THE DETERMINATION OF WATER UTILIZING HEADSPACE GAS CHROMATOGRAPHY AND IONIC LIQUID STATIONARY PHASES

by

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Abstract

THE DETERMINATION OF WATER UTILIZING HEADSPACE GAS CHROMATOGRAPHY AND IONIC LIQUID STATIONARY PHASES

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Water may be the most analyzed compound in the world. It is measured, monitored and controlled in many industries and most consumer products. The moisture is regulated in products intended for human/animal consumption (*e.g.* foods, additives, and pharmaceuticals) since the presence of water can lead to the growth of microorganisms, molds, and spores which cause many diseases. In addition, water can affect the functionality and lifespan of other consumer products. The presence of moisture in petroleum products reduces it caloric value and flow properties. Water can interact with salts present in the petroleum and corrode pipelines and machinery decreasing their lifespan and structural integrity.

Over the past 80 years Karl Fischer Titration (KFT) has been the leading method to determine the moisture content in samples. This method however required knowledge of water content and structural information about the analyte and its matrix in order to utilize the proper sample size, additives, and titration cell type. Moreover the

presence of a thiol, ketone, aldehyde, amine or siloxane functional group on the analyte or matrix can lead to interactions with the iodine titrant causing an artificially higher measurement of the water content. KFT is slow, labor intensive and some compounds have solubility issues which leads to additional sample preparation.

During the past five years, my research focused on the development of a new analytical method for the quantification of water in diverse sample types, encompassing a wide range of water content. The utilization of headspace gas chromatography (HSGC), ionic liquid GC columns, and a duel detector system produced an easy, rapid, accurate and precise method. The resolution of water from all other components found in various raw materials was obtained on three novel ionic liquid GC stationary phases. These were recently commercialized specifically for the measurement of water. The two detectors utilized were a thermal conductivity detector (TCD) and a barrier discharge ionization detector (BID). Both are universal detectors, meaning they can detect virtually any compound, including water. The utilization of these two detectors allowed for a wide range of water concentration/amounts to be measured. A TCD was used for evaluating samples with higher concentrations of water (1% to 95%). A BID has a narrower working range, but is much more sensitive. Therefore, it was used for analysis of samples containing lower amounts of water and for the measurement of trace water content (0.001% to 15%). The overall result is that the method proved to be effective for samples with as low as 10 ppm (0.001%) to as high as 95% water content. An optimized fully automated purging system was created by changing from a gas syringe injection to a sample loop injection. Incorporating a HS-20 autosampler into the set-up allowed for the reduction of residual moisture in sampling vessels by a factor of 10.

Additionally, significant improvements to both reproducibility and the limit of detection were realized. The accuracy of the final, optimized HSGC method was compared to other methods, such as weight loss on drying, refractive index, Karl Fischer titration, by use of standard reference materials with known water contents (obtained from the National Institute of Standards and Technology, (NIST)). Standard addition was performed when feasible to verify the accuracy of all calibration curves. Relative standard deviations were investigated; all were found to be less than 5%. The overall average relative standard deviation of ~3% indicates the method was precise. The method was successfully used to quantify the amount of water in active pharmaceutical ingredients (APIs), final drug dosage formulations (both solid and liquid) pharmaceuticals, food, petroleum and petrochemical samples. When the results from the newly developed ionic liquid HSGC approach was compared to the KFT the HSGC method was found to be faster more accurate, and more precise. The KFT often had adequate precision in producing inaccurate results.

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List of Acronyms

API:	Active pharmaceutical ingredient
ASTM:	American Society for Testing and Materials
BID:	Barrier discharge ionization detector
BMIM FAP:	1-butyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate
BMIM NCNCN:	1-butyl-3-methylimidazolium dicyanamide
DMA	n, n-dimethylacetamide
DMSO	Dimethylsulfoxide
EMIM Cl^-:	1-ethyl-3-methylimidazolium chloride
EMIM FAP:	1-ethyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate
EMIM NTf_2^-:	1-ethyl-3-methylimidazolium bis (trifluoromethylsulfonyl)imide
GC:	Gas chromatography
HID:	Helium ionization detector
HMIM FAP:	1-hexyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate
HSGC:	Headspace gas chromatography
IL:	Ionic liquid
KFT:	Karl Fischer titration
NIR:	Near-infrared spectroscopy
NIST:	National Institute of Technology and Standards
PDHID:	Pulsed discharge helium ionization detector
RI:	Refractive Index
RSD:	Relative standard deviation

TCD: Thermal conductivity detector

TFO^-: trifluoromethylsulfonate

THPC: Tetrakis(hydroxymethyl)phosphonium chloride

Chapter 1 Introduction

1.1 Importance of Water Determination

Water is the most evaluated analyte, it is measured ~500,000 times per day or about 130 million times a year.[1] The reason water is constantly monitored is because it affects most products utilized or consumed by humans. The water content of different substances influences: (1) chemical stability, (2) microbial growth, and (3) purity and (4) quality of products. In addition, the presence of water also has an impact on both economic and industrial applications. The effects of water on chemical stability are specifically highlighted in the pharmaceutical industry since it can lead to hydrolysis, increase formation of molecular complexes and polymorphism. These processes could lower the shelf life of the medication and could cause detrimental effects if byproducts are consumed.[2-5] The critical threshold for microorganism growth is monitored by both food and drug industries since most molds, spores and bacteria are harmful to livestock, animals and humans.[6-9] The overall quality of many products are determined by the water content, as seen by the consistency of honey and the value of crude oil.[10, 11] The economical and industrial impact of water is a concern of the petrochemical industry since it can influence the price of crude oil.[12] In addition, the transportation speed of petroleum in pipelines is decreased as the water content is increased. Water is regularly measured in finished products such as, transformer oil, since water will negatively affect the breakdown voltage making the oil less effective.[12]

1.2 Current Methods for Measuring Water

1.2.1 Loss on Drying

A number of analytical methods have been developed to determine the water in a variety of matrices. The first method is loss on drying where the sample is heated at a specific temperature for a time period.[1, 13, 14] The decrease in mass is associated with the quantity of water vaporized.[1] This method is easy to perform however there are a few disadvantages. This method is not water specific therefore if other volatile compounds are present they will also be measured and will increase the apparent water content.[1, 13, 15-17] Loss on drying is a time consuming method and has low throughput (hours-days) and in addition it is labor intensive.[1] Another drawback is that if the compounds are thermally labile, they will degrade and the water cannot be accurately measured.[1, 13, 15-17]

1.2.2 Karl Fischer Titration (KFT)

The next method, Karl Fischer titration (KFT), is the most common method for measuring water and has been used for over 80 years.[1] The method is based on the Bunsen reaction between iodine, water, and sulfur dioxide in methanol and pyridine.[1] KFT gained popularity since it could measure water over a wide dynamic range (1 ppm - 100% water).[1] It is a water selective method with high precision, however, it has some shortcomings. The first difficulty is solubility; in order to determine all of the water in a sample the analyte must completely dissolve in the "Karl Fischer solution". This can be a problem since many analytes have limited to no solubility in the KFT medium.[1] Solubility can be improved by using additives; however, in some cases, even with additives, the sample is not able to be dissolved.[1] Another problem is samples that have

low water concentrations.[18] One solution is preconcentration where the water from the sample is extracted using a solvent which is immiscible with the sample but is solubilized by the KFT medium.[19] Instead of using a normal sample size of 2-10 mL, another solution for samples with low water content, is to greatly increase the sample size.[20] Many products have colored dyes added to allow the consumer to quickly identify the product or to make them look more aesthetically appealing, however these dyes can interfere with the KFT endpoint.[20] Lastly, side reactions caused by interfering compounds (*i.e.,* aldehydes, ketones, amides, siloxanes, thiols….etc.) reacting with the KFT medium leads to an incorrect, elevated amount of measured water.[13, 15, 16, 21] For example, crude oil contains many sulfur containing molecules (*e.g* methyl sulfurous acids, mercaptans etc.) and in some cases this can inflate the measurement by ~10 times.[22]

There have been many modifications of the KFT in order to accurately determine the water content of complex samples. When the matrix interferences interact rapidly with the I_2 titrant, a mixture of imidazole, potassium iodine, trichloroacetic acid, and sodium thiosulfate in methanol is utilized to reduce the interference.[22, 23] Since the mixture does not contain sulfur dioxide the solution does not interact with water allowing it to react with only the matrix interferences.[22, 23] This is followed by titrating the water using traditional KFT solvents. KFT has also been adapted in a few cases to measure the water content indirectly by heating the sample in a separate cell and either applying a gas to help remove the water from the sample (*e.g.* stripping oven evaporation KFT) or adding toluene to perform azeotropic distillation.[20, 24] In both methods the water is released in a closed system and then bubbled through the KFT solvent and finally is

titrated. [20, 24] These two methods assume that all of the water is available and/or associated with the toluene.[24] In addition, the setup for the oven evaporation procedure requires large vessels which contain atmospheric moisture which increases the background water and leads to a possible positive bias.[25] The residual moisture presence in toluene, 224.9 ppm, is also not discussed with azeotropic distillation; however, it could produce a positive bias.[26]

1.2.3 Headspace Gas Chromatography (HSGC)

Another approach for measuring water is headspace gas chromatography (HSGC). HSGC works by heating a volatile analyte contained in a nonvolatile matrix in a partially filled closed vial, and then analyzing the gaseous headspace of the vial.[27-29] HSGC is utilized versus direct injection since many samples can contain nonvolatile compounds.[30-34] When direct injection is used, the nonvolatile compounds can degrade in the injection port and slowly elute causing unpredictable interfering peaks in the chromatogram and an unstable baseline.[10, 23] In addition, the analysis time is longer since less volatile compounds with longer retention times are also analyzed.[34] The difficulty when HSGC is utilized for water determination is the atmospheric moisture in the headspace will combine with the water from the sample and can interfere with accurately quantifying the sample water content.[34] In order to decrease the effects of residual moisture, vials were purged with helium or argon prior to heating or the samples are prepared in a glove box purged with nitrogen.[27, 29, 35]

1.2.4 HSGC Instrumentation

1.2.4.1 HSGC Injectors

HSGC can utilize several methods for injecting the headspace vapor into the GC. The simplest is using a gas-tight syringe.[36, 37] This can be performed manually or with an automated system. The advantage of using an autosampler is that a heated syringe can be employed to transfer the sample from the vial to the GC and the syringe can be purged with carrier gas between injections to minimize carry over.[36, 37] The use of a gas-tight syringe can lead to small amount of sample loss due to the change of pressure between the vial and the atmosphere, which negatively affects precision and decreases accuracy.[36, 37] Another common injector is a balance-pressure system, where after the sample is heated it is pressurized.[36, 37] A valve is switched to allow the pressure to be relieved and the headspace fills a transfer line and is injected for a specific time.[36, 37] While the method is reproducible the disadvantage of using a balanced-pressure system is the volume injected is not controlled or known.[36, 37] The last system is the pressure-loop system, which is very similar to the balance-pressure system however when the pressure in the vial is relieved it fills a sample loop of a specific size (0.025 mL, 0.05 mL, 0.1 mL, 0.2 mL, 0.5 mL, 1.0 mL or 3.0 mL) then the valve is switched allowing the sample to be introduced into the GC, illustrated in Figure 1-1.[36-38]

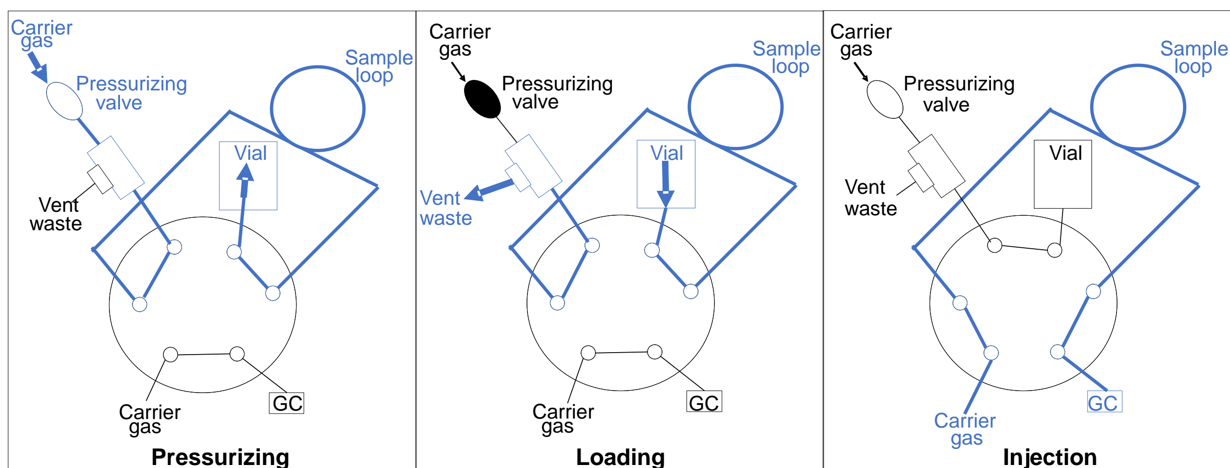


Figure 1-1 Pressure loop system

This system has very high reproducibility, it is easy to change injection volumes and it can be used at high temperatures allowing for the analysis of larger molecules, but sample carryover and ghost peaks are occasionally observed. [36, 37]

1.2.4.2 Solvents

Ionic liquids (ILs) are idea solvents for HSGC.[39-40] An interesting and useful aspect of ILs is that the cation and anion can be modified (*i.e.,* ”tuned”) to provide desired characteristics. ILs are known for dissolving many diverse compounds allowing for complex matrices to be analyzed.[39-40] When ILs are compared to traditional organic solvents in HSGC, they were found to have significant advantages. They have negligible vapor pressure, which means they would not vaporize when heated.[39-40] This reduces competition in the headspace and no solvent peak is observed in the gas chromatogram. In addition, ILs have high thermostablity which allows higher equilibrium temperatures to be utilized without solvent degradation peaks being observed chromatographically.[39-40] Finally, they were found to have a lower concentration of residual solvents than other conventional solvents.

