the top of the cube-shaped heater and the vials were heated to a specific temperature for a sufficient time so that the vial contents reached equilibrium. The sampler then extracts an aliquot of the headspace, which is the directly injected into the gas chromatograph, located behind and to the right of the heater.

Figure 2.1 is a schematic diagram of a contemporary automated system [44]. It shows that the sample vials are in the carousel, which moves the vial of interest into the position of the air-pressurized cylinder. The cylinder then pushes the vial from below into the heater above. When the vial has been heated and set for the desired time, the syringe pierces the septum, and withdraws a sample with the automated plunger. The syringe carrying the sample is then automatically transferred into the injection position in which the plunger pushes the sample into the GC inlet.

In order to resolve the initial problem of headspace sampling of closed sample vials with precise control and reproducibility, it was decided to pressurize the closed and temperature-set sample vial with an inert gas (such as helium), thus allowing the headspace gas to expand for a controlled time into the GC column. This technique allowed for the sample aliquot volume to be accurately and precisely controlled by controlling pressure and time; this technique came to be known as "balanced-pressure sampling."
Figure 2.1- Schematic diagram of contemporary automated system, showing the sample vials in the carousel and the vial of interest in the heater with the syringe.

Reproduced with permission from reference [44].
Another common injection technique incorporated within the instrument is called a “pressure-loop system”. Unlike balanced-pressure, the pressure-loop system uses a known amount of sample. This technique typically uses a six-port valve, and initially sets the vial temperature and then pressurizes the vial. After the vial is pressurized, the valve is turned, and the loop is filled with the sample. After the loop has been filled, the valve is turned again to redirect the gas flow and flush the sample into the transfer line leading to the analytical column.

2.4 Static Headspace Extraction

In static headspace extraction (SHE)-GC, a closed vessel (here, a crimped or screw-capped vial) is inserted into a heater in which it is heated until a thermal equilibrium is reached inside the vial. The sample is sealed in a gas-tight enclosure and held under controlled temperature conditions. Volatile material from a condensed liquid or solid sample enters the headspace of the vial. A single headspace aliquot is removed and injected into a gas chromatographic column at which point it travels with the analytes to the detector where the results are revealed in the form of peaks displayed on an output device.

Thermostatting is the process of heating the vial for a set time at a constant temperature with the intention of bringing the vial and its contents to equilibrium at a given (set) temperature. Equilibrium is considered to be established at the point when the vapor concentration in the headspace and the concentration of the liquid sample phase finally remain constant. Once the contents of the vial have reached equilibrium, an aliquot of the headspace is taken from the vial and injected into the gas chromatograph. The following are the two types of static headspace sampling systems commonly being used.

- Pressure balanced system and
- Pressure loop system.
2.4.1 Pressure Balanced System

In a pressure-balanced or balanced-pressure system, shown in Figure 2.2 [48], the vial is brought to equilibrium, the needle pierces the septum, enters the vial, and pressurizes the vial with the carrier gas (helium) for a fixed time (usually about 2 min) [46, 47]. During pressurization, the pressure inside the vial must be made equal to the carrier gas inlet pressure of the column. Then, once the contents of the vial (headspace) are opened and released into the column gas flow, the content flows forward into the carrier gas flow, into the transfer line, and finally into the column.

Thermostatting Phase: During the thermostatting phase (standby), the sampling needle is in the upper position. The carrier gas flows through solenoid valve, V1, to the column; at the same time the needle cylinder is purged by a small crossflow vented through the second solenoid valve (V2) and needle valve (V2). The crossflow prevents carry over between injections.

Pressurization Phase: After completion of the thermostatting time, the sampling needle moves to the lower position and pierces the sample vial septum. The carrier gas flows into the vial headspace, thus pressurizing it to equal the sampling head pressure (P1).

Injection (or Trap Load) Phase: After the pressurization phase, the solenoid valves, V1 and V2, are closed, which stops the carrier gas flow. The compressed gas in the vial flows into the column or trap. After the pre-selected injection time, the solenoid valves (V1 and V2) are again opened in order to complete the sampling phase. The carrier gas now flows directly into the column and branches to the sample vial, thus preventing additional sample vapor from reaching the column.
Figure 2.2 – Schematic diagram of a balanced-pressure headspace sampling setup. ©2013-2018 PerkinElmer, Inc. All rights reserved. Printed with permission from reference [132].
**Pressure Decay Phase:** This phase allows for the pressure inside the vial to decay into the headspace sampler trap. The column isolation flow prevents branching of the decay flow to the transfer line/GC column and maintains the flow down the column.

### 2.4.2 Pressure Loop System

The other headspace sampler (HS) is the pressure loop system, simply called the loop system. Once the equilibration time is complete after a valve loop injection, a sampling needle is inserted through the vial septum and the sample vial is pressurized to provide a final pressure of 1.5 to 2.0 atmospheres (22.5 to 30 psig). The pressurized vapor is then allowed to escape through a valve sampling loop. In many respects this step is very similar to the pressure-balanced sampling technique described later, except that instead of being diverted directly into the gas chromatographic column or transfer line, the vapor remains in the sampling loop.

The sampling loop has a fixed capacity, which is usually 1 mL. It is held at a temperature that is typically 15 °C above that of the sample vial, which prevents sample condensation. The pressure in the charged loop will be less than that inside the vial and will typically be at ambient pressure at the end of the sampling process. It is possible to connect some restrictor tubing to the loop vent and terminate the sampling early in order to leave a higher residual sample pressure inside the loop that will increase sensitivity of the analytes. Figure 2.3 shows the schematic diagram of various stages of the injection process in a loop system containing a 6-port valve [49].

During *vial pressurization*, the sampling needle punctures the vial after the vial has been thermostatted, and carrier gas pressurizes the vial by maintaining a specified carrier gas flow rate into the vial until the pressure inside the vial reaches the pressure set point. The HS maintains this pressure for the hold time.
Figure 2.3 – Schematic diagram of a loop system with various stages of headspace sampling setup [49]. © Agilent Technologies, Inc. 2011. Reproduced with Permission, Courtesy of Agilent Technologies, Inc.
After the vial is pressurized and has stabilized, the *sample loop filling* mode begins, and the HS will perform the specified extractions. The 6-port valve switches, allowing the pressurized sample to vent through the sample loop. After the specified conditions are met, the loop is considered filled. The *injection mode* begins after the loop equilibration, the HS 6-port valve switches to the injection position, the HS injects the sample, and the HS sends a “Start” command to the GC. The HS vents residual pressure from the vial at this time. After the inject time elapses, the 6-port valve returns to its original position.

### 2.5 Dynamic Headspace Extraction (DHE)

Dynamic headspace extraction (DHE) technique is a continuous gas extraction method that separates volatile sample constituents from the matrix via a continuous flow of an inert gas either above a solid or liquid sample or by bubbling through a sintered glass of high pore density through a liquid sample, preferably an aqueous one; this technique is known as purge and trap (P & T). A high surface is required for rapid mass transfer from the aqueous matrix to the purged gas, and the sintered glass disc provides the necessary small gas bubbles. In an alternative technique, called the chromate-membrane version by Moskvin and Rodinkov [50] that is used for monitoring a continuous aqueous sample flow, sintered Teflon particles packed in a tube of a microporous Teflon membrane provide a three-dimensional porous structure with gaps of open macropores through which the water moves continuously while the purge gas enters the tube through the micropores of the enveloping membrane and those of the sintered particles. The stripped volatiles are finally transferred to the sample loop of a gas chromatograph.

The underlying idea of P&T is to completely separate the volatiles of interest from the sample in order to have them all available for quantitative analysis in the final diluted gas extract. Such an exhaustive extraction proceeds exponentially and therefore takes some time. The purged volatiles are thus present in a diluted gas extract and must be subsequently focused in a trap; this can be a
cold trap, but in general, a cartridge packed with an adsorbent is used from which the trapped compounds are released by thermal desorption and transferred by the carrier gas into the column.

The purpose of using P & T is to completely separate (or purge) volatile components of interest from the sample matrix with the intention of performing quantitative analyses on the final concentrated headspace extract (that was originally concentrated in the headspace during the purge process), which must then be collected in a trap (such as a cold trap). During purging, an inert gas is bubbled through a liquid sample, and the analytes are collected (trapped) in an adsorbent trap. For example, while using a P & T system to determine volatile organic compounds in water, a syringe is used to transfer a controlled volume of sample from a sample container into a glass purge chamber in which it is heated to a desired temperature in order to increase the vapor pressure of compounds of interest. An inert purge gas such as nitrogen or helium is introduced as tiny bubbles through the bottom of a purge chamber, which contains the sample. Smaller bubble sizes, made possible by a fritted disperser, will ensure better gas-water (or solvent/matrix) contact and allow more analyte to be dissolved in the purge gas, which in turn will be collected. The purge gas then passes through a cooled trap with dimensions of about 4 mm internal diameter and 25 cm length that is packed with Tenax or a multi-sorbent trap. Upon completion of the purging, the trap is heated rapidly to between 200°C and 350°C, back-flushed with carrier gas, and the sample is then transferred to the gas chromatograph [51].

2.6 Multiple Headspace Extraction (MHE)

In multiple headspace extraction (MHE), the sample is normally weighed into a sample vial, which is then sealed and thermally equilibrated in the same way as for a regular equilibrium headspace analysis. MHE works by re-analyzing the same sample multiple times. In between each analysis, the headspace is vented so that the vapor equilibration has to be re-established. For each analysis there is less of the analyte in vial, and thus the chromatographic peaks get progressively
smaller. If the same sample is continually reanalyzed, there will come a point at which all of the analyte has been effectively withdrawn from the vial. If we were to add the results from all of these repeated analyses, we would have a measure of the total amount of analyte in the vial and hence in the sample.

To obtain complete extraction of an analyte from a vial may require many repeat analyses (in theory, this will be infinite) and thus, make the technique impractical. However, the study of the way in which the amount of analyte decreases between successful analyses indicates that there is a mathematical trend in which data from just a few runs can be used to predict the results for further analyses. In this way, a few analyses may be performed, and the results from these can be used to estimate the results from all of the analyses needed to extract the total analyte from the vial and thus provide an estimate of the total analyte present in the sample. This is the basis of the MHE technique.

In MHE, successive aliquots are taken from the same headspace of the vial, and this method is then considered to be a dynamic gas extraction carried out in a stepwise manner. The advantage of MHE is the ability to extract virtually the whole amount of analyte from a sample matrix by removing the analytes in parts until there is no analyte left for extraction in the original sample [52]. This technique and associated mathematical model and theory originated with McAuliffe, Suzuki et al., and others [53–60].

MHE is used in instances in which interfering matrices interact with the analyte, such as by partial adsorption or to determine whether a solid analyte contains moisture at a low concentration. Interfering moisture (as an example) has a different vapor pressure from the analyte and would not allow classical quantitative determination using a single point calibration. In cases in which it is impossible to prepare the calibration standard using a matrix identical to that of the actual sample, single point calibration using static headspace techniques will fail. The effect of the
sample matrix is eliminated by extracting the entire analyte amount. Contrary to the situation that exists in single-headspace extraction, it is therefore possible to use calibration standards that do not contain the same matrix as the actual samples. In order for MHE to provide meaningful results, the only prerequisite for using this method is that equilibrium must exist with respect to the analytes with respect to distribution between the phases in the headspace vial.

2.7 Solid Phase Micro-Extraction (SPME)

Solid phase micro-extraction (SPME) was developed in the early 1990s by Pawliszyn and colleagues. The sampling technique involves the use of a fiber coated stationary phase with an extracting phase that can be a liquid or a solid (polymer or sorbent), which extracts both volatile and non-volatile analytes that can be in liquid or gas phase. The quantity of analyte extracted by the fiber is proportional to its concentration in the sample as long as equilibrium is reached or in case of short time pre-equilibrium with the help of convection or agitation.

In SPME, analytes establish equilibria among the sample matrix, the headspace above the sample, and a polymer-coated fused fiber. They then are desorbed from the fiber to a chromatography column. Because analytes are concentrated on the fiber and are rapidly delivered to the column, minimum detection limits are improved, and resolution is maintained. The purpose of using SPME combined with HS-GC instead of HS-GC alone supports the idea that a fiber can discriminate and sample specific analytes of interest that may be in the headspace or liquid sample based on the absorption properties of the surface of the fiber. In addition, difference in solubilities of volatile compounds in the fiber coating also results in selectivity [61]. In one of its earliest published uses in 1992 by Hawthorne et. al., SPME was used in a study in order to determine the amount of caffeine in beverages using fused silica fibers [62]. Also, in a 1992 study by Potter et al., SPME was used with GC-ion trap mass spectrometry in an experiment to detect substituted benzenes in water at the pg/mL level [63]. SPME is a technique that can be
applied to HS-GC in a multiple step process [63–67]. First, a sample vial is prepared the same way as in other static HS-GC techniques. A fiber with a fused-silica coated film of an immobilized stationary phase is attached to the plunger of a gas chromatographic syringe, modified to hold the coated fiber, and moved up and down through the needle. As sampling of the vial begins, the syringe needle pierces the septum, enters the vial and the fiber then protrudes from the tip of the needle and becomes exposed to the contents of the vial.

Once the analyte is absorbed into the fiber, the fiber pulls back up into the needle; the syringe comes out of the vial, and then transfers to the inlet in a manner similar to a normal HS-GC system. Once the syringe is inserted into the hot gas chromatographic inlet, the compound dissolved on the fiber is released by thermal desorption and transferred to the gas chromatographic column via the flow of carrier gas.

In the adsorption step, the fiber is exposed in the headspace of a vial containing the sample in which volatile organic compounds are adsorbed by the fiber. The SPME fiber is retracted into the fiber sheath while the needle is moved to the gas chromatographic inlet port. In the desorption step, the fiber is introduced into the gas chromatographic injection port in which the volatile compounds are released by thermal desorption, and the plunger pushes the SPME fiber back down through the fiber sheath, so that the fiber is exposed to the inside of the inlet of the gas chromatograph in which the sample on the fiber is desorbed and analyzed.