For the analysis of water, the IL solvent should have a low residual water content and not absorb appreciable amounts of atmospheric moisture. Hydrophobic or ultrahydrophobic ILs, are ILs which become saturated at 10,000 ppm of water or less.[41] ILs which contain the tris (pentafluoroethyl) trifluorophsophate ($[FAP]^-$) anion are very hydrophobic containing less than 2030 ppm of water when saturated.[42] The residual water is only 10-15 ppm compared to the residual water in traditional organic solvents, such as dimethylformamide (DMF) with 5594 ppm water or dimethyl sulfoxide (DMSO) which has 773 ppm water.[41,43, 44] Known $[FAP]^-$ ILs contain ~3 times lower amounts of residual water than toluene and 238 times less water than acetone.[43, 44] In fact, the residual water found in acetone is higher than the water content in saturated $[FAP]^-$ containing compounds.[44] Anhydrous organic solvents can be purchased, but they are more costly. Common solvents can be dried to reduce the water content but this process is time consuming or requires specialized equipment such as a glove box. After methanol is dried for 120 hours the residual water is only decreased to 10.5 ppm of water which is the same concentration of residual water found in $[FAP]^-$.[45] Furthermore, once a bottle of anhydrous solvent is opened or the drying method is complete the solvent will absorb atmospheric moisture and the water content will continually increase. Finally, when the $[FAP]^-$ anion is compared to other fluorine-containing anions (*i.e.*, BF_4^- or PF_6^-), it was found to be more thermally stable and hydrolytically stable.[41, 42]

1.2.4.3 Columns

In the past, packed columns for GC were utilized to analyze water. However, the peaks tailed due to non-ideal adsorption isotherms of water on the various supports (*e.g.* molecular sieves, and diatomaceous earth).[35, 47-49] The broadening and poor peak

shape leads to reduced peak area reproducibility and therefore a higher limits of detection. When water and solvents were analyzed concurrently there were often broad overlapping peaks. Lastly, many of these stationary phases degraded in the presence of large quantities of water or air.[50] An improvement in chromatographic methods for measuring water was to utilize poly(ethyleneglycol) (PEG) capillary columns.[27, 28] The water peak still had extensive tailing making it difficult to accurately quantify and decreased precision.[51] Furthermore, the combination of water and elevated temperatures caused rapid column degradation. In order to improve the chromatograms (*e.g.* peak shape, resolution) IL coated open tubular columns were utilized to measure water.[23, 49-56]

The nonmolecular ionic compounds which consist of an organic cation and an organic or inorganic anion are desirable as stationary phases due to their ability to have the cation or anion modified allowing the stationary phase to be tunable.[50,57-61] These stationary phases have been found to give unique selectivity, improved peak shapes and higher reproducibility compared to conventional polyethylene glycol coated capillary columns.[50, 55] The highly polar nature of ILs allows for enhanced separations between polar and nonpolar compounds.[50, 55] These columns have been found to be water and oxygen stable allowing them to be utilized for routine headspace analysis of water containing samples.[2, 50, 52-56] Three IL columns have been shown to retain water while producing good peak shapes with minimal tailing.[50] The Watercol™ 1910 was shown to give the best peak shape for water of the three water columns.[23, 50. 52-57] The Watercol™ 1460 and 1900 were found to have shorter retention times and only require a 30 m column. The Watercol™ columns are utilized to determine water in active

pharmaceutical ingredients, pharmaceutical drug formulations, foods, petrochemical products, alcohols and solvents.[23, 52-57]

1.2.4.4 Detectors

There have been a few different detectors utilized to quantify water (e.*g.* thermal conductivity detector (TCD), mass spectrometry (MS), pulsed discharge helium ionization detector (PDHID), and barrier discharge ionization detector (BID)). Traditionally, the TCD was utilized since it is a common universal detector. It relies on the difference in thermal conductivity between analyte and carrier gas. The only problem with this detector is its relatively low sensitivity compared to other detectors. The use of the MS to determine water can be difficult since water has a low mass to charge ratio (*e.g*. 18 m/z) and the water will be found in the fingerprint region which has increased noise, increasing the limit of detection.[62] In addition, the water present in the carrier gas led the MS to have a high background for water and this negatively affects the limit of detection. The last two detectors are based on a plasma being formed by applying high voltage to rare gases, an idea presented by Lovelock in 1960.[63, 64] The plasma ionizes samples by releasing energy in the form of vacuum ultraviolet light.[63, 64] When helium is the rare gas the ionization energy is 13.5 to 17.5 eV which allows all analytes but helium and neon to be ionized and therefore detected. The newest ionization detector, BID, has placed a dielectric barrier (quartz) between the helium plasma and the electrodes which apply the voltage. The quartz protects the electrodes, increasing the lifespan of the detector and reducing noise associated with the degradation of the electrodes. When the BID was compared to the TCD it was found to be much more sensitive, in some cases ≥ 100 times more sensitive.[64]

1.3 Research Objective and Organization

This dissertation focuses on the development of a new method for the determination of water in diverse samples at virtually any/all concentrations levels. The first portion, Chapters 2-4, of the dissertation focuses on the development of water measurements for pharmaceutical applications. Chapter 2 is the initial development of the method by determining the water content in active pharmaceutical ingredients (APIs) utilizing manual injection and a TCD detector. In Chapter 3 the method has been developed and improved and more complex solid, final drug formulations (APIs and excipient/inert materials) were tested. This research discussed in this includes a comparison of three methods: loss on drying, HSGC, and KFT. It documents the continued development and instrumental improvements in the method by evaluating syringe based autosampler and pressure-loop systems. The last section, Chapter 4, concentrates on liquid pharmaceutical medication which contains a high water content (30 – 90% water) and ethanol (5 – 25%).

The research in chapters 5 and 6 examine the use of HSGC as a facile method for determination of moisture in processed foods. The first section, Chapter 5, focuses on the water content in several different honeys. This chapter also compares two universal detectors, the TCD and the BID, based on their linear range and fthe ability to be utilized for these samples with moderate water contents. Chapter 6 expands the type of samples being analyzed by allowing for all liquid fructose, glucose, and sucralose products. The samples studied in Chapter 6 had a wider range (20% - 95%) of water than honey causing samples with high water content to be evaluated differently from

those with lower water contents. This section utilizes loss on drying, refractive index, and Karl Fischer titration to evaluate the accuracy and precision of the HSGC method.

The last chapter, Chapter 7, focuses on the determination of trace water content (10 ppm to 3500 ppm) in petroleum and petrochemical-based products. The separation of water from the other complex mixture of volatiles was achieved on three unique ionic liquid stationary phases. Finally, the method was evaluated via the determination of water in three NIST standard reference materials.

Chapter 2

WATER DETERMINATION IN ACTIVE PHARMACEUTICAL INGREDIENTS

Abstract

A rapid, accurate, precise and versatile analytical method was developed for the detection and quantification of water in solid active pharmaceutical ingredients (APIs). The headspace gas chromatography (HSGC) method utilized an ionic liquid (IL) based open tubular capillary GC column to increase sensitivity and ruggedness of this method. ILs are also utilized as the headspace solvent because of their low vapor pressure, unique physiochemical properties and high thermal stability. This method is not affected by side reactions and solubility problems which are common with Karl Fischer Titration (KFT) methods. Nor is it as limited as weight loss on drying approaches. The ability to use either/both modern thermal conductivity or barrier ion discharge GC detection provides flexibility, different dynamic ranges and sensitivity. The developed method also was shown to be broadly applicable.

2.1 Introduction

The accurate determination of water content in pharmaceuticals is of importance, and water content is typically controlled in commercial active pharmaceutical ingredients (APIs) and dosage forms. An API's water content can vary during manufacturing, packaging and during the shelf-life of the material. In turn, the water content of an API can be correlated with its chemical stability, the nature of its degradation products, and physical stability (*e.g.,* changes in the crystalline structure and in tablet dissolution profiles).[2-5] Furthermore, too high a water content can facilitate microbial growth.[65] Consequently water levels are specified and controlled. As such, water content is

routinely determined during the development and commercial lifecycle of solid products. The two most commonly used techniques to determine water content are weight loss on drying and the Karl Fischer titration (KFT).

Loss on drying analysis is a relatively straight forward procedure.[1, 13] However, it is not specific, as it measures the change in sample weight after being thermally treated. Any low molecular weight, volatile compounds such as residual solvents or thermal degradation products may be released from the sample and will affect the accuracy of the water quantification.[1,14-16] The most commonly used method for water determination is the KFT, although there are some known disadvantages to this technique.[1, 15] The KFT uses somewhat costly and short lived, limited stability chemicals. The procedure can be labor intensive, slow, and some solid samples do not have good solubility in the working medium leading to additional sample preparation in order to analyze water content.[1, 15, 66] This method can be automated in order to reduce some of the labor required for KFT.[67, 68] When sample preparation is required in order to dissolve solid samples, additional steps to validate and show there is no bias is required, this can be difficult and time consuming.[1, 16, 21] Additionally, water content results can be biased further as KFT is sensitive to atmospheric water content, water content trends higher in summer and lower in winter, thus diligence is required in sample handling, storing, and using solvents and titrants.[1, 14, 66] Also, some samples can have undesired side reactions leading to inaccurate results.[1, 14, 15, 21]

Another method for the determination of water is gas chromatography (GC), this has traditionally been performed using a thermal conductivity detection (TCD) however other detectors such as the helium ionization detector (HID), pulse discharge helium

ionization detector (PDHID), and the barrier ionization detector (BID) also can be utilized. The limiting factor is the vial size, the partition ratio between the solvent and headspace along with the sensitivity of the detector plus the efficiency and stability of the GC stationary phase. TCD, while common, has lower sensitivity therefore the use of the HID, PDHID, or BID can increase the sensitivity of the method. The later ionization detectors, are to a certain degree, analogous in that they all create a helium plasma that ionizes the sample. Early on, a radioactive ionization source was utilized.[69] Later, more acceptable ionization sources were developed including high voltage pulse discharge and high voltage dielectric chambers.[69-72] In 1984 Andrawes used the HID to detect water in solvents and found the limits of detection to be 2 ppm.[71] Later this detection limit was achieved with more advanced TCD detectors.[50] With improvements in the design and ionization source the newer helium plasma detectors should have even lower limits of detection.

In the 1960's, attempts to develop packed column GC into a viable approach to analyze water were presented. However there were numerous problems, such as nonideal adsorption isotherms of water to the diatomaceous earth and various other supports (*e.g.*, molecular sieves) leading to peak tailing and the limited stationary phase matrices that were compatible to such approaches.[35, 37, 46, 48, 49] The broad peak shapes led to poor peak area reproducibility and higher limits of detection and quantitation. Broad overlapping solvent peaks also were problematic. Open tubular capillary columns have been utilized to improved peak symmetry.[35, 37, 49-51] This is particularly seen when ionic liquids (IL) are used as the stationary phases.[49-51] When the anion is trifluoromethylsulfonate (TfO^-) the water peak shape is more symmetric than when the

anion is bis[(trifluoromethyl) sulfonyl] imide (NTf_2^-), BF_4^-, or PF_6^-.[50, 51, 58] In addition, IL stationary phase columns are shown to have high selectivity between water and common residual solvents which may still be present in APIs.[50] When IL stationary phases are used they produce methods that are sensitive, selective, and they do not degrade in the presence of water and oxygen.

Ionic liquids also are ideal solvents for headspace gas chromatography (HSGC), a more sensitive method of analysis compared to direct injection. This is because larger injection volumes of sample and lower split ratios which are commonly used in HSGC. In addition only volatile compounds of interest (and no sample solvent) are injected into the GC leading to lower background noise. ILs are excellent solvents and are now used for analysis of residual solvents in a variety of pharmaceuticals products.[37, 39] Most ILs have negligible vapor pressures even at high temperatures, which minimizes the solvent interference in the chromatogram.[39, 59-61, 74] Furthermore ILs are thermally stable which removes background interferences from degradation products seen with common solvents.[74] Also this extends GC column lifetimes because only the analytes are introduced to the column.[74] The increased stability of ILs allows for higher equilibrium temperatures and increases the sensitivity of the method.[39, 59, 74] The physiochemical properties of ILs can be modified and tuned by altering the structure of the cation and or the anion.[59-61, 73, 74] Due to the tunable nature of the ILs, the variety of organic and inorganic analytes which are soluble in ILs is extensive.[39, 61, 75, 76] Imidazolium trifluorotris(pentylfluoroethyl) phosphate (FAP) ILs, in particular, have unique characteristics which enhance their use for determining water in samples. The FAP anion produces a hydrophobic IL which has low residual water content and low water

uptake upon exposure to ambient conditions.[75] The typical water content in 1-hexyl-3-methylimidazolium trifluorotris(pentylfluoroethyl) phosphate (HMIM FAP) is 10-15 ppm whereas common solvents require time consuming drying processes or costly anhydrous solvents in order to reduce their water concentrations below 50-100 ppm.[75, 76] Anhydrous solvents have higher rates of water uptake causing the water content in samples to change with the age of the solvent. Since the FAP IL has a constant low residual water it would lead to a lower limit of detection and more reproducible analysis. Additionally, FAP ILs have high stability and low viscosity making them ideal solvents for HSGC.[75]

In this work we report the development of a facile procedure to analyze solid samples for residual water content. Compared to existing approaches, small samples sizes can be utilized and the analysis procedure can be automated. This approach is feasible because of the advent of a novel ionic liquids especially designed for enhancing water analysis in HSGC, as well as new and improved GC detectors for water.

2.2 Experimental

2.2.1 Apparatus and Conditions

The analysis with the thermal conductivity detector (TCD) was performed using a 6890N gas chromatograph (Agilent Technologies Inc., Wilmington, Delaware, USA), equipped with Chemstation plus software (Rev.B.01.03). A 1 mL Gastight syringe (Hamilton, Reno, Nevada, USA) was used for all manual injections. A Tracera GC-2010 Plus (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a barrier ion discharge detector (BID), BID-2010 Plus and GC solutions software (version 2.41.00) was used for all analysis performed with the BID detector. The samples were injected

using the Shimadzu AOC-5000 autosampler, with a 1 mL LTN CTC SYR syringe (Hamilton, Reno, Nevada, USA) heated to 50 °C. The vials were agitated at 200 rpm.

A 30 m x 0.25 mm ID x 0.2 μm film coat thickness SLB-IL107, bis-(1-hydroxyethyl imidazole) polyethylene glycol, fused silica capillary column coated with IL synthesized as previously reported or commercially acquired from Supelco/Sigma-Aldrich.[51] The oven temperature was held isothermally at 100 °C with a run time of 7 minutes. The carrier gas for all runs was helium at 1 mL/min (26 cm/sec) with the GC-TCD and 0.8 mL/min (24 cm/sec) for the Tracera GC-BID. The injection port was set at 280 °C and the detector was set at 250 °C. A S8223 Vortex-Genie Mixer (Scientific Products McGaw Parks, Illinois, USA) was used for mixing all samples. The samples were weighed on an AR1140 Adventurer balance (Ohaus Corp., Pine Brook, New Jersey, USA).

2.2.2 Materials

The 1-ethyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (EMIM FAP), 1-butyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (BMIM FAP) and 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (HMIM FAP) were obtained from Merck KGaA (Darmstadt, Germany). Note that the FAP acronym stands for tris(fluoroalkyl)trifluorophosphate. DL-homocysteine thiolactone hydrochloride was obtained from LKT Laboratories Inc. (St. Paul, MN, USA). The α-DL-oxyphene was obtained from Mallinckrodt (Haxelwood, MO, USA). Ascorbic acid, bupivacaine, carnitine, ephedrine, ethylestradiol, ketamine hydrochloride, ibuprofen, lysozyme, penicitamine, promethazine, propranolol, rifampicin, scopolamine, sodium tartrate dihydrate, trimipramine maleate, verapamil hydrochloride, warfarin, 1-Ethyl-3-

methylimidazolium chloride, 1-Ethyl-3-methylimidazoliumbis (trifluoromethylsulfonyl) imide, and 1-butyl-3-methylimidazolium dicyanamide were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

The 15 X 45 mm, 1 dram vials were purchased from Fischer Scientific (Waltham, Massachusetts, USA). The screw-on molded plastic covers were obtained from SKC Inc. (Eighty Four, Pennsylvania, USA). The white silicone/TFE septa were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

2.2.3 Sample Preparation

2.2.3.1 Headspace Gas Chromatography Samples

An uncapped, empty, vial was purged with dry argon for 40 seconds per mL using a 20 G, 1½" long needle. The vial was immediately capped with the molded plastic covers containing two septa. The sample was prepared by adding 400 mg of ionic liquid and 4 mg of analyte to the purged vial. The capped sample was then purged again using a smaller 25 G, 5/8" long needle with dry argon for 15 seconds, a second 25 G, 5/8" long needle was inserted into the septum (2 total needles) in order to alleviate pressure. The two purging needles are removed and then the sample was heated to 125 °C for 30 minutes and 0.6 mL of headspace was extracted and injected into the GC. When there is high humidity additional sample preparation is required. The 1 dram vials are heated to 125 °C for an hour. The vials are then purged with dry argon based on the size of the vial, as before, while cooling to room temperature and then the covers are replaced. The vials are subsequently treated and used as described above.

The calibration curve was produced by making five stock solutions. The following amounts of water, 0.7, 1.4, 2.9, 4.2, and 5.6 µL, was added to 2.8 g of EMIM FAP and

then the solutions were vigorously stirred for three minutes. The solution was divided into 7 parts and analyzed with the HSGC method.

3.2.3.2 Loss on Drying Samples

There are two ways of preforming loss on drying. In the first way a sample of 0.5 grams is weighted and then heated at 50 °C for 2 hours and the mass was measured. This process was then repeated and the mass of the cooled sample was taken again. If the samples lost mass the procedure was repeated until the mass was constant. If the samples did not show mass loss the temperature was increased by 25 °C and the process was repeated until the mass was constant or the compounds degraded. Degradation was determined by a color and consistency changes or a loss of 80% or more of the initial mass. The other method uses a vacuum oven. A 0.5 g sample is weighted and heated at 60 °C for 24 hours. The sample is again weighed and then heated for another 24 hours at 105 °C. The sample is then cooled and weighed again.

3.2.3.3 Solubility of Active Pharmaceutical Ingredients in Ionic Liquids

The solubility of a variety of APIs (e.g. steroids, vitamins, β-blockers, fungicides, etc) in different ILs was determined. 1 mg of sample was added to 400 mg of IL and were heated to 40 °C and stirred for an hour. If the APIs were not visibly dissolved, the samples were subsequently heated to 125 °C and again visually assessed for sample dissolution. The viscosity an important parameter in terms of sample handling. Typically viscosities under 75 Cst are needed for headspace solvents [27, 35]. Higher viscosities (over 1000 Cst) are necessary when using ILs as GC stationary phases [36-38]. The residual water of ILs was examined by heating 0.4 g of the IL to 125 °C for an hour, and then 0.6 mL of headspace is analyzed using the GC.

2.3 Results and discussion

2.3.1 Optimization of Ionic Liquid

Numerous ILs, 1-ethyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate (EMIM FAP), 1-butyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate (BMIM FAP), 1-hexyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate (HMIM FAP), 1-ethyl-3-methylimidazolium chloride (EMIM Cl), 1-butyl-3-methylimidazolium acetate, tetrakis(hydroxymethyl)phosphonium chloride (THPC), tetrabutylphosphonium chloride, 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (EMIM NTf_2^-) and 1-butyl-3-methylimidazolium dicyanamide (BMIM NCNCN) were tested for solubility and viscosity. The subset of FAP ILs was able to dissolve 20 out of the 23 compounds tested; (a few very polar compounds were not well solubilized) and had the lowest viscosities. The other ILs tested were only able to dissolve 13-18 compounds and were much more viscous and thus more difficult to handle. For the ILs with the FAP anion, EMIM FAP provided the highest solubility for the test APIs. As seen in Figure 2-1, as the chain length of the alkyl substituent on the imidazolium cation decreased, the number of APIs solubilized increased. It should be noted that these samples were even less soluble in the KFT medium (sulfur dioxide, imidazole, in methanol) than in the ILs. Also, residual water content, for the ILs with an FAP anion is less than half that of the other 3 ILs studied.

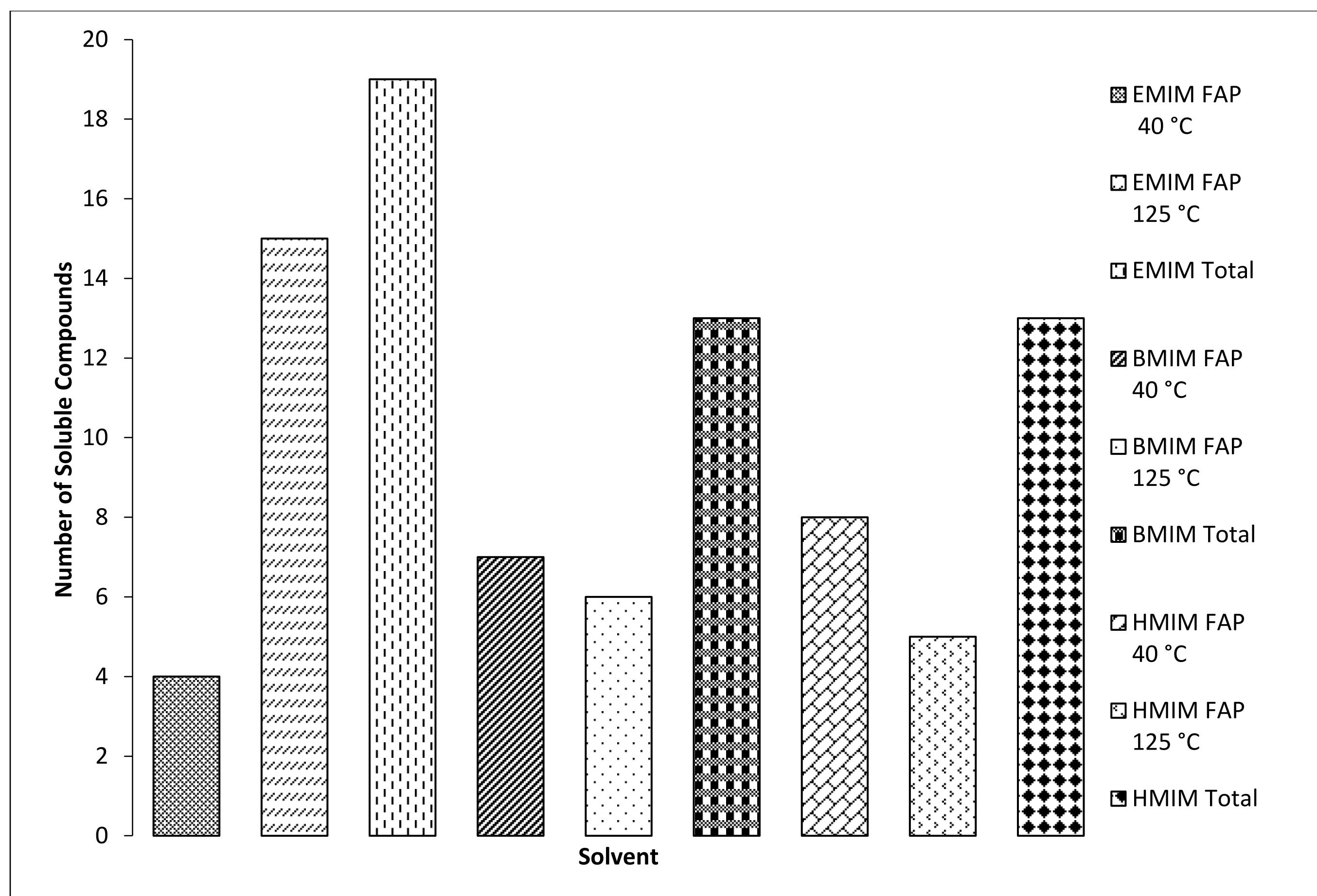


Figure 2-1: The solubility of APIs in Ionic Liquids

A bar graph showing of the number of APIs which are soluble in three different “FAP” ionic liquids: 1-ethyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (EMIM FAP), 1-butyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (BMIM FAP) and 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (HMIM FAP). The samples were heated and stirred at 40 °C for an hour if the sample was not dissolved it was then heated at 125 °C for an hour.

2.3.2 Optimization of Gas Chromatography Separations

The carrier flow rate, split rate, inlet temperature, and oven temperature, were optimized as specified in the Experimental section (see Apparatus and Conditions, Section 2.1). The ionic liquid GC column allows for a rapid, enhanced, robust water detection [17]. The SLB-IL107 ionic liquid stationary phase leads to sharper peaks and

shorter retention times than other ionic liquid columns, or packed columns. The oven temperatures and split ratios for the GC-TCD were; 50, 70, 90, 100, and 110 °C and 1:1, 5:1 and 10:1 respectively, It was determined that 100 °C with a split ratio of 5:1 reduced the overall run time while maintaining baseline separation of the air and water peak, as seen in Figure 2-2 A. Water analysis with the GC-BID was studied with split ratios of 1:1, 20:1, 50:1 and 100:1 and it was determined that a split ratio of 100:1 led to the chromatograms with the best resolution and reduced saturation of the detector.

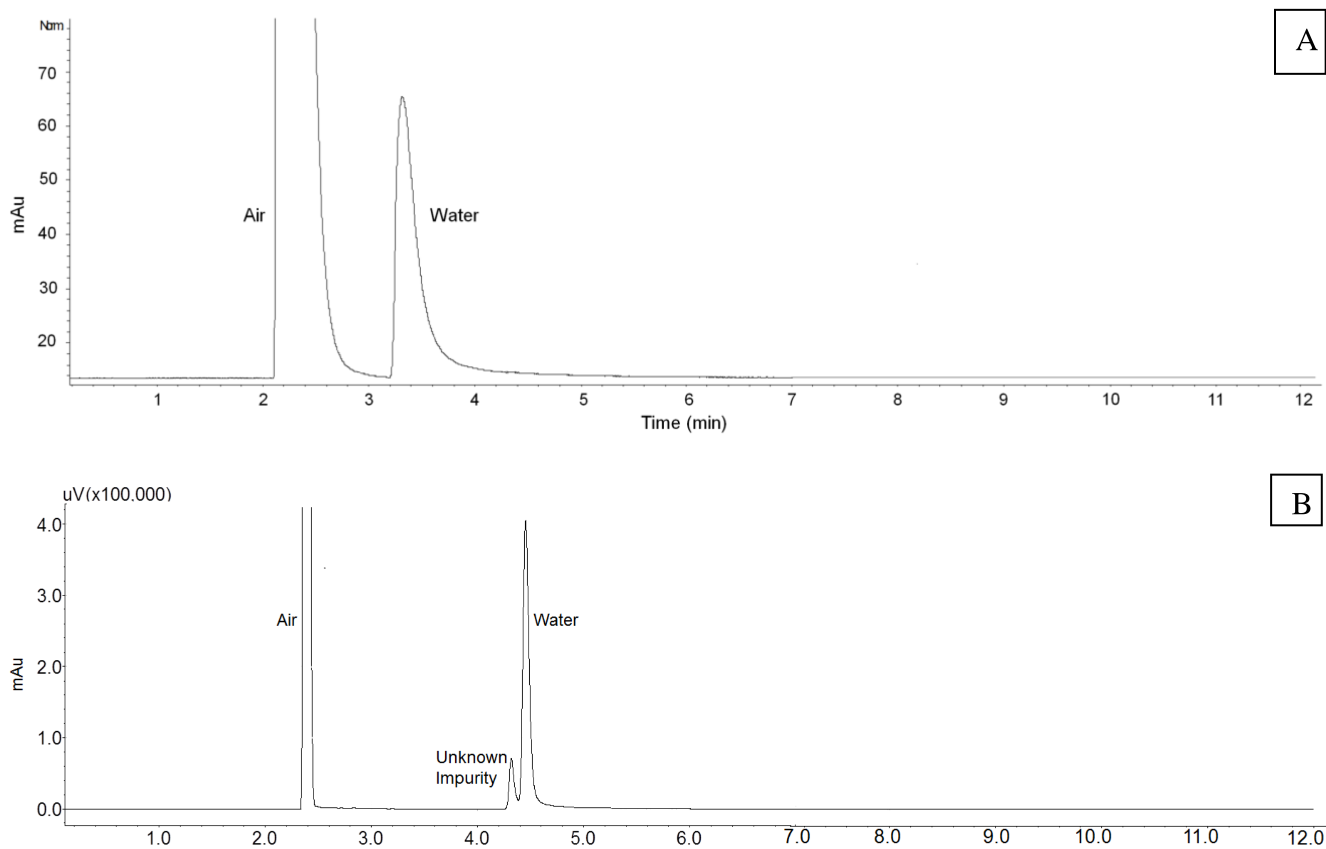


Figure 2-2: Chromatogram of water in pharmaceuticals on the TCD and BID

2-2A, shows the typical chromatogram of a water standard in EMIM FAP at 100 °C with a split ratio of 5:1 on the GC-TCD. 2-2B, shows the typical chromatogram of a water standard in EMIM FAP at 100 °C with a split ratio of 100:1 on the GC-BID.

2.3.3 Optimization of Headspace Conditions

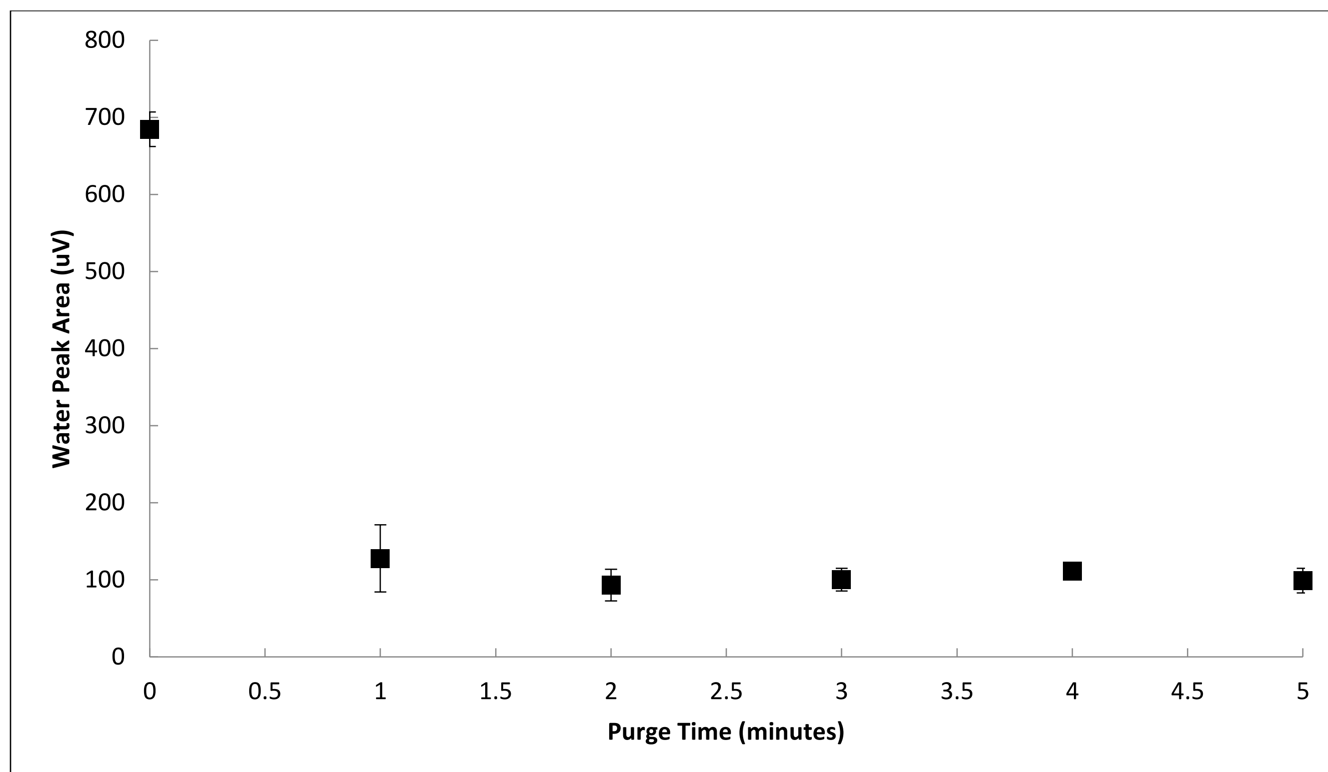


Figure 2-3: Purge effects of argon on an empty vial

The initial purge of empty vials was analyzed in triplicate at 6 different purge times ranging from not purging with argon to a 5 minute purge of argon. The change in the background water content between not purging and purging the vials is easily seen.

The sensitivity, efficiency and recovery of water were optimized by modification of a few parameters that included: the initial purge time, equilibrium time, and equilibrium temperature. The time that the empty vials were initially purged was studied at 0, 1, 2, 3, 4, 5 minutes. The difference between purging and not purging the vials (e.g. 0 minutes) with argon is apparent in Figure 2-3. In addition it can be seen that once the vials have been purged for 2 or more minutes the deviation is greatly reduced. Therefore a purge time of 2 minutes the shortest time to have low background water content along with low standard deviation. The equilibrium temperature was examined

at 50, 75, 100 and 125 °C for both the ionic liquid blank and a representative sample of, ibuprofen dissolved in the ionic liquid.