A three-phase with two equilibrium systems exists when SPME is used, because of the presence of the liquid sample phase, the headspace (vapor phase), and the fiber, which is a solid phase. The first equilibrium system is between the liquid and the vapor phases, and the other equilibrium phase is between the vapor phase and the fiber. Equilibrium is reached when the concentrations at each phase remain constant [68].
2.8 Static Headspace Extraction (SHE) - Theory

The static headspace sampling theory was developed by Kolb and Ettre and described in the book entitled “Static Headspace-Gas Chromatography” [52]. An understanding of fundamental principles governing the mass transfer of analytes in headspace system is indispensable for the rational choice, design, and optimization of sample preparation methods. At equilibrium, the chemical system inside a sealed static headspace vial can be characterized with a conventional theoretical treatment. Figure 2.4 illustrates such a system for a two-phase sample system and names of its more important physiochemical characteristics such as liquid phase volumes \((V_S)\), gaseous phase, \((V_G)\), and the equilibrium concentrations of individual analytes in the liquid phase \((C_S)\) and in the gas phase \((C_G)\) [69]. The figure shows solute molecule migration into and out of the liquid and gas phases in a conceptual manner.

The concentration of each analyte in the gas-phase is the quantity as measured by GC analysis, not their concentrations in the liquid sample phase. The chemical system of the headspace vial has two characteristics that determine the relationships between the measured gas-phase concentrations and the original solute concentrations in the sample.

First, to determine the original analyte concentrations, \(C_0\), in the liquid phase before headspace sampling starts, we need to consider the partition or distribution coefficient at equilibrium, \(K\), which is the ratio of a compound's concentration in the liquid phase to that in the gaseous phase.
Figure 2.4 - Schematic diagram of a sample vial for static headspace extraction (SHE), reproduced with permission from reference [69].
The equilibrium of the analyte A between the gaseous and liquid phases in the headspace vial illustrated in the figure can be written as follows.

\[ A_{\text{solution}} \rightleftharpoons A_{\text{gas}} \]  \hspace{1cm} \text{(Equation 2.1)}

The equilibrium constant, K can be written as follows in terms of analyte concentration.

\[ K = \frac{A_{\text{gas}}}{A_{\text{solution}}} \]  \hspace{1cm} \text{(Equation 2.2)}

If the initial concentration \( C_0 \) of a pure analyte in a sample solution Volume of \( V_S \), and after equilibration the concentration of the analyte in the solution and headspace of the vial is taken as \( C_S \) and \( C_G \) respectively, then the equilibrium can be written as follows.

\[ C_S \rightleftharpoons C_G \]  \hspace{1cm} \text{(Equation 2.3)}

The equation can be written using the distribution coefficient at equilibrium, K as follows.

\[ C_S = K \times C_G \]  \hspace{1cm} \text{(Equation 2.4)}

The equilibrium constant, K can be written as follows.

Partition Coefficient, \( K = \frac{C_S}{C_G} \) \hspace{1cm} \text{(Equation 2.5)}

The above equation can be written using the amount of the analyte present in the gaseous phase and solution as \( W_G \) and \( W_S \), respectively, and the volume of gaseous phase and solution is taken as \( V_G \) and \( V_S \), respectively. The partition coefficient, K, can be written as follows:

\[ K = \left( \frac{W_S}{V_S} \right) / \left( \frac{W_G}{V_G} \right) \]  \hspace{1cm} \text{(Equation 2.6)}

Re-arranging the above equation,

\[ K = \left( \frac{W_S}{W_G} \right) \times \left( \frac{V_G}{V_S} \right) \]  \hspace{1cm} \text{(Equation 2.7)}

After substituting the phase ratio, \( \beta \) for \( V_G/V_S \), the equation can be written as follows.
\[ K = \left( \frac{W_S}{W_G} \right) \times \beta \]  \hspace{1cm} \text{(Equation 2.8)}

In which \( \beta \) is the phase ratio of gaseous to liquid phases.

If the initial amount of the analyte added to the solution before equilibration is taken as \( W_0 \), then the initial concentration can be calculated:

\[ C_0 = \left( \frac{W_S}{V_S} \right) \]  \hspace{1cm} \text{(Equation 2.9)}

\[ W_0 = V_S \times C_0 \]  \hspace{1cm} \text{(Equation 2.10)}

After equilibration, the amounts can be written:

\[ W_S = V_S \times C_S \]  \hspace{1cm} \text{(Equation 2.11)}

\[ W_G = V_G \times C_G \]  \hspace{1cm} \text{(Equation 2.12)}

The initial amount of the analyte, \( W_0 \), can be written using the following equation after equilibration:

\[ W_0 = W_S \times W_G \]  \hspace{1cm} \text{(Equation 2.13)}

Substituting equations 2.10 – 2.13 in equation 2.13, it can be shown that

\[ V_S \times C_0 = (V_S \times C_S) + (V_G \times C_G) \]  \hspace{1cm} \text{(Equation 2.14)}

Substituting \( C_S = C_G \times K \) in the above equation,

\[ V_S \times C_0 = (V_S \times C_G \times K) + (V_G \times C_G) \]  \hspace{1cm} \text{(Equation 2.15)}

\[ V_S \times C_0 = C_G [(V_S \times K) + V_G] \]  \hspace{1cm} \text{(Equation 2.16)}

\[ C_0 = C_G \left[ K + \left( \frac{V_G}{V_S} \right) \right] \]  \hspace{1cm} \text{(Equation 2.17)}
Substituting the phase ratio $\beta = \frac{V_G}{V_S}$ in the above equation (2.17),

$$C_0 = C_G \times (K + \beta)$$  \hspace{1cm} (Equation 2.18)

The peak area ($A$) of the analyte in the headspace analysis is directly proportional to the analyte’s concentration, $C_G$:

$$A \propto C_G = \frac{C_0}{(K+\beta)}$$  \hspace{1cm} (Equation 2.19)

The above equation can be written by including the instrument response factor (RF) term that accounts for the instrumental contribution to the response as follows [127]:

$$A = \frac{(RF)C_0}{(K+\beta)}$$  \hspace{1cm} (Equation 2.20)

Equation 2.20 is one of the key relationships in equilibrium headspace sampling [52,70]. The following conclusions can be made from the above equation: 1) If the sample volume, $V_S$ is increased, there will be a reduction in the headspace volume, $V_G$, in the same vial and so the phase ratio, $\beta$ will be reduced as a result. Decreasing the phase ratio, $\beta$ will increase the concentration of all compounds in the headspace phase. 2) If the partition coefficient $K$, is decreased by raising the vial temperature, then this will have the effect of pushing more analyte into the headspace. Of course more of the sample matrix will also pass into the headspace and there is a risk of increasing the pressure inside the vial that affects the sampling process or even cause leakage or breakage in extreme cases. 3) If the partition coefficient $K$ and the phase ratio $\beta$ is consistent between samples and calibration mixtures, then the compound concentration in the headspace vapor (and thus the chromatographic peak area) will be directly proportional to its concentration in the sample prior to analysis. The above conclusions help to predict the impact of changing $K$ and/or $\beta$ on the observed chromatographic peak size.
**Vapor Pressures and Dalton’s Law**

It is assumed in this discussion that the value of $K$ is constant for a given compound and this assumption should apply when the temperature and the sample matrix are constant [52,70]. Further, this assumption is true for dilute solutions only and inter-molecular interactions may cause deviations at higher concentrations. To understand these interactions further, the relationship between $K$ and vapor pressure should be considered. If we evaluate the headspace vapor from a complex liquid sample that has been sealed and thermally equilibrated inside a suitable vial, it would be shown that a variety of compounds are present. The vapor of each compound will contribute to the total observed pressure inside the vial. Dalton’s Law of Partial Pressures states that the total pressure exerted by a gaseous mixture is equal to the sum of the partial pressures of each individual component in a gas mixture. At equilibrium, the partial pressure of each compound will be equivalent to the vapor pressure of that compound. This relationship can be expressed according to the following equation [52,70]:

$$P_{total} = \sum P_i$$  \hspace{1cm} \text{(Equation 2.21)}

In which:

- $P_{total}$ is the total pressure of the headspace vapor
- $P_i$ is the partial pressure of component, $i$

The partial pressure of each component in the headspace is proportional to the fraction of its molecules in the total number of molecules as shown in Equation 2.22:

$$P_i = P_{total} \times X_{G(i)}$$  \hspace{1cm} \text{(Equation 2.22)}

In which:

- $X_{G(i)}$ is the mole fraction of compound $i$ in the headspace vapor
It can be said that the concentration of a compound is proportional to its partial pressure, because the concentration of a compound in the headspace vapor is directly proportional to the number of the molecules of the compound that are present.

**Raoult’s Law**

As per Raoult’s Law, the vapor pressure of a compound above a solution is directly proportional to its mole fraction in that solution as shown below in Equation 2.23 [52,70].

\[
P_i = P_i^0 \times X_S(i)
\]

(Equation 2.23)

In which:

- \( P_i^0 \) is the vapor pressure of the pure compound \( i \) in the headspace vapor
- \( X_S(i) \) is the mole fraction of compound \( i \) in the liquid phase

Equation 2.23 indicates that the concentration of a compound in the vapor phase is proportional to its concentration in the liquid phase. This relationship may be depicted graphically as shown in Figure 2.5 [70]. The concentration of the compound and the resultant GC peak area will be proportional to its vapor pressure.
Figure 2.5 - Relationship between partial pressures and mole fractions in an ideal binary mixture.

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Activity Coefficient

The above Equation 2.23 assumes that the components in the mixture behave in an ideal manner and this rarely occurs because molecules may interact with each other and have a consequential effect on the vapor pressure [70,52]. Raoult’s Law is modified to include activity coefficients to accommodate these deviations from the ideal, as shown in Equation 2.24 [70].

\[ P_i = P_i^0 \times \gamma_i \times X_{S(i)} \]  
(Equation 2.24)

In which \( \gamma_i \) is the activity coefficient of the compound, \( i \), in the sample mixture.

In a two component system, there are three types of molecular interactions:

- Between A molecules
- Between B molecules
- Between A and B molecules

The value of \( \gamma_i \) would be close to 1.0 if the nature of these interactions is similar in all three instances, and Equation 2.23 and Figure 2.6 [70] would apply. An example would be a mixture of compounds with the same molecular structure but containing different isotopes. If the molecular attractions are stronger between different molecules than within the pure compounds, the value of Compound A would then give rise to a partial pressure relationship as illustrated in Figure 2.6 [70], in which hydrogen bonding is higher between dissimilar molecules in a mixture of chloroform and acetone [73]. The value of \( \gamma_i \) would become positive and give rise to a partial pressure relationship as illustrated in Figure 2.7 [70] if the molecular attractions are weak between different molecules than within the pure compounds for a mixture of n-hexane and ethanol [73]:

\[ K = \frac{P_{total}}{(P_i^0 \times \gamma_i)} \]  
(Equation 2.25)
The partition coefficient (K) is therefore a function of the total pressure in the vial \(P_{total}\), the vapor pressure of the pure analyte and the activity coefficient. The following expression is obtained by combining the Equations 2.20 and 2.25, which includes both instrument response factor and activity coefficient as variables that need to be controlled in SHE-GC method development [127].

\[
A = \frac{(RF)C_0}{\left(\frac{P_{total}}{P^0_i \times \gamma_i}\right) + \beta}
\]  
(Equation 2.26)

The above Equation 2.26 gives the understanding of the dependence of the sensitivity of the analyte to various factors in headspace sampling.
Figure 2.6 - Relationship between partial vapor pressures and mole fractions in a mixture of chloroform and acetone with negative activity coefficients. ©2013-2018 PerkinElmer, Inc. All rights reserved. Printed with permission from reference [70].
Figure 2.7 - Relationship between partial pressures and mole fractions in a mixture of n-hexane and ethanol with positive activity coefficients. ©2013-2018 PerkinElmer, Inc. All rights reserved. Printed with permission from reference [70].
Henry’s Law

It should be noted that the value of $\gamma_i$ may vary with concentration. In a dilute solution with concentrations <approximately 0.1%, a compound’s molecular interactions will be almost exclusively with other molecules in the sample matrix and not with itself [52,70]. This self-interaction has the effect of making $\gamma_i$ (and hence $K$) effectively constant over a range of applied conditions. Under these conditions, Henry’s Law will apply. This law states that at a constant temperature, the amount of a gas dissolved in a liquid is directly proportional to the partial pressure of that gas at equilibrium with that liquid. This can be expressed mathematically by Equation 2.27 as follows.

$$P_i = H_i \times X_{S(i)}$$  \hspace{1cm} (Equation 2.27)

In which $H_i$ is the Henry’s Law constant for the compound, i, in the sample matrix. Although Equation 2.26 looks very similar to Equation 2.23 and Equation 2.24, it will only be equivalent if the activity coefficient is unity. In all other instances, Equation 2.24 will only apply at the extremes of the charts shown in Figure 2.6 [70] and Figure 2.7 [70]. Since the analysis involving headspace sampling and gas chromatography normally involves looking at analyte concentrations well below 0.1%, in the vast majority of applications, Henry’s Law is applicable. It can then be assumed that $K$ will be constant across the monitored concentration range, and thus, the concentration in the headspace should be proportional to the original concentration in the sample. At higher concentrations, some non-linearity in the response curve is expected since activity coefficients do vary; thus, the analysis will require a multi-level calibration with curve fitting for accurate quantification.
2.9 Effect of Temperature on Headspace Extraction

Partition and activity coefficients and vapor pressure are all functions of temperature (and partition coefficients are also indirectly proportional to activity coefficients) [52,70]. The partition coefficient of a pure compound within the sample is related to the inverse of its vapor pressure as shown in Equation 2.25. The vapor pressure of a pure compound is influenced by its temperature, and the relationship is exponential. The relationship between the partition coefficient, $K$, and the temperature can be described by the following generalized relationship:

$$\log K = \left(\frac{B'}{T}\right) - C'$$  

(Equation 2.27)

in which $B'$ and $C'$ are substance-specific constants, and $T$ is the absolute temperature. Vapor pressure increases with temperature and thus the value of $K$ will decrease and more of the compound will pass into the headspace phase. Hot liquids will quickly release dissolved volatile compounds. Partition coefficients ($K$) of various compounds between water and air phases over a range of temperatures are shown in Table 2.1.

The headspace concentration is highly affected by a temperature change for a compound such as ethanol with high values of $K$ when dissolved in water. This emphasizes a requirement for careful temperature control of the vial during the equilibration step. For instance, if the temperature drift of the vial is only 1 °C from a set temperature of 60 °C, the change in ethanol concentration in the headspace will be 5%. In order to achieve quantitative precision of 0.5% (which is typical for a good headspace sampling system), the vial temperature must be controlled to within 0.1 °C.
Table 2.1 - Partition coefficients of various compounds between water and air phases over a range of temperatures [70].