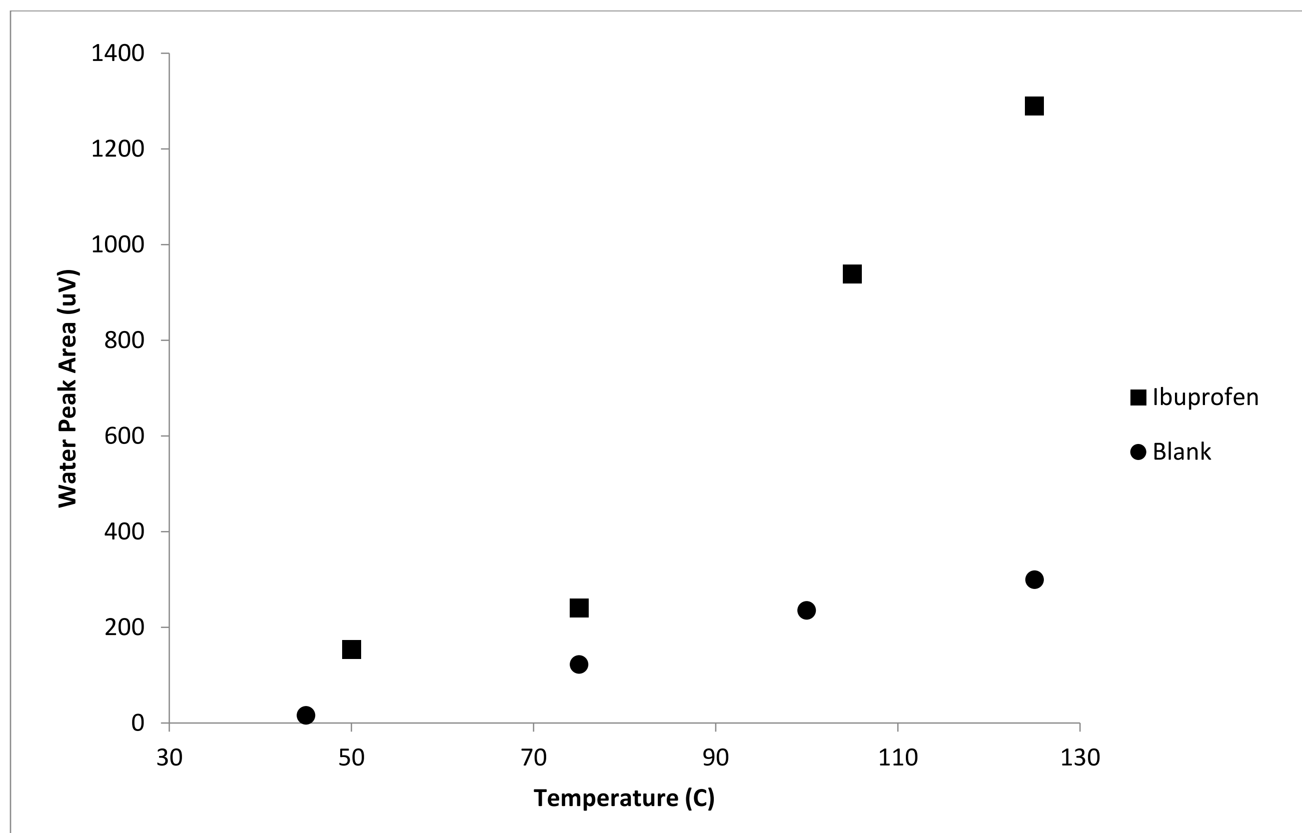


Figure 2-4: The effect of the equilibrium temperature on APIs

The effect of the equilibrium temperature on water detected using a GC-TCD from a sample, ibuprofen, dissolved in EMIM FAP compared to the water in a blank sample of IL, EMIM FAP.

As seen in Figure 2-4, 125 °C led to the largest difference in peak area between the blank ionic liquid and the sample and therefore was determined to be the optimal equilibrium temperature within the limits of the study. The equilibrium time, the time the samples are heated before headspace sampling, also affects the volatilization of the water from the liquid matrix. The equilibrium time was monitored at 2, 5, 10, 20, 40, and 60 minutes. As shown in Figure 2-5, it was determined that 30 minutes produced the largest response.

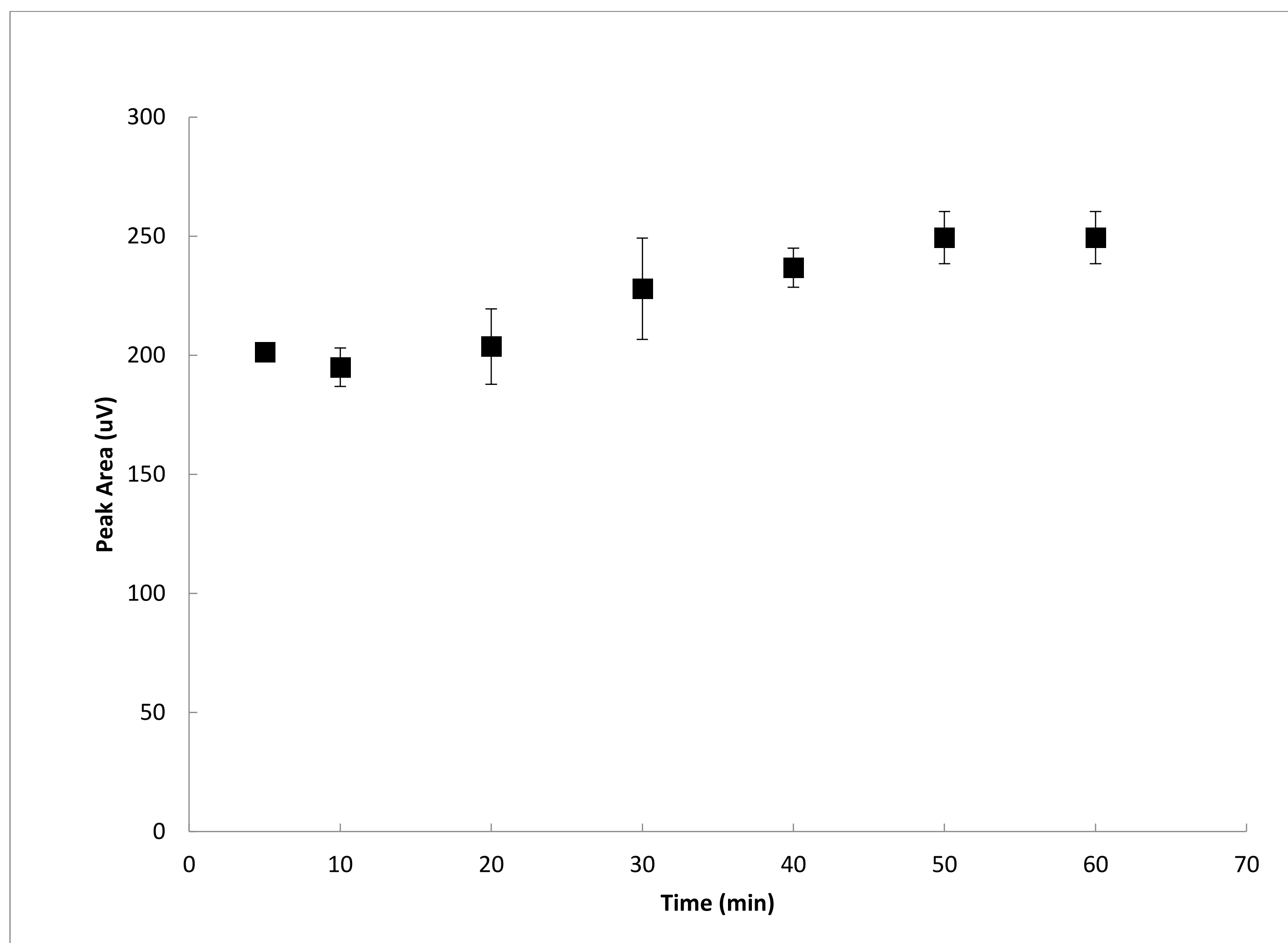
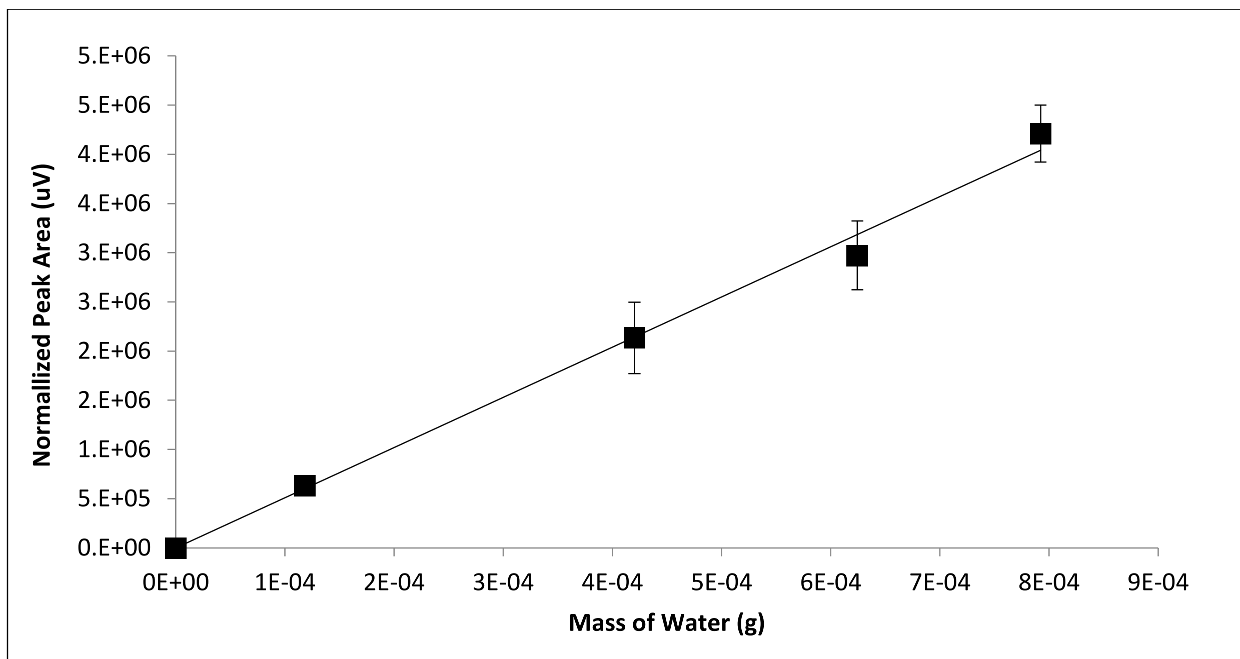


Figure 2-5: The effect of the equilibrium time at 125 °C on water detected using a GC-TCD of a sample of EMIM FAP.

2.3.4 Quantitative Analysis of Water in Active Pharmaceutical Ingredients Samples

A



B

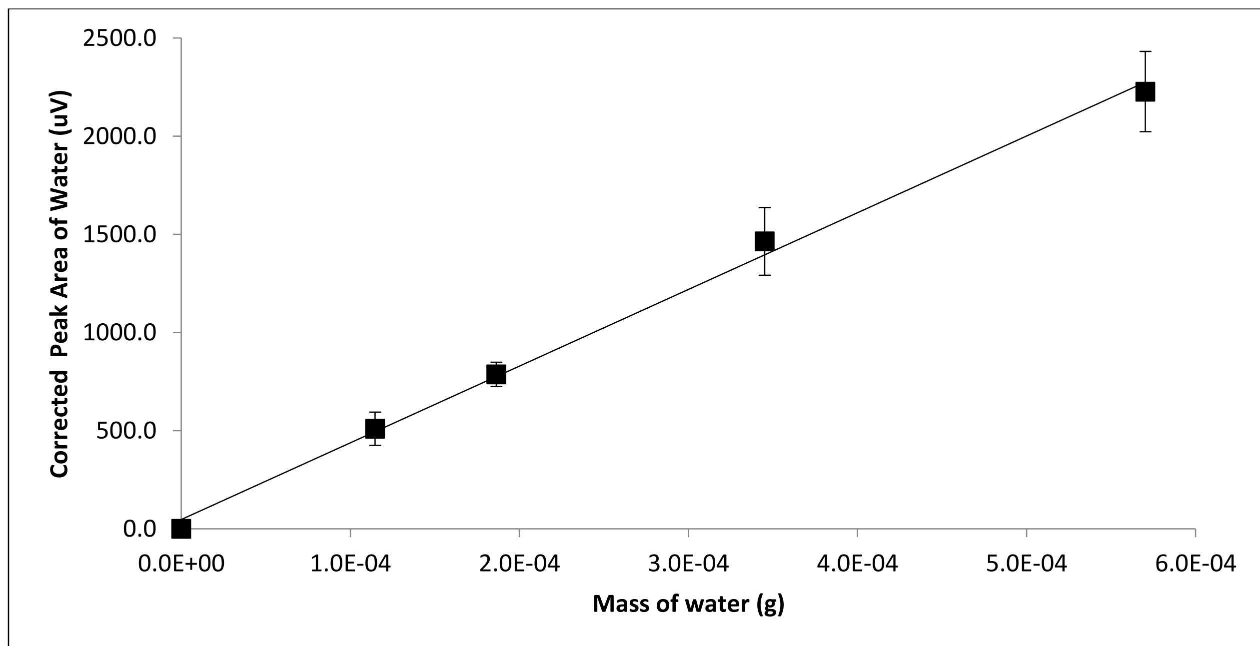


Figure 2-6: Calibration curves for water in EMIM FAP using the TCD and BID

2-6A: Plot of response versus the mass of water in the EMIM FAP using a GC-TCD. The equation for the line is y=3909.3x+46.84 and the correlation is 0.997. 2-6B: Plot of normalized response versus the normalized mass of water in the ionic liquid using a GC-BID. The equation for the line is y=5E+09x and the correlation is 0.9936.

Calibration curves were developed for all three ionic liquids that contained the FAP anion (see Experimental, Section 2.2) in order to quantitatively analyze the water in the solid pharmaceutical samples. The linear relationship between peak area and water concentration in the EMIM FAP, as seen in Figure 2-6A, produced a correlation (r^2) equal to 0.997. Subsequently the water content in 20 APIs were quantified using the indicated calibration curve (Figure 2-6A).

Table 2-1: Detection of water in 20 compounds using HSGC with the TCD and weight loss by drying.

Samples[1]	Percent Water	
	HSGC	Loss on Drying[2]
Ascorbic acid	2.7	a
Bupivacaine	3.8	a
Carnitine	1.9	a
Cholecalciferol	3.6	a
Ephedrine	2.1	a
Ethnylestradiol	3.7	a
Homocysteine	3.4	2.9
Ibuprofen	0.2	a
Ketamine hydrochloride	1.1	1.1
Lysozyme	7.5	a
Oxypene	0.7	0.8
Paclobutrazol	1.0	a
Penicillamine	1.6	a
Promethazine	3.9	3.8
Propranolol	2.7	2.2
Rifampicin	2.9	2.7
Scopolamine	2.1	a
Sodium tartrate dibasic dihydrate	15.9	15.6
Tetracycline	1.0	1.2
Trimipramine maleate	0.8	1.1

Verapamil hydrochloride	1.4	1.9
Warfarin	3.1	2.7

[1] See Experimental for the commercial sources of these compounds
[2] In this column, "a" represents compounds which degraded during the experiment.

The water content in these APIs ranged from 35-650 µg of water which was 1-16% by weight as seen in Table 2-1. Also presented in Table 2-1 are the results obtained (when possible) via the loss on drying method. Note that the loss on drying approach cannot be used for many of these samples (*i.e.,* 50% of the samples) because of degradation and reactions.

2.3.5 Precision

The precision of the manual method was determined by evaluating the relative standard deviation (RSD) of multiple manual injections of the same sample. Three different compounds, ephedrine, oxypene, and sodium tartrate dibasic dihydrate, were analyzed and all gave less than 2% RSD, as seen in Table 2-2, showing that this HSGC method is precise. It should be noted that all three results were obtained manually however the precision of this method can be expected to improve even further by increasing sample size, a headspace autosampler and working in a climate controlled environment.

Table 2-2: The relative standard deviation using the HSGC method for three compounds is shown.

Sample	Percent Water	RSD
Ephedrine	2.8	0.9
Oxypene	0.7	1.9
Sodium tartrate dibasic dihydrate	15.9	1.4

2.3.6 Accuracy

Currently there are no National Institute of Standards and Technology (NIST) standards for water in solid APIs. However the accuracy of this method can determined via use of a few known hydrates or samples with known, accepted literature values of water concentration. These include rifampicin, sodium tartrate dibasic dihydrate, and warfarin. Sodium tartrate dibasic dihydrate while not being an API, was chosen as a standard because it is commonly used to calibrate and determine the accuracy of KFTs because of its consistent water content, 15.6% ± 0.5. The accuracy of this method was determined by analyzing samples with known water content in triplicate and compared to the reported water content. In the case of the sodium tartrate dibasic dihydrate, the water content detected with HSGC was, within experimental error, the same as the reported known water content. These results indicate that the HSGC method is accurate. In addition, the loss on drying method was also used in order to determine the water from the same batch of compounds (Table 2-1), thereby eliminating the possibility of batch to batch differences. The compounds for which the water content was able to be determined, using loss on drying, gave the same results within experimental error as the HSGC method.

2.3.7 Comparison of Detectors

The BID was compared to the TCD detector with the intention of determining if both detectors would be able to determine the low levels of water required for this project and establish if improvements in the method could be made. Both detectors were able to use HSGC to analyze water from 0 – 1000 μg of water. The BID being a more sensitive detector than the TCD was more easily overloaded, often

producing broad or even split peaks. This was alleviated by increasing the spit ratio when using the BID (Figure 2-2). Even with the larger split ratio the BID is still able to detect low concentrations of water because of its tremendous sensitivity. As seen from the calibration curve, Figure 2-6B, 100 µg of water is easily determined. The higher split ratio leads to sharper peaks and a larger separation between the air and water peaks improving the chromatogram (see Figure 2-2). The calibration curve, for the same concentration range of water, produced by the BID detector, Figure 2-6B, had a slope of $2x10^9$ which is steeper than that of the calibration curve produced by the TCD detector which had a slope of $4x10^6$ Figure 2-6A. This shows the considerable sensitivity gained by using the BID over the TCD. Both detectors are able to be used for water determination with HSGC however the BID gives increased sensitivity. The standard deviation of the linear response for the TCD was 148 with a RSD of 11% whereas for the BID the standard deviation was 113000 with an RSD of 15%

2.4 Conclusions

A rapid, accurate, IL-based HSGC method was developed for determination of water in APIs. The HSGC method is not as affected by or biased by the reactivity of the analytes. There are no known deleterious chemical interactions between the APIs and the IL solvents whereas it is known that the solvents and reagents used in KFT can, in some cases, cause side reactions leading to inaccurate determination of water content [7-9]. The sensitivity of the HSGC method is greater than 100 times that of volumetric KFT, allowing very small sample sizes (*e.g.,* 4 mg) to be accurately and reproducibly analyzed. In comparison, a typical sample size of 500-1000 mg is used in KFT [6, 25]. The HSGC method was shown to be applicable to more compounds than loss on drying

and to take less time than loss on drying. The sample preparation in the developed HSGC method is relatively straight forward and the overall method is precise, accurate, and broadly applicable.

2.5 Acknowledgments

We would like to thank Merck for their generous donation of the initial EMIM FAP, BMIM FAP and HMIM FAP.

Chapter 3

WATER DETERMINATION IN SOLID PHARMACEUTICAL PRODUCTS UTILIZING IONIC LIQUIDS AND HEADSPACE GAS CHROMATOGRAPHY

Abstract

A rapid, accurate, and precise headspace gas chromatographic (HSGC) analytical method was developed for the detection and quantification of water in drug products. The analysis is able to be performed in 10 minutes and automated. The HSGC method utilized an ionic liquid (IL) based open tubular capillary GC column to increase the ruggedness of this method and provide improved peak shapes for water. Due to the ILs low vapor pressure, unique physiochemical properties, and high thermal stability, they also make idea solvents for HSGC. Unlike Karl Fischer Titration (KFT) methods, this HSGC method is not affected by side reactions. The developed method was shown to be broadly applicable. The water content in 12 different samples were found to range from 1-7% water. The use of HSGC was highly sensitive and only required 10 mg of sample. In addition it was found to have greater precision and accuracy than KFT and greater precision and speed than loss on drying.

3.1 Introduction

Water content is determined at various stages through the drug manufacturing process and in the final product. When pharmaceutical compounds contain different concentrations of water, it affects the physiochemical properties of the finished drug formula.[2, 3, 5, 81-83] If the water content is increased above a critical threshold, microorganisms are able to grow in drug formulations.[82] Microorganisms can be harmful, causing medications to have adverse effects. During manufacturing the

presence of water on the surface of drug formulation will modify the electrostatic charge and surface energy causing variations in solid flow properties.[2, 3, 81-83] However when there is excessive water, an increase in cohesion and adhesion is observed which decreases the flow properties.[81, 82] When there is a disruption in flow in the hoppers (*i.e.,* arching and bridging) it can lead to halts in production or compound segregation.[81] Segregation in compounds causes composition variations or inconsistent dosages.[81] In addition, the reproducibility of tablet weight and hardness will be reduced with any decrease or inconsistences in flow properties.[81]

Atmospheric moisture can interact with therapeutic drug particles in numerous ways, modifying the water content. Both the active pharmaceutical ingredients and inert materials/excipients which can attract water and modify water content.[2, 3, 15, 82] The drug formulation will only be able to absorb a certain amount of moisture from the atmosphere which is dependent on the temperature at which the finished pharmaceutical product is stored, the size distribution of the particles, and the surface area of the powdered drug formulation.[2,3, 5, 82, 84] When amorphous material is formed via voids or fractures in the crystalline structure a higher content of water is present in the finished drug product.[5, 82] Atmospheric conditions, seasonal effects, along with geographical variations in moisture in which the active pharmaceutical ingredients are synthesized, prepared and stored can also impact the amount of water present in the finished drug products.[82] The process by which the finished drug products are manufactured (*e.g.* wet granulation, spray drying, milling, lyophilization, recrystallization) can increase or decrease the moisture in the drug tablets.[2, 3, 82] Milling can modify the moisture of the powder drug formulation since it decreases the size of the particles and

increases surface area.[81] In addition, fractures to the crystal structure and increases in the formation of amorphous regions are produced when milling.[81] Air milling uses nozzles to form numerous air jets breaking down the particles, which will also dry the newly formed smaller particles.[81]

There are a few ways to measure water content in pharmaceutical products, Karl Fischer titration (KFT) is a method recognized by the US Food and Drug Administration for determination of water in therapeutic drug formulations.[67] This technique is favored since it is a water selective method and has a wide dynamic range, however samples and conditions must be rigorously controlled in order to obtain reliable results.[15, 67, 85] If the atmospheric moisture is not controlled and the titration cell is filled with air then the relative humidity will affect the measurement of water. In one case it has been shown that if air with a relative humidity of 50% is introduced to the titration vessel, it will increase the measurement of water by 1 mg.[85] To reduce the effects of atmospheric moisture, titration cells are heated before analysis, dry gas is purged into the titration cell and only a single sample is analyzed per titration cell.[86] The presence of thiol, ketone, aldehyde, amide and/or siloxane functional groups in the active pharmaceutical ingredient or excipient can lead to interactions with iodine causing the water content measured to be artificially high. A multiple solvent system or additives are utilized when the active pharmaceutical ingredients and excipients have limited solubility in the Karl Fischer solvent/medium.[66] The Karl Fischer medium has limited shelf stability, and being a hygroscopic solvent, it absorbs moisture from the atmosphere which leads to changes in the solvent blank over time.[66] KFT has low throughput and is labor intensive.[67] An automated system can be utilized, where samples are preweighed into

small cells and then titrated, however, they are not sealed from environmental conditions.[67] If the drug (active pharmaceutical ingredient or excipient) is hygroscopic, it will continuously absorb moisture until it is analyzed, giving inaccurate results.[67] Near infrared (NIR) spectroscopy is a recent method developed for determination of water in drug products that contains appreciable amounts of water. However even in the "high water samples" the error is significant compared to the method developed herein.[87, 88]

Ionic liquids (ILs) are used as gas chromatographic (GC) stationary phases and are also exceptional solvents in headspace gas chromatography (HSGC).[34] ILs are ideal due to their tunable nature allowing for selection of desired traits (*e.g.* solubilizing power, thermal stability, viscosity and hydrophobicity).[34, 50, 51, 60, 61, 58, 60, 61, 89-93] The tunable nature of ILs allow for unique selectivity as gas chromatography stationary phases.[89, 90] The columns have high selectivity between common residual solvents and water.[50] The ability to change anions allows for the use of trifluoromethanesulfonate (TFO^-) which results in a better peak shape for water.[50, 34] ILs produce robust stationary phases which do not degrade in the presence of water or air.[50]

The high thermally stability of ILs also makes them useful HSGC solvents.[89] The lack of volatility and degradation products eliminates the solvent peak and reduces the number of contaminant peaks which could interfere with the peaks of interest.[89, 90] In this publication 1-ethyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (EMIM FAP) is utilized as the headspace solvent since it was previously shown to be effective when analyzing water in active pharmaceutical ingredients.[34] The high hydrophobicity provides low residual water and low water uptake.[41, 75, 90] The hydroscopic and hydrophobic nature of EMIM FAP produces low background inference

and therefore a lower limit of detection. The water in common solvents can be removed with time consuming and labor intensive methods or the solvent be purchased as anhydrous solvents, however residual water is still present and tends to increase significantly with age and use.[94, 95] Lastly the EMIM FAP has a relatively low viscosity for ease of handling.[41, 75] The properties stated above makes EMIM FAP an ideal solvent for water analysis with HSGC.

3.2 Experimental

3.2.1 Apparatus and Conditions

All analyses were done with a Tracera GC-2010 Plus (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a barrier ion discharge detector (BID). Two autosamplers were utilized for automated injections, AOC-5000 Plus Autosampler (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a heated 2.5 mL headspace HD-type syringe (Hamilton, Reno, Nevada, USA) or a HS-20 headspace autosampler (Shimadzu Scientific Instruments, Kyoto, Japan) furnished with a 1.0 mL sample loop. The integration was performed with LabSolutions (version 5.71 SP1). All analyses were performed utilizing a split ratio of 100:1 and a constant flow of helium at 1.5 mL/min. The helium was dried with a High Capacity Gas Purifier and an OMI® Purifier Tube (Supelco Bellefonte, PA, USA). The oven, injection port and detector were kept at 150 °C, 280 °C and 250 °C respectively. The Watercol™ 1910 fused silica capillary column coated with ionic liquid, 1,11-di(3-hydroxyethylimidazolium)3,6,9-trioxaundecane trifluoromethanesulfonate, synthesized as previously reported or commercially acquired from Sigma-Aldrich had dimensions of 60 m x 0.25 mm ID x 0.2

µm film coat thickness was utilized for the analysis of water. All samples were weighed on an AR1140 Adventurer balance (Ohaus Corp., Pine Brook, New Jersey, USA).

3.2.2 Materials

The Advil and Gelusil were both purchased from Pfizer (Kings Mountain, NC). Citracal, Claritin D and Equate Aspirin were obtained from Bayer Corporation (Whippany, NJ). Arthritis Pain and Vitamin C were both bought from Costco Wholesale Corporation (Issaquah, WA). Zicam was purchased from Zicam L.L.C (Phoenix, AZ). Excedrin Migraine came from Novartis Consumer Health Inc. (Parsippany, NJ). The 12 Hour Decongestant was obtained from Kroger's Co. (Cincinnati, OH). Acetaminophen was purchased from Walgreen Company (Deerfield, IL) and Target Corporation (Minneapolis MN). The 1-ethyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate (EMIM FAP) was purchased from Merck KGaA (Darmstadt, Germany). The 22 X 75 mm screw-thread vials and the magnetic screw-thread covers for the autosampler were purchased from Restek (Bellefonte, PA, USA).

3.2.3 Sample Preparation

3.2.3.1 Sample Preparation for Headspace Gas Chromatography

First the pharmaceutical products are finely ground then the HSGC samples are made by adding 500 mg of EMIM FAP and 9.8-10.3 mg with an average of 10 mg of the desired pharmaceutical product to an empty 10 mL vial. The vials are immediately capped with a blue PTFE/silicone 1.5 mm thick septum metal cover. When the HS-20 autosampler is utilized to purge the vials the samples are first pressurized to 200 kPa for 2 minutes at room temperature. After pressurizing the diluted headspace is extracted for 1 minute. The samples are then heated at 150 °C for 5 minutes after the vial is then

pressurized to 100 kPa for 1 minute. The sample's headspace is loaded into a 1 mL sample loop for 2 minutes and a 0.5 minute an injection into the GC. When a syringe type autosampler, AOC-5000, is employed the vials are first manually purged for 2 minutes using a 20 G, 1½" long needle. The vials are immediately capped and purged a second time for 15 seconds with 2 smaller 25 G, 5/8" long needles (one to insert argon and one to relieve pressure). The vials are then heated at 125 °C for 20 minutes. After heating, 500 mL of headspace is analyzed with GC.

3.2.3.2 Sample Preparation for Loss on Drying

Samples were prepared for loss on drying by adding 100 mg finely ground pharmaceutical product into an empty preweighed vial. The sample is then heated at 60 °C for 12 hours. The sample is weighed and then heated again at 60 °C for another 12 hours. If after the second heating, the mass of the sample is not consistent, then the sample is heated at 105 °C for another 12 hours. The vials are then weighed and reheated in 12 hour increments until the mass is stable.[11]

3.2.3.3 Sample Preparation for Karl Fischer Titration

The analysis utilizing KFT was performed by Robertson Microlit Laboratories, first the atmospheric and residual moisture in the KFT cell was analyzed by adding 3 mg of sulfosalicylic acid dehydrate to the Hydranal Coulomat AG in the titration cell. The standard is then coulometrically titrated to the electrometric endpoint. The sulfosalicylic acid dehydrate contains 14.17% water therefore any additional water content represents the atmospheric moisture. After the residual moisture is determined, 10 mg of finely ground pharmaceutical product is added to the titration cell and titrated to the

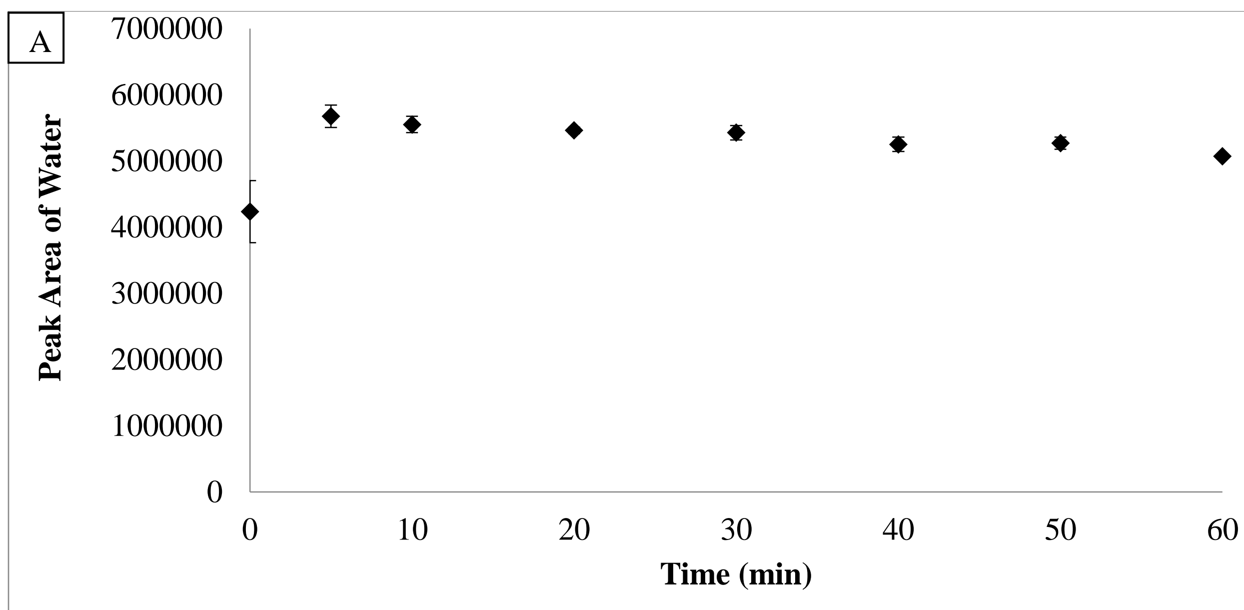
electrometric endpoint. The atmospheric moisture is subtracted from the value reported for the sample to give the water content of the pharmaceutical product.