<table>
<thead>
<tr>
<th>Compound</th>
<th>40 °C</th>
<th>60 °C</th>
<th>70 °C</th>
<th>80 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioxane</td>
<td>1618</td>
<td>642</td>
<td>412</td>
<td>288</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1355</td>
<td>511</td>
<td>328</td>
<td>216</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>825</td>
<td>286</td>
<td>179</td>
<td>117</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>647</td>
<td>238</td>
<td>149</td>
<td>99</td>
</tr>
<tr>
<td>Methyl ethyl ketone</td>
<td>139.5</td>
<td>68.8</td>
<td>47.7</td>
<td>35.0</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>62.4</td>
<td>29.3</td>
<td>21.8</td>
<td>17.5</td>
</tr>
<tr>
<td>n-Butyl acetate</td>
<td>31.4</td>
<td>13.6</td>
<td>9.82</td>
<td>7.58</td>
</tr>
<tr>
<td>Benzene</td>
<td>2.90</td>
<td>2.27</td>
<td>1.71</td>
<td>1.66</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.82</td>
<td>1.77</td>
<td>1.49</td>
<td>1.27</td>
</tr>
<tr>
<td>o-Xylene</td>
<td>2.44</td>
<td>1.31</td>
<td>1.01</td>
<td>0.99</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>5.65</td>
<td>3.31</td>
<td>2.60</td>
<td>2.07</td>
</tr>
<tr>
<td>1,1,1-Trichloroethane</td>
<td>1.65</td>
<td>1.47</td>
<td>1.26</td>
<td>1.18</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>1.48</td>
<td>1.27</td>
<td>0.78</td>
<td>0.87</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>0.14</td>
<td>0.043</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>0.077</td>
<td>0.040</td>
<td>0.030</td>
<td>0.023</td>
</tr>
</tbody>
</table>
For medium values of $K$, the relationship is approximately proportional. There is only a minor change in the headspace concentration as the temperature is raised, when $K$ is low. The important aspect that must be considered when changing temperature is its effect on the vapor pressure of the sample matrix. In the case of water, this effect is present in most sample matrices, and the vapor pressure increases with temperature as shown in Figure 2.8 [70]. It should be noted that headspace sampling is basically a separation technique in which an attempt is made to extract and inject the volatile components while leaving the bulk of the less-volatile sample matrix in the sample vial.

For nearly all compounds, the concentration ratio of a compound in water versus that in the headspace vapor increases proportionally as the temperature of the sample increases. This relative increase is most noticeable in compounds with a low $K$ value. Although increasing the temperature can be a very effective for increasing the concentration of an analyte in the headspace vapor (especially for compounds with a high value of $K$), there will still be significant increases in the amount of water vapor remaining in the headspace vapor. If a column or detector is particularly susceptible to water, caution must then be exercised before increasing the temperature significantly. A heated liquid inside a sealed vial can build up a significant vapor pressure that could easily exceed the pressure rating of a sample vial. Over-pressurizing can also lead to premature injection of the headspace vapor giving it a double peak effect in the chromatography.
Figure 2.8 - Vapor pressure of water versus temperature. ©2013-2018 PerkinElmer, Inc.

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2.10 Effect of Sample Volume on Headspace Extraction

It is clear from Equation 2.19 that the concentration of a compound in the headspace vapor phase is proportional to its original concentration in the sample and the reciprocal of the partition coefficient $K$, that was added to the phase ratio $\beta$ [52,70]. If $K$ is low (the compound prefers the headspace phase), then the value of $\beta$ (hence sample volume) significantly affects the concentration of the compound in the headspace phase. Conversely, if $K$ is high (the compound favors the sample phase), then adjusting $\beta$ will have a minor effect on the concentration of the compound in the headspace phase.

For compounds with a high partition coefficient, (such as ethanol in water), the effects of changing the sample volume makes minimal difference to the concentration in the headspace vapor. In cases, where the sample is in short supply, lower volumes may be used with no significant performance loss. It should be noted that although the concentration and GC response will be largely independent of the sample volume, there will still be proportionality between the sample concentration and the concentration in the headspace vapor. For compound with a medium value of partition coefficient $K$ (such as toluene in water), there is an approximately proportional relationship between sample volume and headspace concentration in the sample volume. With a very low value for $K$ (such as hexane in water), a small change in sample concentration makes a big difference in headspace concentration. In these instances, analytical detection limits are greatly enhanced by an increase in sample volume. It should be noted that it is even possible to create a headspace with a higher concentration of the compound than originally found in the sample.
Chapter 3 – Ionic Liquids (ILs)

3.1 Introduction

An ionic liquid (IL) is a salt in a liquid state. The term has sometimes been restricted to salts with melting points below some arbitrary temperature such as 100 °C (212 °F). More commonly, ILs have melting points below room temperature; some of them even have melting points below 0 °C. Some of these new materials are liquid over a wide temperature range (300–400 °C), encompassing their melting points to the decomposition temperatures. While ordinary liquids such as water and gasoline are predominantly made of electrically neutral molecules, ILs are largely made of ions and short-lived ion pairs. These substances have been called liquid electrolytes, ionic melts, ionic fluids, fused salts, liquid salts, or ionic glasses. They are known as "solvents of the future" as well as "designer solvents".

Room temperature ILs (RTILs) are salts that are liquid at room temperature and exist as ionic species. While metallic salts such as sodium chloride are ionic solids with high melting points, the ILs, on the other hand, are liquid over a wide range of temperatures. The IL has the inability to remain in order in a crystal lattice due to the steric nature of the anion and/or cation, causing them to remain as a liquid [74]. They are also called as non-molecular ionic solvents and it has low vapor pressure which makes them as “green substitutes” compared to conventional organic solvents [75,76,77,78,79].

The physiochemical properties of the ionic liquid can be tailored based on the analytical needs due to the possibility of different combinations of cations/anions and side chains. Therefore, they are called as “designer solvents”. The ease of synthesis and commercial availability of ILs facilitate their application in various fields. The characteristic features of ILs include low volatility, negligible vapor pressure, thermal stability, high viscosity, polarizability, unique
selectivity, surface tension, and wetting properties. These features of ILs, combined with their ease of preparation, have resulted in a remarkable increase in their use.

3.2 Structure of Ionic Liquids

If we compare a typical IL (such as 1-ethyl-3-methylimidazolium ethylsulfate [m.p. < –20 °C]) with a typical inorganic salt (such as sodium chloride [NaCl, m.p. 801 °C]), it is obvious that the IL has a significantly lower symmetry than the regular salt. Furthermore, the cationic and anionic charges are distributed over a larger volume of the molecule via resonance. As a consequence, IL solidification will take place at lower temperatures. In some cases, especially if long aliphatic side chains are involved, a glass transition is observed as opposed to a melting point. Any salt that melts without decomposing or vaporizing usually yields an ionic liquid. NaCl, for example, melts at 801 °C (1,474 °F) into a liquid with mostly sodium cations and chloride anions. Conversely, when an ionic liquid is cooled, it often forms an ionic solid which may be either crystalline or glassy.

In ILs, at least one of the ions has a delocalized charge, and one component is organic, thus preventing the formation of crystal lattice. Typical structure of IL is shown in Figure 3.1 and the figure also shows the structural differences between a salt and an IL, whereas Figure 3.2 shows the structure of a representative IL, 1-methyl-3-methylimidazolium dimethyl phosphate.

In ILs, strong ionic (Coulombic) interactions within the substances result in negligible vapor pressure unless decomposition occurs. ILs contain organic cations, and their anions could either organic or inorganic. Examples of cationic and anionic groups are shown in Figure 3.3 [80].
Figure 3.1: Schematic diagram of difference in the structure between a salt and the ionic liquid
Figure 3.2: Example of an ionic liquid structure, showing the structure of 1-Methyl-3-methylimidazolium dimethyl phosphate
Figure 3.3: Example examples of cationic and anionic groups in ionic liquids
3.3 History of ionic liquids

The discovery date of the "first" ionic liquid along with the identity of its discoverer is disputed. Ethanolammonium nitrate (m.p. 52–55 °C) was reported in 1888 by Gabriel and Weiner [81]. One of the earliest truly room temperature IL was ethylammonium nitrate (m.p. 12 °C), reported in 1914 by Paul Walden. In the 1970s and 1980s, the alkyl-substituted-based ILs, imidazolium and pyridinium cations containing halide or tetrahalogenoaluminate anions, were developed as potential electrolytes in batteries. The “first” RTIL ethylammonium nitrate (EtNH₃NO₃) with a melting point of 120 °C was reported in 1914 [82]. A new class of RTILs that consisted of dialkylimidazolium chloroaluminate was reported by Wilkes et al. in 1982 [83]. These chloroaluminate ILs have not received much interest due to their sensitivity to moisture and many other chemicals.

In 1948, this class of electrical conduction materials (1-butylpyridinium chloride/AlCl3) first drew attention for their use as electrolytes for aluminum electrodeposition. This field is still the subject of current research [84,85,86,87]. Other important applications in this field are RTIL uses in electrolytes for batteries and fuel cells. The major breakthrough came with the advent of less corrosive, air-stable materials in 1992. Wilkes and Zaworotko introduced 1-ethyl-3-methylimidazolium tetrafluoroborate and focused on its potential application as a solvent. Although 1-butyl-3-methylimidazolium hexafluorophosphate and tetrafluoroborate still are frequently discussed in current literature, there are better choices than these two compounds with respect to performance and handling. It was demonstrated in aqueous media the hexafluorophosphate and tetrafluoroborate anions will degrade, resulting in the formation of hydrogen fluoride, a noxious and aggressive acid.
3.4 Liquid range and thermal stability

The liquid range of the ionic liquid is defined as the temperature range between the melting point (or glass transition temperature) and the boiling point (or the thermal decomposition temperature). ILs have wide liquid range over that seen with molecular solvents. For example, 1-alkyl-3-methyl imidazolium salts usually have a glass transition temperature of −70 to −90 °C and thermal decomposition temperature ranging from 320 °C to 520 °C. The high thermal decomposition temperature indicates high thermal stability, which extends IL use at higher temperatures. It has been observed that polycationic and polyanionic ILs show higher thermal stability and find applications in preparation of gas chromatographic stationary phases [88].

3.5 Applications of Ionic Liquids

ILs have been examined for a variety of industrial applications. Their low vapor pressure, thermal stability, and solvation for a wide variety of compounds and gases make them attractive for gas storage and handling applications. Air products use ILs instead of pressurized cylinders as transport media for reactive gases such as trifluoroborane, phosphine, and arsine. The gases are dissolved in liquids at or below atmospheric pressure and are easily withdrawn from the containers by applying a vacuum. The liquid range is defined as the temperature range between the melting point (or glass transition temperature) and the boiling point (or the thermal decomposition temperature).

A relevant and significant methodical advancement for practical routine analytical requirements of pharmaceutical products would be to dissolve the entire final pharmaceutical dosage form in the HS-GC solvent, thus avoiding tedious extractive sample preparation from active pharmaceutical ingredients (API) and finished products. ILs are salts in which the ions are poorly coordinated, resulting in liquid forms <100 °C or as RTILs. At least one of the ions has a
delocalized charge, and one component is organic, thus preventing the formation of a crystal lattice. The advantages of using ILs as solvent consist of several characteristics: (1) they exhibit higher boiling points; (2) clean liquids (“green substitute”); (3) dissolves both polar and non-polar molecules; (4) can be used to determine solvents such as DMSO, DMF, and DMAC; and (5) higher partition coefficient, thus less vapor pressure is generated in the headspace vial; (6) they are often called “designer solvents” as solvents with the desired physical properties such as viscosity and boiling point can be obtained by changing the ionic groups in the molecule; and (7) highly stable even at higher temperature.

Several ionic liquids were tried as solvents and the IL, 1-butyl-3-methylimidazolium tetrafluoroborate (Bmim BF4) was finalized as the solvent to dissolve the sample and the analytes. The ionic liquid, Bmim BF4, exhibited better characteristics suitable for use as a solvent. Method validation and the application of the method using real samples were carried out using Bmim BF4.

3.6 Ionic Liquids as Stationary Phase for GC Columns

The column plays an important role in the separation of compounds in the chromatography. The column dimension, the operating temperature range of the stationary phase, the type of coated stationary phase, the retention adjustment of relative peak positions based on “phase polarity”, and the longevity of the column in order to ensure consistent results are all important considerations to achieve satisfactory separation efficiency and performance in GC [89]. Selectivity has the greatest influence on resolution more than either capacity or efficiency. Selectivity is governed by the mechanisms and the relative strengths of these mechanisms to each other. Interaction mechanisms are dependent on the base structure of the stationary phase in addition to any pendent groups.
The most widely used GC phase platforms are based on polysiloxane polymers or polyethylene glycols and the drawbacks for these phases includes active hydroxyl (-OH) groups at the polymer termini make these phases susceptible to a back-biting reaction if exposed to moisture and oxygen and can lead to phase degradation and contribute to column bleed; the limited capability to modify the phase limits the capability to alter selectivity; and a major limitation of PEG phases is their thermal limit of around 280 °C.

ILs are attractive stationary phase materials for GC and offer several benefits, including high polarity, high viscosity, and low vapor pressure, resulting from their ionic nature; these factors help improve GC column coatings. The chemical flexibility of their organic part and thermal stability of their inorganic part all offer great potential for research and development scientists to develop new IL-based stationary phases.

ILs consists of two or more linked, anion-associated organic cations, which can be either inorganic or organic. ILs differs physically and chemically from other phases. They are much smaller compared to big, bulky polysiloxane polymer and polyethylene glycol phases, and there are no active hydroxyl groups. These features can lead to greater phase stability, even in the presence of moisture and/or oxygen. Many modifications are possible in order to prepare columns with unique selectivity. The base structure can be di-cationic or poly-cationic, and there are numerous cation, linkage, and anion choices available. Also, the pendant groups can be added to cations and/or linkages. The Figure 3.4 shows the structure of the IL, 1,9 Di(3-vinylimidazolium) nonane bis-(trifluoromethylsulfonyl)imide being used as stationary phase in Supelco, SBL IL-100, the first commercially available ionic liquid GC column [90].
Figure 3.4 - Structure of the Ionic Liquid, non-bonded, 1,9 Di(3-vinylimidazolium) nonane bis-(trifluoromethylsulfonyl)imide being used as stationary phase in the capillary column, Supelco, SBL IL-100, the first commercially available ionic liquid GC column [90].
3.7 Ionic Liquids as Sample Solvent in Headspace Sampling

Static headspace (SH) injection is the most commonly used sampling technique for residual solvents testing in pharmaceuticals with GC [2]. In SHE-GC, only volatile components are introduced into the GC system; this type of introduction reduces interference caused by substances decomposed from samples in addition to diluent impurities. Solvent matrix choice is an important step for SH-GC. For water-soluble samples, water is the matrix medium of choice, and for water-insoluble samples, solvents such as DMSO, DMF, DMA, and benzyl alcohol are used as solvent matrix media. The peak intensity of different analytes is strongly affected by the equilibration temperature of the sample and the type of matrix medium in the headspace vials.

The main disadvantage for using conventional solvents in SHE-GC is the high vapor pressure that is generated due to lower boiling points, which causes flooding of the sample solvent in the injector/column. Flooding causes broad solvent peaks on the chromatogram. RTILs are generally accepted as potential environmentally benign solvents [75,76] with several unique properties [77,78] such as the wide liquid range, negligible vapor pressure, good thermal and chemical stability, and exceptional dissolution properties for both organic and inorganic compounds. The application of ILs as sample solvent in the SH-GC has been reported. ILs exhibit special characteristics, including high boiling points and capability of dissolving both polar and non-polar molecules. The major problem in using ILs as sample solvents is the presence of volatile impurities in them. In chapter 6 of this thesis, a method for determining high boiling USP class 2 solvents by SHE-GC using the IL, Bmim BF4, as diluent has been described. Figure 3.6 shows the structures of some of the ILs that can be used as sample solvents in SHE-GC analysis.
Figure 3.6 - Structures of Ionic Liquids used as sample solvents in headspace GC analysis.
The potential of ionic liquids as solvents for headspace gas chromatography was investigated and reported in the past. The ILs, 1-n-butyl-3-methylimidazolium hydrogen sulfate and 1-n-butyl-2,3-dimethylimidazolium dicyanamide were successfully used for the determination of compounds with boiling points above 200 °C such as 2-Ethylhexanoic acid, formamide, and tri-n-butylamine by SHE-GC with mass spectrometer as detector [128].

It was reported that the IL, 1-n-Butyl-3-methylimidazolium dimethyl phosphate (BMIM DMP) generated very low vapor pressure. Therefore it used as a solvent for the headspace gas chromatographic analysis of solvents with such as dimethylsulfoxide, N-methylpyrrolidone, sulfolane, tetralin, and ethylene glycol in a matrix of excipients such as carboxymethylcellulose, magnesium stearate, guar flour, and corn starch in pharmaceutical products by SHE-GC with mass spectrometer as detector [129].

It was also reported that a procedure was developed by SHE-GC with FID as detector for the determination of the analytes, acetonitrile, dichloromethane, N-methyl-2-pyrrolidone (NMP), toluene, dimethylformamide (DMF), n-butyl ether using the IL, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF4) as sample solvent [123]. Detection limits (DL) were found to be ppm level for all the analytes using this method [123].

Recently, two ionic liquids (ILs), 1-butyl-3-methylimidazolium bis[(trifluoromethyl) sulfonyl]imide ([BMIM][NTf2]) and trihexyltetradecylphosphonium bis[(trifluoromethyl) sulfonyl]imide ([P66614][NTf2]) were successfully used as diluents for the determination of residual solvents using static headspace gas chromatography (SHS-GC) coupled with flame ionization detection (FID) [130]. It was reported that by employing the [BMIM][NTf2] IL as a diluent, a 25-fold improvement in limit of detection (LOD) was observed with respect to traditional HS-GC diluents, such as N-methylpyrrolidone (NMP).
The main issue of using ILs as sample solvent is the presence of impurities which could interfere with the peak of interest and the degradation at higher temperature. The presence of impurities can be removed by heating the IL at elevated temperatures by continuously sparging with inert gases such as nitrogen and helium.

Another issue is relatively higher viscosities of the ILs which could make the handling of these solvents in the laboratory more challenging. The relationship between the viscosities and the ionic structure of the ionic liquids (ILs) has been studied and reported [131]. A direct correlation was observed on the effects of ionic structure with temperature, pressure, and impurity on the viscosity was established. Viscosities of the ILs should be considered as one of the factors while choosing an individual IL as a solvent matrix for SHE-GC.
4.0 Chapter 4: Effect of Varying Sample Matrix and Headspace Sampler parameters

4.1 Introduction

In chapter 2, the theoretical aspects of the headspace sampling for GC were discussed with respect to basic physiochemical processes that determine the contents of the gaseous headspace above a sample in a sealed vial. Although it is possible, and sometimes even desirable, to sample and inject headspace directly into a gas chromatographic system using a hand-held syringe, most of the time analysts will use an automated headspace sampler to acquire and transfer suitably sized headspace fractions into the inlet of a gas chromatographic system. Aside from providing the benefits of better accuracy and repeatability (similar to what is gained using a liquid autosampler compared to manual injections), automated headspace samplers control crucial variables other than timing. Temperature, pressure, flow rates, and timing parameters strongly influence the headspace composition as it appears at the gas chromatographic column or inlet. Headspace autosamplers and their associated sampling methods are more sophisticated than their liquid autosampler counterparts in that they consist of a more extensive set of choices that chromatographers should use in order to obtain the best possible results. As such, the headspace sampler instrument parameters such as temperature, thermostating and injection times, or volume should be controlled in order to obtain precise and accurate results with maximum sensitivity.

Apart from instrument parameters, the sample matrix plays a crucial role in determining the sensitivity of the analysis. First, the selected solvent must completely dissolve the sample and analytes of interest. An excellent solvent choice is water due to ease of handling and the fact that it is non-toxic. If a sample is insoluble in water, an organic solvent may be required, in which case, solvents such as DMSO, DMF, and DMA are commonly used. Applications using
solvents such as benzyl alcohol (BA), N-methyl-2-pyrrolidone (NMP), and ILs have also been reported. Use of mixed diluent systems (such as H₂O/DMSO mixtures) have also been studied and shown to affect responses.

It was also found that adding salt to the sample raises the boiling point of water by affecting the equilibrium of the solvent with its vapor. Because headspace also depends on solvent-vapor equilibria, such techniques can also be used to increase vapor concentration in a headspace GC sample. Salts have been used in headspace analysis. The activity coefficients discussed in Equation 2.24 may be adjusted in many cases by the addition of salts or solvents to the sample matrix. These modifiers are chosen to increase activity coefficients and thus, decrease the partition coefficients and cause more of the compound to pass into the headspace phase.

4.2 Objective

The objective of this study was to determine the effect of variations of the following headspace sampler instrument parameters and the variations due to changing the sample matrix:

- Sample solvents (diluents)
- Matrix effect – *salting out*
- Sample volume / change in phase ratio (β)
- Thermostatting Time
- Injection time / volume in pressure balanced system

The key objectives of these experiments were to determine the effects on the sensitivity of analytes, including methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF), and pyridine.
4.3 Materials

All the solvents used as analytes, including methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine were of ACS reagent grade (>99.5%) obtained from Millipore Sigma (St. Louis, MO, USA). The DMSO used as a solvent was of ACS reagent grade (purity: >99.9%) and sodium sulfate, anhydrous, ACS reagent grade (purity: >99.0%) were also obtained from Millipore Sigma (St. Louis, MO, USA).

4.4 GC Conditions

The instrument model used was a Perkin Elmer Autosystem XL system with the capillary column, DB-624 (Agilent), 60 m length x 530 µm diameter, with 3 µm film thickness, installed on the GC system. The initial temperature of the GC oven was set at 50 °C for 5 min, then raised to 130 °C at 5 °C/min and maintained at this temperature for 1 min, and then raised to 230 °C at 30 °C/min and maintained at this temperature for 5 min. The injector and detector temperatures were set at 230 °C and 250 °C, respectively. Helium was used as the carrier gas with a flow rate of 4.5 mL/min was maintained through the column, with a column head pressure of 6.5 psi. The split flow was maintained at 45 mL/min with a split ratio of 10:1, and the run time for the analysis was 30 min.

4.5 Headspace Sampler Conditions

The instrument model was PE Turbomatrix HS 110 with headspace flow (helium) maintained at 20 psi (138 kPa), and the vial shaker was set as “disabled”. The headspace oven temperature was set at 90 °C, while the needle and the transfer line temperatures were set at 100 °C and 110 °C, respectively. The vials were equilibrated for 30 min in the oven prior to injection with injection time was set at 0.05 min. The vial pressurization time was set at 2.0 min, and the GC cycle time was set at 37 min.
4.6 Diluent Solutions preparation:

**Water/DMSO–50% solution:** Equal volumes of purified water and DMSO were mixed and equilibrated to room temperature.

**Sodium Sulfate–2.5% solution:** 5.0 g of sodium sulfate was weighed precisely and placed in separate 200 mL volumetric flasks. The contents were dissolved and diluted to a set volume with water, 50% water/DMSO solution, or DMSO. The contents were mixed well and equilibrated to room temperature.

**Sodium Sulfate – 7.5% solution:** 15.0 g of sodium sulfate was weighed precisely and placed in a 200 mL volumetric flask. The contents were dissolved and diluted to a set volume with water. The contents were mixed well and equilibrated to room temperature.

4.7 Standard Solutions preparation:

**Standard Stock Solution:** A standard stock solution was prepared in water by transferring the analytes, including methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF), and pyridine individually with the aid of gas-tight syringe into a 25 mL volumetric flask that was half-filled with water. The volumetric flask was weighted using an analytical balance after the addition of each analyte into flask and the weight of each analyte was calculated. The volumetric flask was diluted to the desired volume with water and then mixed well.

**Working Standard Solutions:** Working standard solutions were prepared by transferring 1.0 mL of standard stock solution, using class A pipettes, to seven separate 100 mL volumetric flasks. Each volumetric flask was diluted to volume with water, 50% DMSO in water, 100% DMSO, 2.5% sodium sulfate in water, 2.5% sodium sulfate in 50% DMSO solution, 2.5% sodium sulfate in DMSO, or 7.5% sodium sulfate in water.
The final concentrations of individual analytes are presented in the Table 4.1. Standard vials were prepared by adding accurately measured quantities (3.0 mL or otherwise specified in the experiment) of working standard solution into 22 mL headspace sampler vials and sealed by crimping using an aluminum cap with septa and analyzed by SHE-GC using the instrument conditions described above. A representative chromatogram of a working standard solution (3.0 mL) is shown in Figure 4.1.
Table 4.1 – Concentrations (µg/mL) of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in the Working Standard solution

<table>
<thead>
<tr>
<th>No.</th>
<th>Analyte</th>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>396</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>197</td>
</tr>
<tr>
<td>3</td>
<td>Acetone</td>
<td>79</td>
</tr>
<tr>
<td>4</td>
<td>Acetonitrile</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>Methylene Chloride</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>Tetrahydrofuran (THF)</td>
<td>89</td>
</tr>
<tr>
<td>7</td>
<td>Pyridine</td>
<td>147</td>
</tr>
</tbody>
</table>
Figure 4.1 – Example of a chromatogram of standard solution in water. The standard vials were prepared by transferring 3.0 mL of working standard solution into 22 mL Headspace Sampler vials. All the known analyte peaks are well separated from their neighbouring peaks.
4.8 Results and Discussions

4.8.1 Effects of the variation of diluent and diluent composition

Often, analyses require detection of small amounts of an analyte in the matrix. One of the options for increasing the sensitivity of an analyte is to vary the solvents in the diluent. Many organic compounds have higher solubilities in organic solvents rather than in water; therefore, their partition coefficient is higher in an organic solvent than in water, indicating that their headspace sensitivity is poorer. Upon addition of water, the solubilities of an analyte changes, the partition coefficient is reduced, and thus, headspace sensitivity is increased. In order to study the sensitivity variations, standard solutions containing methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine were prepared using one of three diluents:

- Water
- 50% Dimethyl sulfoxide (DMSO) in water
- 100% Dimethyl sulfoxide (DMSO)

Standard solution vials were prepared from all of the above standard solutions and analyzed using the above GC/headspace parameters. Six injections were prepared from the standard solution preparations. The average of the peak areas of analytes from the standard solution with water as diluent were taken as 100%, and a chart was prepared for comparison. Peak responses of analytes with different diluents are presented graphically in Figure 4.2. Table 4.2 shows the physical properties of analytes and the solvents, which includes boiling point, polarity index, and solubility in water. The graph shows that higher peaks responses were obtained for the analytes in 50% DMSO for all analytes except for methylene chloride and pyridine.
Figure 4.2 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine using water, 50% DMSO and 100% DMSO as diluents
Table 4.2 – Physical properties of analytes and the solvents used for the study, adapted from reference [52], page# 30

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Boiling Point in °C</th>
<th>Polarity Index*</th>
<th>Solubility in Water In %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>65</td>
<td>5.1</td>
<td>100</td>
</tr>
<tr>
<td>Ethanol</td>
<td>79</td>
<td>5.2</td>
<td>100</td>
</tr>
<tr>
<td>Acetone</td>
<td>56</td>
<td>5.1</td>
<td>100</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>82</td>
<td>5.8</td>
<td>100</td>
</tr>
<tr>
<td>Methylene Chloride</td>
<td>40</td>
<td>3.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>66</td>
<td>4.0</td>
<td>100</td>
</tr>
<tr>
<td>Pyridine</td>
<td>116</td>
<td>5.3</td>
<td>100</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
<td>10.2</td>
<td>100</td>
</tr>
<tr>
<td>Dimethyl Sulfoxide</td>
<td>189</td>
<td>7.2</td>
<td>100</td>
</tr>
</tbody>
</table>
Addition of water increases intermolecular activity and this, in turn, increases the activity coefficient. As a result, the partition coefficient (K) values decreases, and the sensitivity increases. The solubility of methylene chloride in water is poor, and its partition coefficient in water is very low. Therefore, it tends to move to the headspace more easily when water is used as a diluent. With 50% DMSO in water and 100% DMSO as diluents, the solubility of methylene chloride was increased, and thus, it stayed with the diluent rather than moving into the headspace. Sensitivity of methylene chloride was reduced in the presence of DMSO. Similarly for pyridine, the intermolecular activity was high when 100% water was used as a diluent. With the headspace sampler temperature of 90 °C, the intermolecular activity was poor when 50% and 100% DMSO were used as diluents since the boiling points of pyridine and DMSO are 116 °C and 189 °C, respectively. Thus, the partition coefficients of pyridine in 50% and 100% DMSO were higher than in water; therefore, there was a decrease in sensitivity of an analyte’s analysis.