3.3 Results and Discussion

3.3.1 Optimization of Headspace Gas Chromatographic Conditions

The HSGC method was optimized in order to produce the highest response for water compared to the background response and to produce the highest throughput. In order to achieve this, the equilibrium time (the length of time the sample is heated in the headspace vial before being analyzed) and equilibrium temperature (the temperature to which the sample is heated before being analyzed) were studied. Figure 3-1A shows the effect of sample equilibrium time on the signal response of water. After 5 minutes of equilibration, the response of water levels off and becomes independent of equilibration time. The response from water in relationship to increasing the equilibrium temperature is shown in Figure 3-1B. It can be seen that at 150 °C, the response difference between the blank (EMIM FAP) and the sample dissolved in EMIM FAP was greatest. At temperatures higher than 150 °C the samples began to show discoloration.

The chromatographic conditions were also optimized to allow for fast separation of the air and water peaks allowing complete analysis in under 5 minutes as seen in Figure 3-2. A 60 m Watercol™ 1910 capillary column used at a temperature of 150 °C and a flow rate of 1.5 mL/min produced sharp water peaks that are accurately and reproducibly integrated. Further this IL stationary phase is inert to water and shows no degradation or change after 1000 injections.



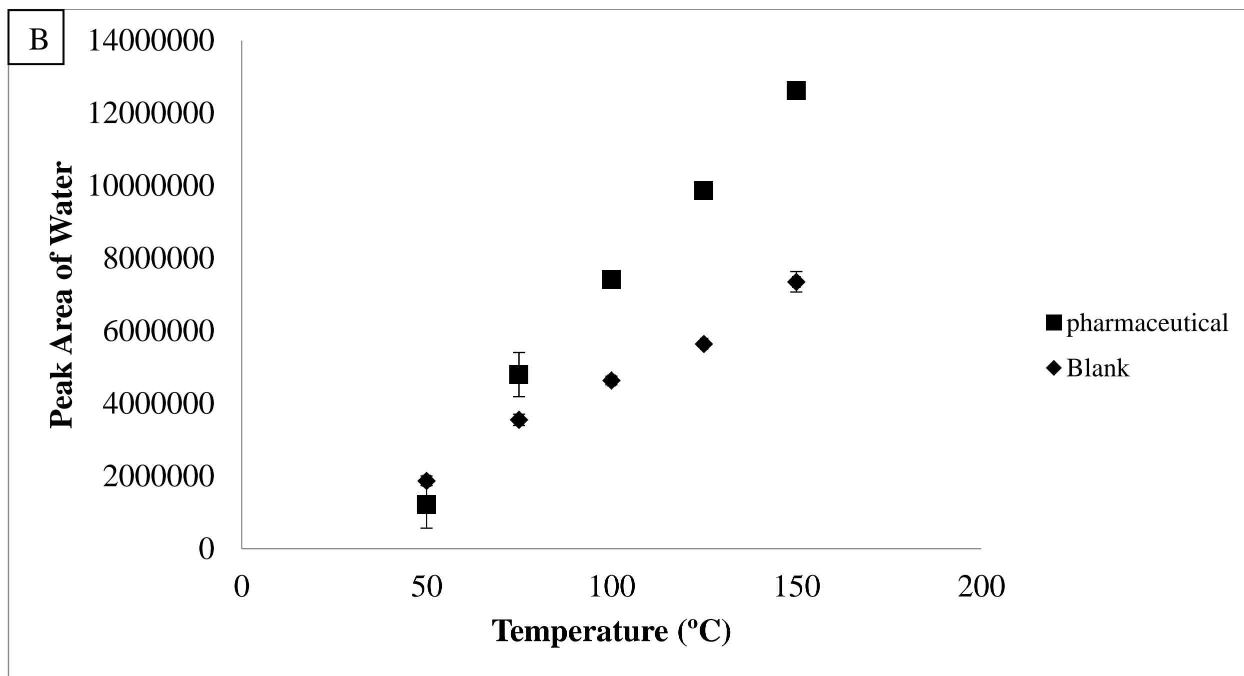


Figure 3-1: The effects of equilibrium time and temperatures on the response of water in pharmaceutical samples

(3-1A) As shown, the equilibrium time was evaluated from 0 – 60 minutes at 125 ° C. It was found that after 5 minutes the increased heating time did not increase the response of water. (3-1B) Th equilibrium temperature was evaluated by comparing the response of Gelusil dissolved in EMIM FAP to the response of blank EMIM FAP from 50 – 150 ° C. The largest change in response between the sample and the blank was at 150 ° C. At temperatures above 150 °C many of the samples showed discoloration.

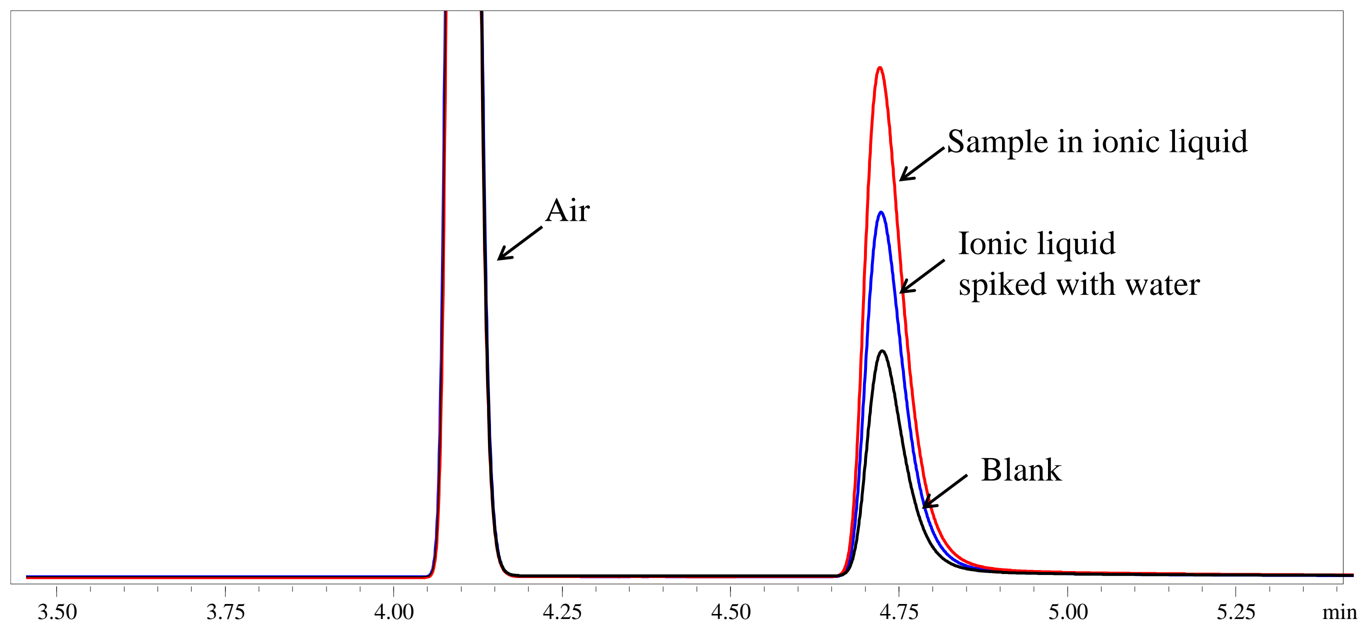


Figure 3-2: A typical chromatogram of water in Excedrin Migraine dissolved in 1-ethyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (EMIM FAP) at 170 °C with a split ratio of 100:1 on the GC-BID.

3.3.2 Method Quantification, Precision and Accuracy

An external calibration curve was produced for a range of water from 0-7.5 mg. The calibration curve produced had a slope of 9.05 X 10^9. The linearity (R^2) between the content of water and response was $R^2 = 0.984$. This curve was used to quantify the water content in 13 solid pharmaceutical samples, see Table 3-1. The water content in these diverse pharmaceutical products ranged from 1-7%.

The water content for all 12 pharmaceutical products was determined utilizing HSGC and loss on drying and KFT (see Experimental) in order to determine the accuracy of the HSGC method. The water quantities measured with HSGC and loss on drying were found to be the comparable for all 12 compounds as seen in Table 3-1.

Table 3-1: Comparison of the water content, standard deviation (SD) and relative standard deviation (RSD) for 12 solid pharmaceutical compounds utilizing loss on drying, headspace gas chromatography (HSGC), and Karl Fischer titration (KFT).

Solid Pharmaceuticals	Loss on Drying [a]		HSGC [a]		KFT [b]	
	Percent Water	RSD	Percent Water	RSD	Percent Water	RSD
Gelusil	3.1 ± 0.3	8.9	2.9 ± 0.1	3.6	1.6 ± 0.2 [e]	12.7
Zicam	3.5 ± 0.05	1.4	4.0 ± 0.1	2.8	3.3 ± 0.2	4.9
Vitamin C	2.3 ± 0.1	3.1	2.9 ± 0.08	2.6	9.7 ± 0.1 [d]	1.4
Citracal	2.5 ± 0.2	9.3	2.8 ± 0.08	2.8	5.2 ± 0.5 [d]	9.5
12 Hour Decongestant	5.1 ± 0.4	7.0	5.2 ± 0.09	1.8	3.1 ± 0.2 [e]	6.8
Excedrin Migraine	2.0 ± 0.4	17.9	1.9 ± 0.09 [c]	4.7	1.3 ± 0.2 [e]	15.3
Claritin D	6.6 ± 0.04	0.6	6.4 ± 0.2	3.7	5.0 ± 0.3 [e]	5.9
Advil	2.5 ± 0.7	28.5	3.1 ± 0.1	3.7	3.1 ± 0.06	1.9
Arthritis Pain	2.3 ± 0.2	10.3	2.2 ± 0.02	0.7	1.9 ± 0.05 [e]	2.5
Acetaminophen (Target)	2.5 ± 0.3	13.1	2.4 ± 0.07	3.0	0.6 ± 0.07 [e]	11.1
Acetaminophen (Walgreen)	1.0 ± 0.03	3.0	0.9 ± 0.03	3.3	1.6 ± 0.1 [d]	8.0
Equate Aspirin	1.4 ± 0.3	17.8	1.6 ± 0.07	4.3	1.3 ± 0.3	21.8

[a] The samples were analyzed in quadruplicate
[b] The samples were analyzed in triplicate
[c] The sample was analyzed in septuplicate
[d] Values are artificially high (based on a t-test at 95% confidence) because the active pharmaceutical ingredients and/or excipients react with KFT medium.
[e] Values are artificially low (based on a t-test at 95% confidence) because of sample insolubility in KFT medium.

The precision of the three methods was compared by analyzing the relative standard deviation (RSD). HSGC had an average RSD of 3.1% which was lower than the standard deviations of KFT and loss on drying method with RSDs of 8.5% and 10.1% respectively. The RSD was lower than 5% for 11 of the 12 samples when HSGC was utilized whereas loss on drying method and KFT only had 4 compounds with an RSD below 5%. The largest RSD was 28.5% which was produced when the water content was measured in Advil with loss on drying. The results of the three different methods show when the HSGC and loss on drying method were compared with the T-test they

gave similar results for all but three compounds. The T-test (at 95% certainty) was also utilized to compare KFT to loss on drying and HSGC where only 3 and 2 compounds respectively were found to be equivalent to KFT. The KFT was found to give significantly higher water content for three of the pharmaceutical compounds; vitamin C, Citracal, and acetaminophen (Walgreen). In the case of vitamin C, it is known that ascorbic acid reacts with the Karl Fischer titrant. It can be seen that the KFT method is precise with low standard deviation and RSD of 1.4%. However it has low accuracy due to the side reactions, that is, it produces the wrong answer in a reproducible fashion. Six of the compounds were reported to have lower water concentrations with KFT compared to loss on drying and HSGC, the most likely cause is the limited solubility of the pharmaceutical products in the KFT medium. In the case of Equate Aspirin, one of the few compounds where all three methods produced the same water content, 1.3-1.6% water, the standard deviations are significantly different. HSGC was the only method which had < 5% RSD. While KFT was able to give the correct average water content it produced a wider range of water amounts from the individual samples of (*i.e* 0.86-1.61%) and an RSD of 21.8%. KFT can be performed more rapidly than loss on drying, however, depending on the sample it often was shown to give the incorrect water content. Loss on drying was shown to give more accurate results, however the method is labor intensive and slow. Thus it was found that the HSGC method was more precise and likely more accurate for determining water content, than both loss on drying and KFT.

3.3.3 Comparison of Autosamplers

A comparison of two different headspace autosamplers was done by comparing results obtained for five compounds. The use of the HS-20 autosampler allowed for automated purging of the vials whereas the AOC-5000 autosampler required manual purging with argon. The manual purging increased the standard deviation compared to the automated method. The RSD for vials purged with the HS-20 was 0.5% whereas the RSD for manual purging was 6.3%. The RSD for 4 of the samples analyzed with the HS-20 autosampler were under 5% with only one compound having 6% RSD. The HS-20 autosampler produced an average RSD of 3.5% whereas the AOC-5000 has an average RSD of 10.3% which is about three times the relative standard deviation. In addition to the improved precision achieved with the HS-20 autosampler the equilibrium time was 6 times shorter. When the AOC-5000 autosampler was utilized, it took 30 minutes for the maximum response to be reached whereas the HS-20 autosampler took only 5 minutes.

Table 3-2: Comparison of the quantity of water and standard deviation achieved utilizing the AOC-5000 and HS-20 autosamplers for 5 samples (n=4).

Solid Pharmaceuticals	Percent Water	
	AOC-5000	HS-20
Claritin D	6.7 ± 1.4	6.4 ± 0.4
Arthritis	1.8 ± 0.2	2.2 ± 0.02
Acetaminophen (Target)	1.5 ± 0.1	2.4 ± 0.1
Acetaminophen (Walgreen)	1.0 ± 0.03	0.9 ± 0.03
Equate aspirin	2.0 ± 0.2	1.6 ± 0.1

3.4 Conclusions

A rapid, effective, automated method for the determination of water in solid pharmaceutical products was developed. The use of HSGC was found to be accurate and have good precision. There were no side reactions between the IL and therapeutic drug formulation allowing for a more accurate determination of water. This method was used to analyze samples in a total of 10 minutes, 5 minutes to heat the samples before analysis and a 5 minute chromatographic run time. In addition, this method only requires 10 mg of sample due to the high sensitivity of the BID. Conversely loss on drying takes many hours to complete. The KFT was found to produce inaccurate results for most samples. The use of a HS-20 autosampler was found to decrease deviation compared to the AOC-5000 autosampler. In addition the HS-20 allowed for automatization of purging samples. This entire method can be automated.