4.8.2 Effects of adding salt to the diluents

An analyte may be salted out of a solution – that is, made less soluble by adding salt to the solution. If the solute molecules are nonpolar and have not dissociated, the main forces holding them into the solution are the intermolecular forces between solute molecules and polar water molecules (such as hydrogen bonds). The salt dissociates into ions after dissolving and these ions are more attractive to polar water molecules than to nonpolar solute molecules, which reduces the intermolecular forces holding them into the solution. Solubility of an analyte and that of any other organic/nonpolar molecules is then reduced.

Adding salt to a sample solution can also have the beneficial result of reducing humidity. As described above, the extra ions from the salt attract the water molecules in the solution, making them less likely to evaporate out into the headspace. As a result, the activity coefficient
of the analytes increases and thus the partition coefficient of polar analytes in polar matrices can be significantly reduced after addition of a salt. In order to study the variation in sensitivity, standard solutions containing the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine were prepared using the following diluents.

- Water
- 50% Dimethyl sulfoxide (DMSO) in water
- 100% Dimethyl sulfoxide (DMSO)
- 2.5% of Sodium sulfate in water
- 7.5% of Sodium sulfate in water
- 2.5% of Sodium sulfate in 50% Dimethyl sulfoxide (DMSO) in water
- 2.5% of Sodium sulfate in 100% Dimethyl sulfoxide (DMSO)

Standard solution vials were prepared from all of the above standard solutions and analyzed using the above GC/headspace parameters. Six injections were prepared from standard solution preparations. The average of peak areas of the analytes from the standard solution using water as diluent was taken as 100%, and a chart was prepared for comparison. The peak response of each analyte with a different diluent is presented graphically in Figures 4.3, 4.4 and 4.5. The graph in the Figure 4.3 shows that higher peak responses were obtained for all analytes in 7.5% sodium sulfate in water followed by 2.5% sodium sulfate in water than the peak responses in water. It also shows that increasing the salt concentration caused an increase in the sensitivity of the analytes. As explained above, addition of salt increases the intermolecular activity and thereby increasing activity coefficient. Equation 2.25 indicates that increasing the activity coefficient caused a decrease in the partition coefficient (K) value, and thus the analytical sensitivity was increased.
Figure 4.3 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine using water, 2.5% sodium sulfate and 7.5% sodium sulfate as diluents
Figure 4.4 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine using 50% DMSO in water and 2.5% sodium sulfate in 50% DMSO as diluents
Figure 4.5 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine using 100% DMSO and 2.5% sodium sulfate in DMSO as diluents
The graph in the Figure 4.4 shows peak response obtained for all the analytes in 50% DMSO in water and 2.5% sodium sulfate in 50% DMSO that there is no significant change in the sensitivity of the analytes when the electrolyte (salt) is added. A very small increase in the sensitivity of the analytes was observed by the addition of sodium sulfate (electrolyte), except for methylene chloride. This is due to the fact the concentration of salt is very low and also due to the fact that concentration of the polar solvent - water which contains the active hydrogen is only around 50% in the diluent and so there is insignificant change in the activity coefficient for the analytes. Addition of the salt caused an increase in the viscosity of the sample solution and reduced the ability of the analytes to escape from the solution into the headspace for lesser polar analytes such as methylene chloride which was dissolved in the DMSO present in the diluent.

The graph in the Figure 4.5 shows peak responses obtained for all of the analytes in 100% DMSO and 2.5% sodium sulfate in 100% DMSO. It can be seen that there was no significant change in the sensitivity of the analytes when an electrolyte (salt) is added, except for a small increase in the pyridine sensitivity. This was due to the absence of highly polar solvents such water, which contains active hydrogen atoms in the molecule. Thus, there were no significant changes in the activity coefficients of the analytes. Furthermore, the analytes were well dissolved in 100% DMSO and an addition of a small concentration of salts does not have any effect on the partition coefficient of the analytes in the DMSO. In pyridine, the nitrogen atom is nucleophilic, because the lone pair of electrons on nitrogen cannot be delocalized around the ring. In the absence of highly polar solvents such as water in the diluent, the addition of salt caused an increase in the intermolecular activity of the polar pyridine molecule, and thus caused an increase in the activity coefficient. This increase in activity coefficient, in turn, caused a decrease in the partition coefficient (K) value, and thus, the sensitivity increased.
4.8.3 Effects of varying the sample volume or change in phase ratio (β)

Headspace sample vials typically come in 10-mL and 20-mL sizes. Often, it may be required to analyze a very small amount of a residual solvent in a matrix, and this small quantity raises sensitivity issues. Traditionally, it is expected that increasing the concentration of a sample or injecting more sample into a column would yield a better signal, thereby increasing signal strength. With headspace, more sample volume does not always provide the expected increase in area counts because the greater the sample volume, the smaller the actual headspace volume becomes. The liquid sample volume, in the form of the phase ratio (Equation 2.19), influences the gas-phase concentration more strongly for compounds that are less soluble. As per this equation, increasing sample volume will not significantly affect the headspace concentration for analytes with high $K$ values [52].

4.8.3.1 Experiment to determine the actual Vial Volume, $V_v$

The vial volume is the critical parameter in the determination the effect of varying the sample volume or change in phase ratio (β) on the sensitivity of analytes. In order to determine actual vial volume of the 20 mL headspace sample vial (Perkin Elmer part number: N9306079), an experiment (as described below) was conducted.

Five empty headspace sampler vials were individually weighed on an analytical balance. The vials were then filled carefully with water, making sure to fill the vials completely up to the top. The headspace sampler vial with water reweighed, and the volume of the vial was determined using the density value of water (0.997 g/mL at 20 °C). The results were tabulated and are shown in Table 4.3.

The results in the Table 4.3 clearly demonstrate that the actual volume of the 20 mL headspace sampler vial was 22.3 mL, and this value was used in this study.
Table 4.3 – Vial volume $V_v$, of the 20 mL headspace sampler vials

<table>
<thead>
<tr>
<th>No.</th>
<th>Weight (g)</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.48103</td>
<td>22.41359</td>
</tr>
<tr>
<td>2</td>
<td>22.44149</td>
<td>22.37417</td>
</tr>
<tr>
<td>3</td>
<td>22.36479</td>
<td>22.29770</td>
</tr>
<tr>
<td>4</td>
<td>22.29159</td>
<td>22.22472</td>
</tr>
<tr>
<td>5</td>
<td>22.49207</td>
<td>22.42459</td>
</tr>
</tbody>
</table>

Mean Volume (mL): 22.34695 ~ 22.3 mL
4.8.3.2 Experiment to determine the effect of varying the phase ratio

Experiments were conducted in order to study variations in the sensitivity for the changes in sample volumes, and standard solutions containing the analytes, including methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine were prepared using the following diluents:

- Water
- 50% Dimethyl sulfoxide (DMSO) in water
- 100% Dimethyl sulfoxide (DMSO)

Standard solution vials were prepared by adding 1.0 mL, 2.5 mL, 5.0 mL and 7.5 mL in 20-mL headspace vials (actual volume: 22.3 mL) from all of the above standard solutions and analyzed using the above GC / headspace parameters. The peak areas of the analyte from the standard solution with a 1.0 mL sample volume were taken as 100%, and a chart was prepared for comparison and presented graphically in Figures 4.6–4.8.

The graphs in Figures 4.6 and 4.7 represent peak responses obtained for all of the analytes in 100% water and 50% DMSO in water, respectively. It is clear that only the peak response of methylene chloride changed with sample volume increase. The graph in Figure 4.8 represents the peak response obtained for all the analytes in 100% DMSO. The phase ratio (β) can be defined as the relative volume of the headspace compared to volume of the sample in the sample vial. Lower values for β (such as larger sample size) will yield higher responses for volatile compounds (Equation 2.19). Table 4.4 shows the phase ratio, β, with the sample volume in 20 mL headspace vial (actual volume: 22.3 mL).
Figure 4.6 – Normalized peak response of the analytes, methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in water with the sample volumes of 1.0 mL, 2.5 mL, 5.0 mL and 7.5 mL.
Figure 4.7 – Normalized peak response of the analytes, methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in 50% DMSO in water with the sample volumes of 1.0 mL, 2.5 mL, 5.0 mL and 7.5 mL
Figure 4.8 – Normalized peak response of the analytes, methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in 100% DMSO with the sample volumes of 1.0 mL, 2.5 mL, 5.0 mL and 7.5 mL.
Table 4.4 – Phase Ratio (β) for the sample volume in a 20 mL headspace vial

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample Volume (mL)</th>
<th>Phase Ratio (β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>21.300</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>7.920</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>3.460</td>
</tr>
<tr>
<td>4</td>
<td>7.5</td>
<td>1.973</td>
</tr>
</tbody>
</table>
The significant increase in the sample volume-related peak response for methylene chloride in Figures 4.6 and 4.7 is most likely due to the fact that the partition coefficient of methylene chloride in water is very low. As seen in Equation 2.19, the denominator of the equation contains the partition coefficient, \( K \), plus the phase ratio, \( \beta \), and these two combined factors are responsible for the change in analyte sensitivity in a headspace sampling system. Partition coefficients and phase ratios work together to determine the final volatile compound concentrations in the headspace of sample vials. Striving for the lowest values for both \( K \) and \( \beta \) will result in higher concentrations of volatile analytes in the gas phase and therefore, will yield better sensitivity. If the partition coefficient, \( K \), is large as in the case of all of the analytes except methylene chloride, (Figures 4.6 and 4.7), the change in phase ratio, \( \beta \), does not significantly affect the combined value of the denominator in the Equation 2.19. On the other hand, if the partition coefficient, \( K \), of an analyte in a diluent at a particular temperature is small as in the case of methylene chloride, (Figures 4.6 and 4.7), then the change in the phase ratio, \( \beta \), significantly affects the analyte’s sensitivity.

The graph in Figure 4.8 shows that there is no significant change in the peak response with the change in the sample volume, including that for methylene chloride. This shows that the partition coefficient, \( K \), for all of the analytes in 100% DMSO is larger than the change in phase ratio, \( \beta \), but these changes do not significantly affect the combined value of the denominator in the Equation 2.19. Thus, the peak responses for all of the analytes did not change significantly due to the change in sample volume. It should be noted that for intermediate values of \( K \) (\( \sim 10 \)), the increase in sample volume was approximately linear, and for analytes with low values of \( K \), an increase in sample volume will give a large proportional increase in headspace concentration. The study shows that for analytes having good solubility in the sample matrix, the
sensitivity cannot be significantly improved by increasing sample volume. In addition, decreasing the β value does not always produce the required increase in response that is needed to improve sensitivity. When β is decreased via a concurrent increase in sample size, compounds with high $K$ values partition less into the headspace compared to low $K$ value compounds and yield correspondingly smaller sensitivity changes.

4.8.4 Effects of varying the headspace sampler on thermostatting time

The vial equilibration or thermostatting time plays an important role in solvent-vapor equilibria in headspace analysis. The more readily a solvent can be evaporated into the headspace, the more of that particular solvent will be injected onto the column. If the sample is equilibrated for too short a period of time, less of the analyte will be in the headspace, which can affect overall area amounts. However, after a while, the analyte and its solution will settle into equilibrium; more equilibration will not result in any more samples entering the vapor phase and may result in sample degradation or cause secondary reactions.

The partition coefficient of a compound in the sample is related to the inverse of its vapor pressure when it is a pure compound as shown in Equation 2.25. Vapor pressure increases with temperature and so the value of $K$ will decrease and more of the compound will pass into the headspace phase. As described in section 2.9, the headspace concentration is highly affected by a change in temperature for a compound such as ethanol with high $K$ values in water. It underlines the need for carefully controlling the vial’s temperature during the equilibration step. In order to achieve a quantitative precision of 0.5% (which is typical for a good headspace sampling system) the temperature of the vial must be controlled to within 0.1 °C. In order to achieve the precision in the temperature, the sample solution should attain the set temperature prior to
injection. This experiment was conducted in order to determine the thermostating time required to achieve the equilibrium in the headspace vial system.

In order to study sensitivity variations over the thermostating period, standard solutions containing the analytes, which included methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine, were prepared using the diluents: (1) Water; (2) 50% DMSO in water; and (3) 100% DMSO.

Standard solution vials were prepared from all of the standard solutions and analyzed using the GC/headspace parameters with vial equilibration times of 10, 20, 30, 45, and 60 min. The peak areas of the standard’s analytes with an equilibration time of 10 min for individual diluents were taken as 100% and presented graphically for comparison in Figures 4.9–4.11.
Figure 4.9 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in water with the thermostatting times of 10, 20, 30, 45 and 60 minutes.
Figure 4.10 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in 50% DMSO in water with the thermostatting times of 10, 20, 30, 45 and 60 minutes.
Figure 4.11 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in 100% DMSO with the thermostatting times of 10, 20, 30, 45 and 60 minutes.
The graph in Figure 4.9 represents the normalized peak responses of methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine in water with the thermostatting times of 10, 20, 30, 45, and 60 minutes. It also shows that the peak response increased slightly with an increase in thermostatting time, except for methylene chloride in which the peak response increased to >140% for the thermostatting time of 60 min in comparison to 10 min. For all other analytes, which includes methanol, ethanol, acetone, acetonitrile, THF, and pyridine, the slight increase in peak response shows a normal trend as it takes time to transfer the heat from the headspace oven to the vial. This transfer stabilized after about 20 min. After 20 min, the increase in peak response was very negligible and was within experimental error. Thus, it can be concluded that for methanol, ethanol, acetone, acetonitrile, THF, and pyridine, the minimum thermostatting time required to attain equilibrium in water was 20 min.

Table 4.2 shows that the solubility of methylene chloride is very poor, and this compound is almost insoluble in water. Also, it should be noted that the density of methylene chloride is higher than that of water, and methylene chloride moves to the bottom of the headspace vial when water is used as the diluent. Thus, it takes longer for the methylene chloride to move into the headspace of the vial and attain equilibrium at a particular temperature. Hence, the methylene chloride peak response increased significantly over the experimental time course.

The graph in Figure 4.10 represents the normalized peak responses of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine in 50% DMSO in water with thermostatting times of 10, 20, 30, 45, and 60 min. It shows there is slight increase in peak response with an increase in the thermostatting time. The increase in peak response for methylene chloride is more pronounced than the other analytes due to the reason cited above. After 20 min, the increase in peak response is very negligible and is within experimental error.
The graph in Figure 4.11 represents the normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine in 100% DMSO with thermostatting times of 10, 20, 30, 45, and 60 min. The variation in the peak responses over the range of thermostatting times for all the analytes is within the experimental variation, and there is no significant change for any of the analytes.

It should be noted that the United States Pharmacopeia National Formulary (USP–NF) General Chapter <467> on Residual Solvent specifies the vial equilibration time (thermostatting time) as 60 min for headspace operating parameters procedure 1 and for procedures 2 and 3, the equilibration time (thermostatting time) is specified as 45 min. [104]

4.8.5 Effects of varying the headspace sampler injection time (volume)

One of the parameters in the headspace sampling that affects the sensitivity of the analytes is the injection volume, which consists of the amount of sample from the headspace vial that is transferred to the GC injector for subsequent analysis. It is expected that the sensitivity of the analytes should be directly proportional to the injection volume of the sample from the headspace sample. There are various ways of taking headspace vapor out of a sample vial and injecting just a small fraction of it into a GC column. As discussed in section 2.4, the following two types of headspace sampling systems are presently used predominantly for the instrumentation:

- Pressure Balanced Sampling or Balance Pressure System
- Pressure Loop System or Loop System

In the pressure loop system, the volume of the loop is fixed, and in most of the headspace sampler instruments, a loop with a volume of 1.0 mL is installed to the 6-port valve in the injector assembly. This loop is not generally changed or replaced. However, in the pressure
balanced system, the injection volume could be changed by setting the injection time; changing the injection time could affect the sensitivity of the analytes. Pressure-balanced sampling has the significant advantage of being a single-stage injection technique in which sample vapor from the headspace vial flows directly into the GC column. Methods can be established in which the sample stream is not subject to dilution or loss during the transfer process, and it is easy to adjust the sample volume as it is directly proportional to the sampling time entered in the method.

The following experiment was conducted in order to study sensitivity variations with respect to the change in injection time in a pressure balance system. Standard solutions containing the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, THF, and pyridine were prepared using the following diluents:

- Water
- 50% DMSO in water
- 100% DMSO

Standard solution vials were prepared from all of the above standard solutions and analyzed using the GC/headspace parameters by setting the injection times to 0.02, 0.05, 0.10, 0.25, and 0.50 min. The peak areas of the analytes from the standard solution with an injection time of 0.02 min was taken as 100% and presented graphically for comparison in Figures 4.12–4.14 for the analytes in water, 50% DMSO in water, and 100% DMSO as diluents, respectively.

The graphs in Figures 4.12–4.14 show that there is a general trend with respect to increasing peak responses with the increase in injection time, except for acetonitrile at 0.50 min; however, there is no linear relationship found between the injection time and the peak response.
Figure 4.12 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in water with the injection times of 0.02, 0.05, 0.10, 0.25 and 0.50 minutes.
Figure 4.13 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in 50% DMSO in water with the injection times of 0.02, 0.05, 0.10, 0.25 and 0.50 minutes.
Figure 4.14 – Normalized peak response of the analytes methanol, ethanol, acetone, acetonitrile, methylene chloride, tetrahydrofuran (THF) and pyridine in 100% DMSO with the injection times of 0.02, 0.05, 0.10, 0.25 and 0.50 minutes.
It is clear from the graphs that the trend in the increase in the peak response with the increase of injection volume is same for all the analytes irrespective of the type of diluents used as the trend is similar for all the three diluents (water, 50% DMSO in water and 100% DMSO). It can be concluded that the partition coefficient, $K$, of the analytes does not play any role in the sensitivity with the changes on the injection time. It is important to determine the injection volume, which is the actual sample volume that is transferred to the GC injector with respect to the injection time set in the instrument. The injection volume of the sample can be calculated using the following equation [70]:

$$V_{sampled} = F_{column} \left[ \frac{F_{vial}}{(F_{vial} + F_{GC})} \right] \times \left( \frac{P_{ambient}}{P_{vial}} \right) \times \left( \frac{T_{vial}}{T_{ambient}} \right) \times t_{inject} \quad \text{(Equation 4.1)}$$

In which

$V_{sampled}$ is the equivalent volume of headspace vapor at the pressure and temperature inside the sample vial that is actually injected into the GC column

$F_{column}$ is the flow rate of carrier gas at the outlet of the GC column measured at ambient temperature and pressure

$F_{vial}$ is the flow rate of sample vapor from the sample vial measured at ambient temperature and pressure

$F_{GC}$ is the flow rate of the additional carrier gas added by the GC controller measured at ambient temperature and pressure

$P_{ambient}$ is the absolute ambient pressure under which $F_{column}$ was measured

$P_{vial}$ is the absolute ambient pressure of the headspace vapor inside the vial

$T_{ambient}$ is the absolute temperature under which $F_{column}$ was measured

$T_{vial}$ is the absolute temperature of the headspace vapor inside the vial
\( T_{inject} \) is the injection time set in the method

Based on Equation 4.1, the injection volume was calculated corresponding to the injection time under the instrument conditions/instrument parameters specified in sections 4.4 and 4.5, and the results are graphically presented in Figure 4.15. It is clear from the figure that the trend using as increasing injection volume with increasing injection time is same as that of the increasing trend for the peak response with respect to the injection time presented in Figures 4.12–4.14. From this study, it can be concluded that the increase in peak response is linearly proportional to the injection volume of the analytes.

The chromatograms of the standard injections with different injection times were reviewed with respect to the peak shapes of the analytes. These chromatograms are presented in Figures 4.16–4.20. It is clear from the chromatograms that the peak shapes deteriorate as the injection time increases, and the earlier eluting peaks are broader for the injection times of 0.25 and 0.50 minutes. The chromatograms show that the acetonitrile and methylene chloride peaks were not well separated from the methylene chloride peak at the injection times of 0.25 and 0.50 min. Since there was no baseline separation between acetonitrile and methylene chloride peaks at the injection time of 0.50 minutes, the acetonitrile peak was not properly integrated, and thus the peak area response was found to be lower than the expected trend. The out-of-trend value for acetonitrile peak for injection volume at 0.50 min as shown in Figures 4.12–4.14 is due to the partly merged peak of acetonitrile with the neighboring methylene chloride peak. It is concluded that an injection time of >0.10 min is not recommended.
Figure 4.15 – Graphic presentation of change in injection volume with respect to the change in injection times of 0.02, 0.05, 0.10, 0.25 and 0.50 minutes.
Figure 4.16 – Example of a chromatogram of standard solution in water with the injection time of 0.02 minutes. All the known analyte peaks are well separated from their neighbouring peaks and the peak shapes of the analytes are good.
Figure 4.17 – Example of a chromatogram of standard solution in water with the injection time of 0.05 minutes. All the known analyte peaks are well separated from their neighbouring peaks and the peak shapes of the analytes are good.
Figure 4.18 – Example of a chromatogram of standard solution in water with the injection time of 0.10 minutes. All the known analyte peaks are well separated from their neighbouring peaks, but the peak shapes of the analytes are found to be slightly broadened.
Figure 4.19 – Example of a chromatogram of standard solution in water with the injection time of 0.25 minutes. Acetonitrile peak is not well separated from methylene chloride peak and the peak shapes of the analytes are found to be broadened.
Figure 4.20 – Example of a chromatogram of standard solution in water with the injection time of 0.50 minutes. Acetonitrile peak is not separated from its neighbouring methylene chloride peak and the peak shapes of the analytes are found to be very much broadened
5.0 Chapter 5: Experimental – Partition Coefficient Determination of Ethanol

5.1 Introduction

As discussed in section 2.2, the evolution of “Headspace analysis”, started with the analysis of alcohol in water and body fluids as reported in the abstract of a paper presented by R.N. Harger, E.G. Bridwell, and B.B. Raney of the Department of Biochemistry and Pharmacology of Indiana University School of Medicine (Indianapolis, Indiana) published in the year 1939 using “aerometric method” [105]. The paper described a method representing a combination of static and dynamic sampling; the headspace above the liquid sample was conducted through a sulfuric acid/permanganate reagent in order to rapidly determine alcohol in water and body fluids, including blood and urine. Air/water partition coefficients of alcohol were also determined in this and a later paper in 1950 by the same author and compared to values published by other authors in the temperature range of 0 °C to 40 °C [21–31]. Partition coefficients were used to calculate the alcohol concentration in the original sample based on the amount present in the gas phase.

In the early 1960s, G. Machata of the University Forensic Institute in Vienna, Austria, developed a way to overcome the problem of non-volatiles in the blood sample by using a pre-column for the blood alcohol analysis [106]. Machata also started to investigate the possibility of using the headspace as the mode of injection by placing the blood sample with the added internal standard into a serum vessel, thermostatting it at 60 °C, and injecting 1–2 mL of the headspace into the gas chromatograph with a heated syringe [107]. His work led to the development of first headspace sampler, F-40, by Perkin Elmer in 1967.

As discussed previously, the partition coefficient, \( K \), is the fundamental parameter that determines the ability of an analyte to “escape” into the headspace of the vial. Ethanol’s partition
coefficient, \( K \), in blood was used in the “Breath Analyzer (Breathalyzer)” instrument, which is used to determine the blood alcohol content (BAC) in a breath sample. Breath analyzers do not directly measure blood alcohol contents or concentrations, which require a blood sample analysis. Instead, the BAC was estimated indirectly by measuring the amount of alcohol in an individual’s breath. The Breathalyzer measures the ethanol content in breath (BrAC), and from this value, the blood alcohol content (BAC) can be calculated by multiplying this value with the \( K \) value of 2100 for ethanol in air-blood system. This measurement is non-intrusive and used by the law enforcement if an individual is suspected of driving under the influence of alcohol.

In 1983, A.W Jones published a paper that used HS-GC and determined the partition coefficients, \( K \), for water/air, whole blood/air, and plasma/air as 2133, 1756, and 2022, respectively, at 37 \(^\circ\)C, which is the body temperature of humans [108]. The following two types of methods are used to determine the partition coefficient of analyte in a headspace sampling system:

- Vapor Phase Calibration (VPC) method and
- Phase Ratio Variation (PRV) method

Experiments were conducted to determine the partition coefficient \( K \) of ethanol in water using the above two procedures.

### 5.1.1 Vapor Phase Calibration (VPC) method

This procedure, the vapor phase calibration (VPC) method, was developed in 1992 by Kolb et. al [109]. In the VPC method, two sets of vials are prepared. One set is known as the calibration set since it contains a known volume of pure analyte in the vials, which is then totally vaporized and analyzed under the set conditions of analysis. This is known as the total vaporization technique (TVT) [52]. The TVT is used in order to create a vapor-phase only
system, which allows the sample to be used as a calibration standard. In the second set of vials, the same volume of analytes used in the first set is added to the vials containing the liquid phase, and this second set is called as the sample set of vials. The equation to calculate the partition coefficient is derived as follows:

When an amount, \( m^0 \), of the volatile analyte is transferred into the sample vials containing the sample matrix with volume \( V_S \), the amount is distributed between the gaseous and liquid phases (\( m_S \) and \( m_G \), respectively), and the distribution coefficient, \( K \), is calculated as described below in Equation 5.1:

\[
K = \frac{C_S}{C_G} = \left( \frac{m_S}{m_G} \right) \times \left( \frac{V_G}{V_S} \right) \quad \text{Equation 5.1}
\]

in which \( C_S \) and \( C_G \) are concentrations of the analyte in liquid and gaseous phases, respectively. The amount in the sample, \( m_S \), after equilibration is the difference between the initial amount added (\( m^0 \)) and the amount in the gaseous phase, \( m_G \).

\[
m_S = m^0 - m_G \quad \text{Equation 5.2}
\]

The peak area, \( A_S \), obtained from the analysis of the sample vials corresponds to the gas phase concentration, \( C_G \), with the calibration factor denoted with the symbol, @. To determine the calibration factor, @, the standard vials containing the amount of analyte \( m^0 \) in empty vials is vaporized totally and analyzed using the same conditions as that of the samples. The resulting peak area, \( A_C \), is then used to calculate the concentration \( C_G^C \) of the analyte vapor with a vial volume of \( V_V \) as shown in equation 5.3:

\[
C_G^C = \frac{m^0}{V_V} = @A_C \quad \text{Equation 5.3}
\]

\[
m^0 = @A_C \times V_V \quad \text{Equation 5.4}
\]
In the sample vials, the concentration of the analyte in the gas phase \((C_G)\) is calculated as shown in equation 5.5, and the amount of analyte in the sample phase is determined as shown in the next series of equations:

\[
C_G = \frac{m_G}{V_G} = @A_S \tag{Equation 5.5}
\]

\[
m_G = @ \times A_C \times V_G \tag{Equation 5.6}
\]

\[
m_S = @ \times (A_C \times V_V) - (A_S \times V_G) \tag{Equation 5.7}
\]

The equation for the partition coefficient, \(K\), is derived from equations 5.1, 5.2, and 5.7 as shown in the next series of equations:

\[
K = \left(\frac{m_S}{m_G}\right) \times \left(\frac{V_G}{V_S}\right) = \frac{@\times V_G[(A_C\times V_V)-(A_S\times V_G)]}{@\times A_S\times V_G\times V_S} \tag{Equation 5.8}
\]

\[
K = \frac{[(A_C\times V_V)-(A_S\times V_G)]}{A_S\times V_S} \tag{Equation 5.9}
\]

Equation 5.9 is used for the determination of the partition coefficient, \(K\), for ethanol in the air/water system.