3.5 Acknowledgments

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Chapter 4

THE DETERMINATION AND QUANTIFICATION OF WATER AND ETHANOL IN MEDICINAL SYRUPS AND ELIXIRS

4.1 Introduction

Liquid medications are beneficial when patients find swallowing a pill difficult.[96] This is readily seen in children's medications where nearly 80% are in liquid form versus as a tablet or capsule.[96] The water content in the liquid pharmaceuticals will influence the solubility of the active pharmaceutical ingredients (APIs) and the homogeneity of the product. If the water content is not rigidly controlled microorganisms are able to grow in the solutions.[65]

Given the variety and complexity of modern APIs it is not surprising that many of them have limited solubility in water.[97] Consequently there is an increasing need of a cosolvent or cosolvents which have "inert" behavior but will allow for a homogenous fluid mixture.[97, 98] When selecting a cosolvent, a class 3 solvent is desired since it is the least toxic of the three classes. In addition, "Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals".[99] Ethanol, a class 3 solvent, is ideal since it increases the solubility of hydrophobic APIs in water.[96, 99] Also, ethanol has been found to decrease the growth of microorganisms thereby increasing the shelf life of the medicinal elixir.[96]

While ethanol can be useful in medications it is known to have some negative effects when combined with certain APIs. For example, the uptake of some APIs can be inhibited by ethanol. There can be an increase or decrease of the metabolic rate of drugs when taken in concurrence with ethanol, which can increase the blood

concentrations of specific APIs.[98, 100] A disulfiram-like reaction (*i.e.,* nausea, vomiting, cardiac arrhythmias, and convulsions) has been observed when ethanol containing medicinal liquids were used simultaneously with antibacterial drugs (*i.e.,* moxalactam, metronidazole, and sulfonamide).[96, 98] A large concern in developing pediatric medications is the quantity ethanol and the regulations governing its use.[98] If children are taking medication regularly the ethanol content in the blood can elevate to undesirable levels. The concentration of ethanol in the blood that will cause acute toxic effects (reduced coordination of muscles, decreased reaction times, and behavioral changes) or even death remains controversial and additional study is still necessary.[98, 101]

The method recommended by the European Pharmacopoeia for the quantification of ethanol in liquid medicinal syrups measures the density of the distillate (ethanol and water).[96, 102] The method is difficult since liquid pharmaceutical drug products frequently contain large quantities of sweeteners which greatly increase viscosities and densities.[96] In order for this method to be accurate, the samples must contain a higher quantity of ethanol. In addition, it is assumed that the distillate only contains water and ethanol, the presence of other violate compounds can lead to difficulties characterizing ethanol when a pycnometer is used.[102]

In this work we present a headspace gas chromatography (HSGC) method utilizing ionic liquid capillary columns to produce sharper water peaks which are able to be separated from common alcohols associated with medicinal syrups. Two different detectors, a thermal conductivity detector (TCD) and a barrier discharge ionization detector (BID), were utilized and compared for the measurement of ethanol and water in

ten products. Two different headspace sampling devices were compared as well. This approach was found to be rapid, reproducible, accurate and able to be fully automated.

4.2 Method

4.2.1 Materials:

The 22 X 75 mm screw-thread vials and the magnetic screw-thread covers with a blue PTFE/silicon septa were purchased from Restek (Bellefonte, PA, USA). The products were obtained at a local store. BPM-DM-PHEN Syrup was obtained from Cintex Services, LLC (Suwanee, GA). Pepto-Bismol and Nyquil were from Procter & Gamble (Cincinnati, OH). Children's Dimetapp Cold and Cough is produced by Wyeth (Philadelphia, PA). Chloraseptic Sore Throat is from Prestige Brands Holdings, Inc. (Tarrytown, NY). Equate Tussin DM and Tussin Chest Congestion were produced by Perrigo (Allegan, MI). Sevier Cold and Fever was obtained from CVS Pharmacy (Woonsocket, RI). S. S. S. Tonic is produced by Midway Importing (Huston, TX). Listerine is from Johnson & Johnson (New Brunswick, NJ). *N*-*N*-dimethylacetamide (DMA) was purchased from Sigma-Aldrich (St. Louis, MO).

4.2.2 Apparatus and Conditions

A Tracera GC-2010 Plus (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a barrier ion discharge detector (BID) and thermal conductivity detector (TCD), and LabSolutions (version 5.71 SP1) was used for all sampling. An AOC-5000 Plus autosampler (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a 2.5 mL headspace HD-type syringe (Hamilton, Reno, Nevada, USA) or a HS-20 autosampler with a 0.2 mL sample loop (Shimadzu Scientific Instruments, Kyoto, Japan) were utilized for all automated samples. A split ratio of 100:1 was used for all analyses.

The injection port was set to 280 °C, the BID detector was set at a temperature of 250 °C and the TCD was kept at 200 °C with a current of 80 mA. The analysis was completed in under 6 minutes with all three stationary phases, a 30 m x 0.25 mm ID x 0.2 um film thickness Watercol^{TM} 1460 and Watercol^{TM} 1900 along with a 60 m x 0.25 mm ID x 0.2 um film thickness Watercol^{TM} 1910 fused silica capillary columns (Supelco, Bellefonte, PA). All samples were weighed on an AR1140 Adventurer balance (Ohaus Corp., Pine Brook, New Jersey, USA).

4.2.3 Sample Preparation

4.2.3.1 Sample Preparation of Headspace Gas Chromatographic AOC-5000 Samples

Samples are prepared by adding 125 mg of sample and 375 mg of *N*-*N*-dimethylacetamide (DMA) to an empty 10 mL vial. The vials were manually purged for two minutes a using a 20G 1½" needle with dry argon. The samples were then immediately capped. Two smaller 25G 5/8" needles (one to insert argon and one to relieve pressure) were used to purge the vials a second time. After 15 seconds both needles were removed. Then the sample is heated and 500 mL of headspace was removed with the AOC-5000 autosampler and analyzed using the Tracera GC.

4.2.3.2 Sample Preparation of Headspace Gas Chromatographic HS-20 Samples

When the HS-20 autosampler was utilized the sample can be automatically purged. In this case 125 mg of sample and 375 mg of DMA is added to a 10 mL vial and capped. The autosampler oven was kept at room temperature (25 °C) and the transfer line and sample loop are heated to 170 °C. The samples were pressurized to 200 kPa for 2 minutes. Then a 1 minute load time was used to extract a fraction of the diluted headspace. Samples were heated and then pressurized to 100 kPa for 1 minute. The

sample's headspace was loaded into the sample loop and injected into the GC for 2 minutes and 0.5 minute respectively.

4.2.3.3 Preparation of Calibration Curves

The HSGC had two calibration curves produced to quantify both water and ethanol. The first external calibration curve was made by adding 0.125, 0.25, 0.375, and 0.5 grams of water to 2.375, 2.25, 2.125, and 2.0 grams of DMA respectively. This produced samples with a water content of 5, 10, 15 and 20% water. The calibration curve is produced by adding ethanol to DMA to produce samples with 1-5% ethanol. This was achieved by combining 0.03, 0.05, 0.08, and 0.1 grams ethanol to 2.48, 2.45, 2.43, and 2.40 grams DMA. The calibration curve standards are then produced in 0.5 gram aliquots and the headspace is purged with the HS-20 the same as the samples.

4.2.3.4 Sample Preparation for Karl Fischer Titration

The KFT analysis was performed by Robertson Microlit Laboratories. The residual moisture in the KFT cell was measured by adding 3 mg of sulfosalicylic acid dehydrate to the titration cell which contains Hydranal Coulomat AG. The standard is then coulometrically titrated and the variation from the 14.17% water content is associated with residual moisture. Then 10 mg of sample is measured in the same manner and the residual moisture is subtracted from the evaluated water content.

4.3 Results and Discussion

4.3.1 Separations

The separation of ethanol and water with three ionic liquid columns is shown in Figure 4-1. When a 30 m Watercol 1460 was used, the ethanol and water were separated with a resolution of 1.7 at 150 °C. Ethanol and water were able to be

separated at 130 °C with a resolution of 2.5 on a 30m Watercol 1900. When the samples were analyzed on a 60 m Watercol 1910 capillary column the optimized analysis temperature was determined to be 140 °C. A separation between ethanol and water was achieved in less than 6 minutes with a resolution of ~4.0. It can be seen in Figure 4-1 that of the three columns, the Watercol 1460 produced sharp peaks for both the water and ethanol. In addition it provided the shortest analysis time.

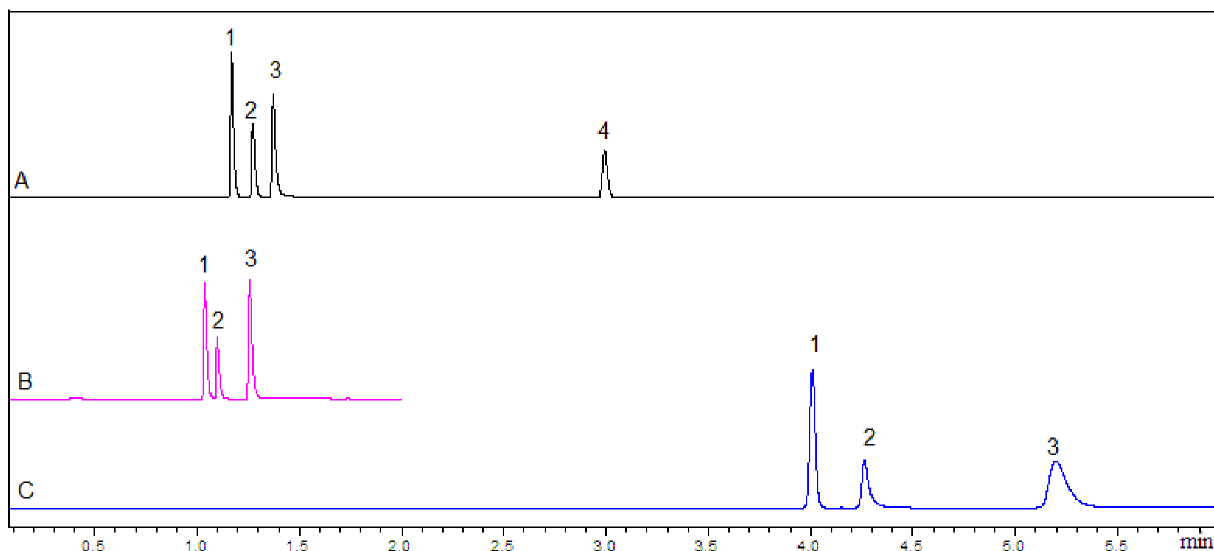


Figure 4-1: A typical chromatogram of Listerine dissolved in DMA and separated on three ionic liquid columns. In 1A the sample was analyzed on the Watercol 1460 at 150 ºC, in 1B it is analyzed on the Watercol 1900 at 140 ºC and in 1C the analysis is performed at 140 ºC on the Watercol 1910. (1) Air, (2) Ethanol, (3) Water, (4) DMA

4.3.2 Comparing Autosamplers

Two autosamplers with different heating apparatuses, and injection systems were compared. The first autosampler is the Shimadzu AOC-5000 which uses a gas-tight 2.5 mL syringe and a heated metal mantle. The second autosampler, Shimadzu HS-20, utilizes a pressure-loop system furnished with a 0.2 mL sample loop. In addition, it has an insulated oven equipped with a fan and operates similarly to a convection

oven. The throughput was approximately six times greater with the HS-20. The HS-20 adds helium to the vials, pressurizing the vial to 200 kPa, and then extracting the headspace for 30 seconds. Since the HS-20 can dilute and remove residual moisture it also can be used for automatic purging of the headspace of the vial. The AOC-5000 is unable to purge the vials; therefore to decrease the residual moisture, vials need to be manually purged.

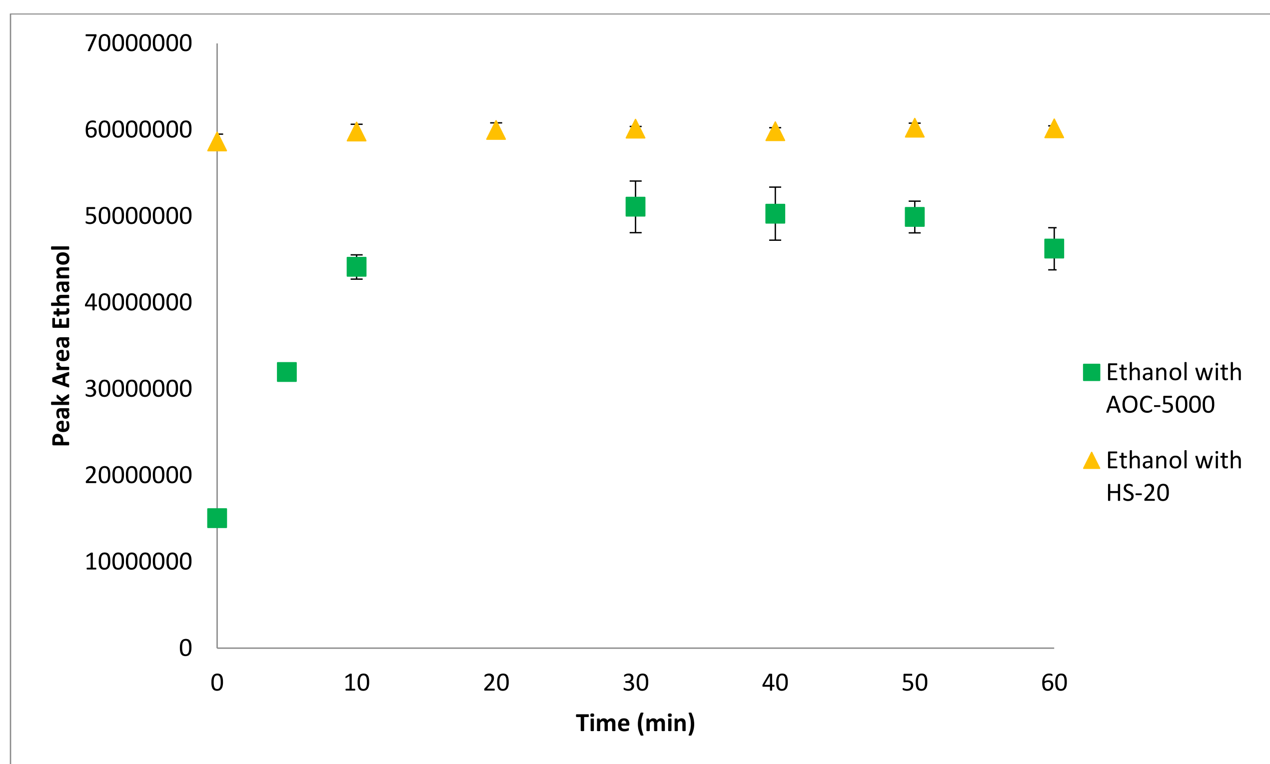


Figure 4-2: The effect of equilibrium time on the response of ethanol in Listerine using the HS-20 and AOC-5000 autosamplers.

When the equilibrium time (*i.e.,* the length of time that the samples are heated before extracting the headspace for analysis) was evaluated it was found that the HS-20 had an equilibrium time of five minutes, whereas the AOC-5000 required 30 minutes, as seen in Figure 4-2. The equilibrium time needed for the HS-20 was six times shorter than the AOC-5000, which allows the HS-20 to have a higher throughput.