5.1.2 Phase Ratio Variation (PRV) method

The phase ratio variation (PRV) method was developed in 1993 by Ettre et al [110]. In the phase ratio variation (PRV) method, using the same standard solution containing a known analyte concentration, the phase ratio of the system was changed and analyzed. The PRV method is based on the relationship between the reciprocal peak area and the phase ratio, \(\beta\), of the sample solution in the vial. If aliquots of a solution are placed in several headspace vials containing different volumes and thus different phase ratios, the resulting peak areas from the headspace analysis of a volatile solute will be different; this difference enables the determination of the prevailing partition coefficient \(K\). The equation for the determination partition coefficient, \(K\),
using the PRV method is explained in Chapter 9 in the book entitled “Static Headspace – Gas Chromatography – Theory and Practice” by Bruno Kolb and Leslie S. Ettre. The equation is derived from the linear regression analysis of phase ratio β against the reciprocal peak area of the respective sample solution (1/A_G):

\[ K = \frac{b'}{a'} \]  

Equation 5.10

In which a’ is the slope, and b’ is the intercept of the linear regression.

There are limitations in calculating partition coefficients using the PRV method. This method for the determination of the partition coefficient depends on peak area differences resulting from changing the phase ratio values and the partition coefficient value will be accurate only if the differences in the peak area values are as large as possible. In the case of large \( K \) values, the relative area differences become small, and the partition coefficient value will not be accurate in this case. The analytical data in section 4.8.3, “Effects of varying the sample volume or change in phase ratio (β)” demonstrates that the peak response for ethanol does not increase with the increase in sample volume in the headspace vial even at the thermostatting temperature of 90 °C. This lack of increase is due to that fact that the partition coefficient of ethanol in air-water is very high, and thus a relatively smaller change in the value of phase ratio β does not result in any significant change in ethanol’s peak sensitivity. Therefore, the PRV method was not used to determine the partition coefficient of ethanol in water at 37 °C.

5.2 Objective

The objective of this study was to determine the partition coefficient, \( K \), of ethanol in an air/water system using the vapor phase calibration (VPC) method and to verify if the value agrees with the previous findings.
5.3 Materials

The solvent used in this study, ethanol, was of ACS reagent grade (>99.5 %) obtained from Millipore Sigma (St. Louis, MO, USA). Purified water from the Milli-Q purified water system (Millipore Sigma, MA) was used as a sample diluent.

5.4 GC Conditions

The instrument model used for analysis was the Perkin Elmer Autosystem XL system with the capillary column, DB-624 (Agilent), 60 m length x 530 µm diameter, 3 µm film thickness installed on the gas chromatographic system. The temperature of the gas chromatographic oven was set at 120 °C (isothermal) for 5 min. The injector and detector temperatures were set at 180 °C and 250 °C, respectively. The flow rate of the carrier gas (helium) was maintained at 7.0 mL/min through the column with a column head pressure of 11.5 psi. The split flow was maintained at 105 mL/min with a split ratio of 15:1, and the run time for the analysis was 5 min.

5.5 Headspace Sampler Conditions

The instrument model used was PE Turbomatrix HS 40 with headspace flow (helium) maintained at 18.5 psi (127 kPa) and the vial shaker was set as enabled. The headspace oven temperature was set at 38 °C (equivalent to 37 °C in the vial), while the needle and the transfer line temperatures were set at 105 °C and 120 °C, respectively. The vials were equilibrated for 30 min in the oven prior to injection with the injection time was set at 0.04 min. The vial pressurization time and withdrawal times were set at 2.0 min and 0.5 min respectively. The gas chromatographic cycle time was set at 7.5 min.
5.6 **Calibration Standard and Sample vials preparation:**

**Calibration Standard Vials:** The calibration standard vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of anhydrous ethanol, precisely measured using a gastight syringe into separate 20 mL headspace sampler vials and sealed. Five sets of calibration standard vials were prepared for analysis.

**Sample Vials:** The sample vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of anhydrous ethanol, precisely measured using a gastight syringe into separate 20 mL headspace sample vials containing 1.0 mL of purified water, measured using class A pipette and then sealed. Five sets of sample vials were prepared for analysis.

These vials were analyzed by using the instrument conditions above. A representative chromatogram of a sample solution is shown in Figure 5.1.
Figure 5.1 – Example of a chromatogram of sample solution in 1.0 mL of water. The sample vial was prepared by transferring 1.0 μL of ethanol into 20 mL headspace sampler vial containing 1.0 mL of water.
5.7 Results and Discussions

5.7.1 Experiment to determine the actual temperature of the content in the vial

One of the critical parameter in the determination of the partition coefficient is the temperature of analyte/sample matrix. As discussed in section 2.9, the temperature is inversely proportional to the partition coefficient. In other words, the sensitivity of the analyte increases with an increase in the temperature of the sample solution. Table 2.1 contains the partition coefficient values of solvents over a range of temperatures.

In order to determine the actual temperature of the sample solution in the vial for the set temperature of the headspace sampler oven, the following experiment was performed. One milliliter of purified sample was transferred to 20 mL headspace vials, sealed, and thermostatted for 30 min. in the headspace oven by setting at temperatures at 37 °C and 38 °C for the vial and oven, respectively. The temperatures of the vial content were measured immediately using a calibrated thermometer. The results of the temperature readings are tabulated in Table 5.1 below.

It is clear from the results in the Table 5.1, for the set temperature of 38 °C in the headspace sampler oven, the actual temperature of the sample solution in the headspace sampler vial is found to be 37 °C. So, all the experiments in this study was performed by setting the headspace sampler oven temperature at 38 °C in order to obtain the vial temperature of 37 °C.
Table 5.1 – Temperature measurements of the content of the vials for the set temperature in the headspace sampler oven

<table>
<thead>
<tr>
<th>Number</th>
<th>Temperature set in the oven: 37 °C</th>
<th>Temperature set in the oven: 38 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading# 1</td>
<td>36.1 °C</td>
<td>37.2 °C</td>
</tr>
<tr>
<td>Reading# 2</td>
<td>36.3 °C</td>
<td>37.1 °C</td>
</tr>
<tr>
<td>Reading# 3</td>
<td>36.1 °C</td>
<td>37.0 °C</td>
</tr>
<tr>
<td>Reading# 4</td>
<td>36.2 °C</td>
<td>37.2 °C</td>
</tr>
<tr>
<td>Reading# 5</td>
<td>36.2 °C</td>
<td>37.1 °C</td>
</tr>
<tr>
<td>Mean</td>
<td>36.2 °C</td>
<td>37.1 °C</td>
</tr>
</tbody>
</table>
5.7.2 Experiment to determine the partition coefficient of ethanol

The calibration standards and the sample solutions prepared were analyzed using the chromatographic parameter/conditions as specified above in order to determine the partition coefficient of ethanol in air-water system using vapor phase calibration (VPC) method. A linear curve was obtained by plotting peak area against the analyte’s volume, and the partition coefficient values of five sets were calculated using the formula under Equation 4.9. In addition to calculating the partition coefficient using peak area, the slope from the regression analysis of the linear curve was used for the calculation of the partition coefficient, $K$, using the following formula under Equation 5.11:

$$K = \frac{[(a_C \times V_V) - (a_S \times V_G)]}{a_S \times V_S}$$  \hspace{1cm} \text{Equation 5.11}

In which $a_C$ and $a_S$ are the slopes of the linearity curves of the calibration standard and sample solution injections, respectively, and $V_V$, $V_G$, and $V_S$ are the volumes of the headspace sampler vial and gaseous and liquid sample phases, respectively. It has been experimentally determined in section 4.8.3.1 that the total vial volume of the 20 mL headspace sample vial (Perkin Elmer part number: N9306079) is 22.3 mL, and this value was used for the calculation in this study. The peak area of the five sets of calibration standard and sample solutions are tabulated in Tables 5.2 and 5.3, respectively. The linear curves for five sets of calibration standards are shown in Figures 5.2–5.6 and the linearity curves of five sets of sample solutions are shown in Figures 5.7–5.11. The final calculated results of partition coefficient are tabulated in Table 5.4.
Table 5.2 – Peak area of ethanol from the calibration standards with anhydrous ethanol volumes of 1.0 µL, 2.0 µL, 3.0 µL and 4.0 µL

<table>
<thead>
<tr>
<th>Set</th>
<th>1.0 µL</th>
<th>2.0 µL</th>
<th>3.0 µL</th>
<th>4.0 µL</th>
<th>Correlation Coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>313324</td>
<td>652547</td>
<td>920282</td>
<td>1254034</td>
<td>0.9990</td>
</tr>
<tr>
<td>2</td>
<td>298209</td>
<td>627054</td>
<td>951635</td>
<td>1216725</td>
<td>0.9988</td>
</tr>
<tr>
<td>3</td>
<td>300630</td>
<td>606170</td>
<td>938193</td>
<td>1242896</td>
<td>0.9999</td>
</tr>
<tr>
<td>4</td>
<td>288106</td>
<td>616682</td>
<td>955673</td>
<td>1278618</td>
<td>1.0000</td>
</tr>
<tr>
<td>5</td>
<td>322391</td>
<td>632565</td>
<td>937389</td>
<td>1269964</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

Mean: 304532 627004 940634 1252447

%RSD: 4.4 2.8 1.5 1.9
Figure 5.2 – Linearity curve of ethanol in calibration standard set - I. The vials were prepared by adding 1.0–4.0 μL (in 1.0 μL increments) of ethanol in empty vials and analyzed.
Figure 5.3 – Linearity curve of ethanol in calibration standard set - II. The vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of ethanol into empty vials and analyzed. 

\[ y = 308012.9000x + 3373.5000 \]

\[ R^2 = 0.9975 \]
Figure 5.4 – Linearity curve of ethanol in calibration standard set - III. The vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of ethanol into empty vials and analyzed.

\[ y = 315882.1000x - 17733.0000 \]
\[ R^2 = 0.9997 \]
Figure 5.5 – Linearity curve of ethanol in calibration standard set - IV. The vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of ethanol into empty vials and analyzed.
Figure 5.6 – Linearity curve of ethanol in calibration standard set - V. The vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of ethanol into empty vials and analyzed.
Table 5.3 – Peak area of ethanol from the sample solution containing 1.0 µL, 2.0 µL, 3.0 µL and 4.0 µL of ethanol in 1.0 mL of water

<table>
<thead>
<tr>
<th>Set</th>
<th>1.0 µL</th>
<th>2.0 µL</th>
<th>3.0 µL</th>
<th>4.0 µL</th>
<th>Correlation Coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3082</td>
<td>6791</td>
<td>9940</td>
<td>13181</td>
<td>0.9993</td>
</tr>
<tr>
<td>2</td>
<td>3183</td>
<td>6780</td>
<td>9713</td>
<td>12952</td>
<td>0.9992</td>
</tr>
<tr>
<td>3</td>
<td>3414</td>
<td>6600</td>
<td>9576</td>
<td>12986</td>
<td>0.9997</td>
</tr>
<tr>
<td>4</td>
<td>3338</td>
<td>6453</td>
<td>9536</td>
<td>13045</td>
<td>0.9995</td>
</tr>
<tr>
<td>5</td>
<td>3205</td>
<td>6608</td>
<td>9888</td>
<td>13055</td>
<td>0.9999</td>
</tr>
<tr>
<td>Mean:</td>
<td>3244</td>
<td>6646</td>
<td>9731</td>
<td>13044</td>
<td></td>
</tr>
<tr>
<td>%RSD:</td>
<td>4.1</td>
<td>2.1</td>
<td>1.9</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.7 – Linearity curve of ethanol in sample solution set - I. The vials were prepared by adding 1.0–4.0 μL (in 1.0 μL increments) of ethanol into vials containing 1.0 mL of water and analyzed.
Figure 5.8 – Linearity curve of ethanol in sample solution set - II. The vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of ethanol into vials containing 1.0 mL of water and analyzed.
Figure 5.9 – Linearity curve of ethanol in sample solution set - III. The vials were prepared by adding 1.0–4.0 μL (in 1.0 μL increments) of ethanol into vials containing 1.0 mL of water and analyzed.
Figure 5.10 – Linearity curve of ethanol in sample solution set - IV. The vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of ethanol into vials containing 1.0 mL of water and analyzed.
Figure 5.11 – Linearity curve of ethanol in sample solution set - V. The vials were prepared by adding 1.0–4.0 µL (in 1.0 µL increments) of ethanol into vials containing 1.0 mL of water and analyzed.
Table 5.4 – Final results of Partition Coefficient K of ethanol in air-water system at 37°C

<table>
<thead>
<tr>
<th>Volume (µL)</th>
<th>Partition Coefficient, K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I Set</td>
</tr>
<tr>
<td>1</td>
<td>2305</td>
</tr>
<tr>
<td>2</td>
<td>2049</td>
</tr>
<tr>
<td>3</td>
<td>2089</td>
</tr>
<tr>
<td>4</td>
<td>2091</td>
</tr>
<tr>
<td>Mean:</td>
<td>2133</td>
</tr>
<tr>
<td>%RSD:</td>
<td>5.5</td>
</tr>
<tr>
<td>K, By Slope:</td>
<td>2039</td>
</tr>
</tbody>
</table>
5.8 Conclusion

The results in Table 4.4 demonstrate that this method gave very precise results and the resulting partition coefficient ($K$) value agreed with the previously determined value of 2133. The procedure described in this section provided a convenient method for the determination of partition coefficients ($K$) determination in gas-liquid systems. This method provided practical results and may be useful in helping to establish criteria for equilibrium headspace measurements or for the general GC analysis of volatile compounds. This method can also be used to determine the partition coefficient ($K$) of ethanol in blood/air and thereby can be used to determine the blood breath ratio (BBR).
6.1 Introduction

Currently, solvents including dimethylsulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA), benzyl alcohol (BA), and water are used as diluents in the headspace gas chromatographic analysis of pharmaceutical residual solvents [111,112,113,114,115]. As a downside, these diluents preclude the analysis of high-boiling solvents. Due to this, headspace gas chromatography cannot be employed for the residual solvents with very low vapor pressures, formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, N-methylpyrrolidone, and sulfolane. These are designated as Class 2 residual solvents as per United States Pharmacopeia National Formulary (USP–NF) General Chapter <467> on Residual Solvents and the limited applicability of HSGC for very high boiling residual solvents is stated expressly in the U.S. Pharmacopoeia as it states, “[NOTE—The following Class 2 residual solvents are not readily detected by the headspace injection conditions described in the section Identification, Control, and Quantification of Residual Solvents in this general chapter: formamide, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, N-methylpyrrolidone, and sulfolane. Other appropriate validated procedures are to be employed for the quantification of these residual solvents. Such procedures shall be submitted to the USP for review and possible inclusion in the relevant individual monograph. In addition, USP Residual Solvent Class 2—Mixture C RS can be used to develop an alternative procedure.]”[104]. XiaoZhen Feng et al developed a method using petroleum ether (60–90 °C), acetone, tetrahydrofuran, ethyl acetate, methanol, dichloromethane (DCM) and pyridine as diluent [116].
The advent of ionic liquids (ILs) in analytical chemistry separation techniques and sample preparation has brought new possibilities and opportunities \[117,118,119,120,121\]. An ionic liquid is a salt in which the ions are poorly coordinated, which results in these solvents being liquid below 100 °C, or even at room temperature (room temperature ionic liquids, RTIL's). At least one ion has a delocalized charge and one component is organic, which prevents the formation of a stable crystal lattice. Ionic liquids exhibit characteristics including high boiling point and dissolving both polar and non-polar molecules. The use of ionic liquids as solvents of samples for HSGC residual solvents has been demonstrated recently \[122,123,124\].

The major problem in using ionic liquids as sample solvents is the presence of volatile impurities in ionic liquids. In this study, 1-Butyl-3-methylimidazolium tetrafluoroborate (Bmim BF4) was used as a sample solvent and the structure of this ionic liquid is given in Figure 6.1 below. This IL is a liquid at room temperature with a melting point of \(-81 \, ^\circ C\), density of 1.17 at 30 °C, viscosity of 180 mPas at 25 °C and is freely miscible with water \[123\].

Static headspace extraction coupled to GC (HS-GC) is an indispensable technique for analyzing volatile organic compounds and enables the analyst to assay a variety of sample matrices while avoiding the costly and time-consuming preparation involved with traditional GC.

As discussed earlier, headspace chromatography is based on the partitioning of a volatile compound between a liquid or solid sample and the surrounding gas phase followed by transfer of the volatile compound in the gas phase to the gas chromatograph for analysis. The partitioning process is governed by the distribution coefficient, which in cases of gas-liquid systems is termed as the partition coefficient \((K)\) \[125\].
Figure 6.1 – Structure of the Ionic Liquid (IL) 1-Butyl-3-methylimidazolium tetrafluoroborate (Bmim BF4)
6.2 Materials:

The solvents used as standards included 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, formamide, N-methylpyrrolidone, and sulfolane. These were of ACS reagent grade (≥99.5 %) obtained from Millipore Sigma (St. Louis, MO, USA). Caffeine (melting point: 233 °C) used as a sample matrix was Reagentplus® grade (purity: ≥ 99.0 %) was obtained from Millipore Sigma (St. Louis, MO, USA) and Bmim BF4 with a purity of >99 % was obtained from io-li-tec, Ionic Liquid Technologies.

6.3 GC Conditions:

The instrument model used was Agilent 5977A GC/MS system with the capillary column, DB-624 (Agilent), 60 meter length, 530 µm diameter with 3 µm film thickness, installed on the GC system. The initial temperature of the gas chromatographic oven was set at 80 °C for 10 min and then raised to 230 °C at 10 °C/min and kept for 5 min. The injector and detector temperatures were set at 230 °C and 250 °C respectively. The carrier gas, helium flow was maintained at 6.0 mL/min through the column with the split ratio of 2 : 1. The run time for the analysis was 30 min. Data acquisition and processing on HS-GC was conducted by using Agilent MassHunter® software.

6.4 Headspace Sampler Conditions:

The instrument model used was Agilent 7697A headspace sampler connected to the gas chromatograph using the transfer line type, DB-ProSteel® with 0.53 mm diameter and 1 mL loop was installed on the instrument. The vial fill pressure (helium) was maintained at 15 psi, and the vial shaking level was set at 2. The headspace oven temperature was set at 210 °C, while the loop and the transfer line temperatures were set at 215 °C and 225 °C, respectively. The vials were equilibrated for 30 min in the oven prior to injection with an injection time set at 1.0 min.
The loop equilibration and the vial pressurization times were set at 1.0 and 2.0 min, respectively. The GC cycle was set at 40 min.

6.5 Solvent Matrix

The IL, Bmim BF4, was used as the solvent matrix. Blank vials were prepared by adding 5.0 mL of Bmim BF4, using positive displacement pipette from Eppendorf® into 22 mL headspace vials and sealed by crimping using aluminum cap with septa. A representative chromatogram is shown in Figure 6.2.

6.6 Standard preparation:

A standard solution with six analytes was prepared in Bmim BF4 by weighing the individual analytes in a 50 mL volumetric flask containing about 10 mL of Bmim BF4, using analytical balance, and then diluting to volume with Bmim BF4. Further diluted 5.0 mL of this solution using positive displacement pipette from Eppendorf® to 100 mL in a class A volumetric flask with Bmim BF4 and the final concentrations presented in the Table 6.1. Standard vials were prepared by precisely adding measured quantities (5.0 mL) of standard solution using positive displacement pipette from Eppendorf® into 22 mL headspace sampler vials, sealed by crimping using aluminum caps with septa, and analyzed using the instrument conditions above. A representative chromatogram is shown in Figure 6.3.
Figure – 6.2: Chromatogram of 1-Butyl-3-methylimidazolium tetrafluoroborate (Bmim BF4) used during validation study.
Table 6.1: Properties of the six residual solvent analytes and it’s concentrations in the Standard preparation

<table>
<thead>
<tr>
<th>No.</th>
<th>Solvent</th>
<th>Boiling Point (°C)</th>
<th>Standard Conc. (µg/mL)</th>
<th>Equivalent amount in sample (ppm)</th>
<th>Permitted Daily Exposure (mg/day) as per USP [6]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-Methoxyethanol</td>
<td>125</td>
<td>6.0</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>2-Ethoxyethanol</td>
<td>135</td>
<td>19.3</td>
<td>160</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>Ethylene Glycol</td>
<td>197</td>
<td>100</td>
<td>833</td>
<td>6.2</td>
</tr>
<tr>
<td>4</td>
<td>Formamide</td>
<td>210</td>
<td>50.0</td>
<td>417</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>N-Methylpyrrolidone</td>
<td>203</td>
<td>63.5</td>
<td>530</td>
<td>5.3</td>
</tr>
<tr>
<td>6</td>
<td>Sulfolane</td>
<td>285</td>
<td>30.0</td>
<td>250</td>
<td>1.6</td>
</tr>
</tbody>
</table>
Figure – 6.3: Chromatogram of Standard in IL, 1-Butyl-3-methylimidazolium tetrafluoroborate (Bmim BF4)
6.7 **Sample preparation:**

Sample vials were prepared by adding precisely measured quantities of IL into 22 mL headspace sampler vials containing the sample. Caffeine was chosen to represent the drug substance sample in the sample solution preparation. For sample preparations, 600 mg of caffeine was weighed into a 22 mL headspace vial. Five milliliters Bmim BF4 was measured using positive displacement pipette from Eppendorf® and added to the headspace vial, sealed by crimping using an aluminum cap with septa, and analyzed by SHE-GC using the instrument conditions described above. Spiked sample vials were also prepared by spiking the standard solutions at different levels in order to verify the accuracy of the procedure during the validation study. Representative chromatograms are shown in Figures 6.4 to 6.5.
Figure – 6.4: Chromatogram of Sample in IL, 1-Butyl-3-methylimidazolium tetrafluoroborate (Bmim BF4).
Figure – 6.5: Chromatogram of Sample of caffeine, spiked with standard solution in IL, 1-Butyl-3-methylimidazolium tetrafluoroborate (Bmim BF4).
6.8 Results and Discussion

A standard solution containing the six analytes was prepared in Bmim BF4. The analyte peaks were very well separated from each other and any neighboring peaks. It was observed that there were impurity peaks found in the blank chromatogram. These impurity peaks were observed below the retention time of 6.0 min, and there were no interfering peaks found at or close to the analyte peaks.

Caffeine was used as a sample matrix to represent the drug substance for the determination of high boiling USP class 2 solvents. Sample vials were prepared by weighing 600 mg of caffeine directly into 22 mL Headspace Sampler vials and adding accurately measured quantity of ionic liquid (5.0 mL). None of the analyte peaks were present in the sample solution preparation. It was observed that caffeine was completely dissolved in 5.0 mL of Bmim BF4 after undergoing heating in the headspace sampler during the analysis. The spiked sample vials were prepared by weighing 600 mg of caffeine directly into 22 mL headspace sampler vials and adding accurately measured quantity of standard solution (5.0 mL) was accurately added. All of the known analyte peaks were well separated from neighbouring peaks.

6.8.1. Validation

The analytes, 2-ethoxyethanol, 2-methoxyethanol, ethylene glycol, formamide, N-methylpyrrolidone, and sulfolane were easily separated. Peak selectivity and identification were addressed by preparing the analytes individually in the IL and injecting them using the same GC headspace conditions. Blank runs (See below) were used to check analyte carryover. Caffeine, used as a sample matrix dissolved readily in BmimBF4 upon heating in the headspace sampler and no interfering peaks were observed in the sample chromatogram. The method precision and accuracy were established by preparing six samples spiked with the analytes at the working
standard concentration levels. The %RSD of the peak area of individual analytes from the six injections was < 3.0 %, and the % recovery for sample preparations was 98%–101%. The test method accuracy was established by preparing three samples that were spiked with the analytes at the levels of the limit of quantitation (LOQ) and 120% levels. The % recovery obtained was 97%–108%. The LOQ and linearity of the detector response was done by preparing analyte solutions at various levels from LOQ to 150% of the working standard solution concentration levels. The %RSD of the peak area of the individual analytes from the six injections of LOQ solution was < 5.0%, and the R-squared values of individual analytes from the linearity solutions were >0.99. The results are shown in Table 6.2.
**Table 6.2: Validation data**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>2-Methoxy ethanol</th>
<th>2-Ethoxy ethanol</th>
<th>Ethylene glycol</th>
<th>Formamide</th>
<th>N-Methyl pyrrolidone</th>
<th>Sulfolane</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Precision / Accuracy – 100%</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (µg/mL)</td>
<td>6.72</td>
<td>19.56</td>
<td>100.16</td>
<td>51.36</td>
<td>63.18</td>
<td>28.27</td>
</tr>
<tr>
<td>Equivalent ppm in sample</td>
<td>56</td>
<td>163</td>
<td>835</td>
<td>428</td>
<td>527</td>
<td>236</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.4</td>
<td>1.5</td>
<td>1.8</td>
<td>2.4</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>%Recovery</td>
<td>100.4</td>
<td>98.4</td>
<td>100.2</td>
<td>99.8</td>
<td>99.7</td>
<td>100.9</td>
</tr>
<tr>
<td><strong>Accuracy – LOQ</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (µg/mL)</td>
<td>0.34</td>
<td>0.39</td>
<td>10.02</td>
<td>10.27</td>
<td>1.90</td>
<td>4.24</td>
</tr>
<tr>
<td>%Recovery</td>
<td>98.6</td>
<td>100.4</td>
<td>102.7</td>
<td>108.4</td>
<td>103.3</td>
<td>98.6</td>
</tr>
<tr>
<td><strong>Accuracy – 120%</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (µg/mL)</td>
<td>8.06</td>
<td>23.47</td>
<td>120.19</td>
<td>61.63</td>
<td>75.82</td>
<td>33.92</td>
</tr>
<tr>
<td>%Recovery</td>
<td>100.4</td>
<td>104.7</td>
<td>102.5</td>
<td>100.2</td>
<td>100.7</td>
<td>97.3</td>
</tr>
<tr>
<td><strong>Precision-LOQ</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (µg/mL)</td>
<td>0.3360</td>
<td>0.3912</td>
<td>10.0160</td>
<td>10.2720</td>
<td>1.8954</td>
<td>4.2405</td>
</tr>
<tr>
<td>% Level</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>20</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Equivalent ppm in sample</td>
<td>3</td>
<td>3</td>
<td>83</td>
<td>86</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td>%RSD</td>
<td>2.4</td>
<td>1.1</td>
<td>3.2</td>
<td>4.1</td>
<td>3.2</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Linearity</strong>&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Level</td>
<td>5 - 150</td>
<td>2 - 150</td>
<td>10 – 150</td>
<td>20 - 150</td>
<td>3 - 150</td>
<td>15 - 150</td>
</tr>
<tr>
<td>R-Squared</td>
<td>0.9998</td>
<td>0.9996</td>
<td>0.9998</td>
<td>0.9991</td>
<td>0.9997</td>
<td>0.9986</td>
</tr>
<tr>
<td><strong>Inter day - Precision – 100%</strong>&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (µg/mL)</td>
<td>6.55</td>
<td>20.08</td>
<td>100.86</td>
<td>51.22</td>
<td>65.14</td>
<td>29.33</td>
</tr>
<tr>
<td>Equivalent ppm in sample</td>
<td>55</td>
<td>167</td>
<td>841</td>
<td>427</td>
<td>543</td>
<td>244</td>
</tr>
<tr>
<td>%RSD</td>
<td>2.4</td>
<td>2.0</td>
<td>1.9</td>
<td>1.6</td>
<td>2.1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

---

a – Prepared six samples spiked with standard solution

b – Prepared three samples spiked with standard solution at limit of quantitation (LOQ) level

c – Prepared three samples spiked with standard solution at 120% level

d – Prepared six standard solution at limit of quantitation (LOQ) level

e – Prepared one standard solution at each levels

f – Prepared six samples spiked with standard solution on a different day
6.9. Conclusion

RTILs are promising solvents and can be used as solvents to determine higher boiling points of trace solvents using SHE-GC. This finding is significant for water-insoluble samples, especially for increasing the sensitivity of higher boiling point analytes. With the present method, we were able to establish the LOQ level of equivalence to as low as 3 ppm each for 2-methoxy ethanol and 2-ethoxy ethanol in the samples. Also, the accuracy of the test procedure was established at the LOQ level and at the 100% and 120% levels of the standard preparation with the % recovery within 97%–108% for each analyte. The linearity of the test method was established from LOQ to 150% levels of the standard preparation with obtained R-squared value higher or equivalent to 0.999 for each analyte. RTILs will be better alternate solvents than the traditional solvents such as water, DMSO, DMF, DMA, and benzyl alcohol. It was demonstrated that BmimBF₄ is an excellent matrix medium for the determination of all the residual solvents employed in this study. GC determination of trace levels of residual solvents with low vapor pressure in a well-defined, realistic matrix of common excipients using an IL as solvent for headspace equilibration has been accomplished with excellent validation results.
7.0 LIST OF PUBLICATIONS


8.0 REFERENCES


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203.