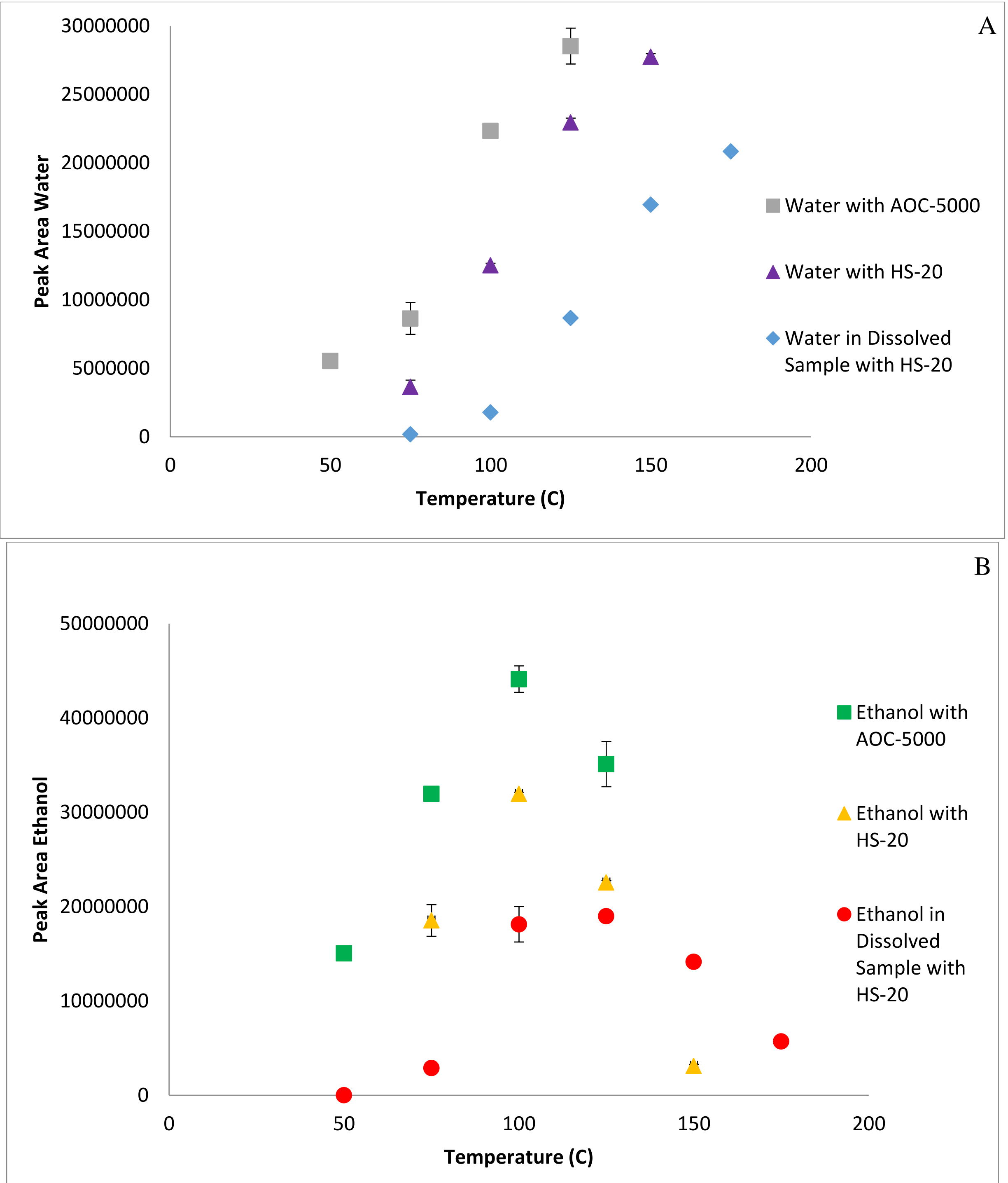


Figure 4-3: The effect of equilibrium response on water (A) and ethanol (B). When the sample (Listerine) is dissolved in a solvent the highest response of ethanol is increased from 100 ºC to 125 ºC.

The equilibrium temperature (the temperature the samples are heated before extracting the headspace for analysis) was found to be dependent on the ethanol. As seen in Figure 4-3A, the response for water was still increasing after 175 °C however, the ethanol (Figure 4-3B) substantial decreased. The amount of ethanol present in the vials, 25 mg ethanol or 100 mg, affects the optimal equilibrium temperature as will be discussed subsequently. The optimized equilibrium temperature was the same for both of the autosamplers and followed the same trend for ethanol (*i.e.,* an increased followed by decreased ethanol response). When the samples were first studied 500 mg of sample were added to the vials (neat) however due to the linear dynamic range of the detector, the samples were later dissolved in DMA. When the dissolved samples (25 mg ethanol) were compared to the samples which were not dissolved into a solvent (100 mg of ethanol) it can be seen in Figure 4-3B that the equilibrium temperature is increased from 100 °C to 125°C. Due to the ability of the HS-20 to automatically purge vials, it increased throughput and resulted in better precision, it was used for the analysis of 10 medicinal syrups.

4.3.3 Quantification

Two external calibration curves were developed in order to quantify ethanol and water in medicinal syrups. The TCD detector was used to determine the content of water since the products contained high water contents. The TCD was unable to be used to measure the ethanol content since there is only ~ 6-30 mg of ethanol present in the samples when the products were dissolved in DMA. Therefore the BID was used for measuring the ethanol content. The calibration curves were then used to measure the water content in the ten samples and ethanol content in six of the medicinal liquids.

It was found that the water content ranged from 30 – 98% as seen in Table 4-1. The evaluation of water content also was performed with Karl Fischer titration (KFT) and HSGC utilizing a BID and TCD detector. It was found that the BID was only able to quantify samples with lower water contents. When the water content increased above 70% the detector became saturated and the response was nonlinear. When the TCD was used to determine the water content in the 10 samples it was found to produce equivalent values and precision as KFT.

Table 4-1: The water content in 10 samples using Karl Fischer titration (KFT) and headspace gas chromatography (HSGC) with two different detectors (*i.e* .barrier discharge ionization detector (BID) and thermal conductivity detector (TCD))

Sample	KFT		HSGC-BID		HSGC-TCD	
	Percent Water	RSD	Percent Water	RSD	Percent Water	RSD
BPM-DM-PHEN Syrup	61 ± 0.6	1.0	60 ± 0.6	1	64 ± 0.8	1.2
Children's Dimetapp Cold and Cough	69 ± 0.5	0.7	63 ± 2.0	3.1	64 ± 2.4	3.8
Chloraseptic Sore Throat	97 ± 0.8	0.9	76 ± 1.9[a]	2.5	93 ± 4.4	4.7
Equate Tussin DM	35 ± 0.6	1.6	33 ± 0.4	1.3	36 ± 1.5	4.1
Listerine	74 ± 1.4	5.6	62 ± 2.4[a]	3.8	73 ± 4.2	5.8
NyQuil	43 ± 0.9	2.1	37 ± 0.3	0.8	41 ± 2.0	4.9
Pepto-Bismol	95 ± 2.1	2.2	73 ± 2.4[a]	3.3	92 ± 1.8	1.9
Severe Cold and Fever	40 ± 1.2	2.9	36 ± 0.3	0.8	42 ± 2.0	4.8
S. S. S. Tonic	86 ± 5.4	6.2	73 ± 3.0[a]	4.1	85 ± 3.9	4.6
Tussin Chest Congestion	37 ± 1.8	4.8	34 ± 1.4	4.3	42 ± 2.2	5.2

[a] Samples saturated the detector and the results therefore underestimate the water content.

The six samples were found to contain a range of 0.01 - 22% ethanol (See Table 4-2). In two of these products, S.S.S. Tonic and Listerine, the ethanol content is reported by the manufacture and when studied with HSGC, ethanol levels were found to be comparable to those given on the label (see Table 4-2).

Table 4-2: The ethanol content in four samples using headspace gas chromatography (HSGC) compared to the commercially reported numbers when provided.

Sample	Reported Value	HSGC	
		Percent Ethanol	RSD
Children's Dimetapp Cold and Cough	[a]	0.01 0.001	5.0
Listerine	22	20.7 ± 0.4	2.1
NyQuil	[a]	8.7 ± 0.2	2.2
Pepto-Bismol	[a]	0.02 ± 0.002	6.3
Severe Cold and Fever	[a]	7.1 ± 0.3	4.9
S. S. S. Tonic	12	12.0 ± 0.01	0.1

[a] Manufactures do not report the actual alcohol content in these samples.

4.4 Conclusions

A method was developed for simultaneously measuring ethanol and water in liquid pharmaceutical products. Ionic liquid columns effectively separate water and ethanol and provide sharp narrow peak shapes. The utilization of headspace reduces the degradation of the sugar in the inlet of the GC when direct injection is done. It was found that the pressure-loop based autosampler provided the highest precision, and throughput and was able to automatically purge the vial's headspace. It was found that both the BID and TCD were required in order to quantify both the ethanol and the water in the medicinal syrups. Finally the method was found to be precise, accurate and fast.

Chapter 5

THE UTILIZATION OF TWO DETECTORS FOR THE DETERMINATION OF WATER IN HONEY USING HEADSPACE GAS CHROMATOGRAPHY

Abstract

A headspace gas chromatography (HSGC) method was developed for the determination of water content in honey. This method was shown to work with five different honey varieties which had a range of water from 14-16%. It also utilized two different detectors, the thermal conductivity detector (TCD) and the barrier discharge ionization detector (BID). This method needs no heating pretreatment step as in the current leading method, (i.e., the measurement of refractive index). The solvent free procedure negates the possibility of solvent compound interactions as well as solubility limitations as is common with Karl Fischer titrations (KFT). It was also apparent that the classic loss on drying method consistently and substantially produced results that were lower than the correct values. This approach is shown to be rapid, with an analysis time of 4 minutes when using TCD detector and under 3 minutes when utilizing the BID detector. HSGC is feasible for the determination of water due to the new PEG-linked geminal dicationic ionic liquid coated GC capillary column. In addition it provides accurate and precise determinations of the water content in honey. When using the sensitive BID detector, other trace volatile compounds are observed as well.

5.1 Introduction

Honey is a natural, viscous, stable sweetener, consisting mainly of fructose, glucose and water. Since honey is a saturated sugar solution it is able to absorb moisture from the atmosphere.[6] The water content in honey is influenced by a number of factors including botanical origin, atmospheric conditions and seasonal variations.[6, 103-105] In addition to natural variations, assorted human modifications (*e.g.* treatments, production and storage conditions) also affect the water content.[6] The content of water

affects the quality, marketability, and physical properties of the honey. The moisture content of honey must not exceed 21% however it should remain above 14% water.[6, 106] When the water content is too low, under 14%, the viscosity is increased and crystalline entities appear. However, if the water content exceeds 21%, it can support microbial growth. In these cases the contaminated honey can cause severe illness if consumed by humans.[6, 105. 107]

The water content of honey has traditionally been analyzed in one of three ways, refractive index (RI) measurement, gravimetric determination of water loss after drying, or Karl Fischer titration (KFT). Refractive index measurement is the most common method for water determination in honey. While this is simple, fast and reproducible there are some problems with this approach.[107] The procedure requires a thermal pretreatment step for the honey sample which leads to some loss of water content and therefore inaccurate results.[6] In order for the refractive index to be used as a method for water determination a relative conversion table has to be utilized as well. The conversion table however may not be accurate for all types of honey due to significant variations in the ratio of different sugars and other minor components.[107] These differences in composition are known to affect the refractive index, thereby decreasing the accuracy of the method.[6, 108]

Loss on drying is not used nearly as often due to difficulties with this method.[107, 109] After heating, a highly viscous product is formed leading to slow diffusion of water, and tightly bound water which is difficult to vaporize. This approach usually produces numbers that are lower than the true water content of the samples.[110] Also, this method is time consuming and labor intensive. Additionally, honey can have other volatile

components which can vaporize leading to errors in the estimation of water content.[107, 109]

More recently, attempts have been made to adapt Karl Fischer titration (KFT) for determining the water content in honey. While KFT has better reproducibility compared to the loss on drying or RI, it does have an increased cost in solvents and can be time consuming.[107] Furthermore, honey has limited solubility in the KFT medium (typically methanol solutions). In order to overcome this problem formamide and methanol are combined with the working solvent, and the titration cell is heated. This method is known to have poor laboratory to laboratory reproducibility making it a less than ideal technique.

The water content in a few select foods has been determined with a fourth method, headspace gas chromatography (HSGC). In this method the samples are first dissolved in methylglycol (*i.e.,* 2-methoxyethanol). Since few samples can be completely dissolved suspensions are usually obtained. It is labor intensive method because of the need for standard addition, multiple standards and multiple headspace extractions.[47] GC has been utilized in the past with packed columns in order to measure water, however there were numerous problems (*e.g.,* broad tailing peaks, peak overlap, irreproducibility).[35, 46] In addition many of these columns degraded in the presence of water. In 1999, a new class of open tubular column stationary phases was introduced, which consisted of ionic liquids.[51, 61, 77, 93, 111] These stationary phases are stable in the presence of water and oxygen. Further, water is easily separated from other solvents and volatile substances as a relatively efficient, symmetrical peak. The water peak had improved peak area reproducibility due to the narrowing of the peak and improved peak

symmetry.[50] Consequently water analysis using capillary GC became feasible. Recently, this approach was used to measure the water content of active pharmaceutical ingredients and the, water/ethanol content of various consumer products.[34, 56]

In this work, we developed a simple HSGC method for the determination of the water content in honey. Since this method directly quantifies water, it does not require a conversion table, is not impacted by solid particles, has no preheating treatment, does not have any solubility issues and does not require multiple extractions. In addition, the method is fast and straight forward.

5.2 Experimental

5.2.1 Apparatus and Conditions

All manual injections were performed utilizing a 6890N gas chromatograph (Agilent Technologies Inc., Wilmington, Delaware, USA) with thermal conductivity detector (TCD). The 6890N GC-TCD was equipped with Chemstation plus software (Rev.B.01.03). The oven temperature was held isothermally at 110 °C while a split ratio of 10:1 was used. The injection port and detector were set to 280 °C and 250 °C respectively. Helium at 1 mL/min was used for all runs. A typical analysis was completed in 7 minutes. A 1 mL gas tight syringe (SGE Analytical Science, Melbourne, Australia) was used for all manual injections. A Tracera GC-2010 Plus (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a barrier ion discharge detector (BID), LabSolutions (version 5.71 SP1) and an AOC-5000 Plus Autosampler (Shimadzu Scientific Instruments, Kyoto, Japan) was used for all automated sampling. The oven was kept isothermally at 110 °C with a split ratio of 100:1. The injection port

was set at 280 °C and the detector was set at 200 °C. A 2.5 mL headspace HD-type syringe (Hamilton, Reno, Nevada, USA) was used for all automated injections. The analysis was completed in 5 minutes. A 30 m x 0.25 mm ID x 0.2 um film coat thickness SLB-IL107 fused silica capillary column.[51] This column is now commercially available as the Watercol™ 1910 column from Supelco (Bellefonte, PA). All samples were measured on an AR1140 Adventurer balance (Ohaus Corp., Pine Brook, New Jersey, USA).

5.2.2 Materials

Fructose was obtained from Sigma Aldrich (St. Louis, Missouri, USA). Buckwheat blossom honey was purchased from Dutch gold (Littleton, NH, USA). Organic white raw honey was obtained from Whole Foods Market (Austin, Texas, USA). Wild flower honey was acquired from Madhava Natural Sweeteners (Longmont, CO, USA). Mandarin blossom honey was bought from Rigoni di Asiago (Miami, Florida, USA). Raw honey was obtained from Mountain Gold Honey (Ogden, UT, USA).

The 15 X 45 mm, 1 dram vials were purchased from Fischer Scientific (Waltham, Massachusetts, USA). The screw-on molded plastic covers were obtained from SKC Inc. (Eighty Four, Pennsylvania, USA). White silicone/TFE septa were obtained from Sigma Aldrich (St. Louis, Missouri, USA). The 22 X 75 mm screw-thread vials and the magnetic screw-thread covers for the autosamper were purchased from Restek (Bellefonte, PA, USA).

5.2.3 Sample Preparation

5.2.3.1 Preparation of Samples for Headspace Gas Chromatography

The samples analyzed on the GC-TCD were prepared by adding 400 mg of the honey to the vial. All weights were recorded to 0.1 mg using an analytical balance. The

vials were then purged with dry argon for 2 minutes using a 20 G 1½ " needle and were immediately capped with the molded plastic covers containing two white silicone/TFE septa. The capped samples were then purged again using a smaller 25 G, 5/8" long needle with dry argon for 15 seconds, while a second 25 G, 5/8" long needle was inserted into the septum. The two purging needles were removed and the sample was heated to 55 °C for 30 minutes. Finally, 600 μL of headspace was manually extracted with a gas tight syringe and injected into the GC-TCD. When honey samples were analyzed using the Tracera GC-BID 500 mg of the analyte honey was added to the vial. All weights were recorded to the nearest 0.1 mg. The vials were then purged in the same way as the vials used for manual analysis. After purging the vials, the samples were heated at 55 °C for 20 minutes and had 250 μL of headspace automatically injected with the autosampler (Section 2.1) into the GC-BID. The samples analyzed with the GC-BID had different equilibrium times due the difference in agitation and heating. The difference in the amount of headspace vapor injected in the two different approaches is attributed to the increased sensitivity of the BID detector compared to the TCD detector.

The calibration curve for the manual injection on the GC-TCD was produced by using solutions of 0.36, 0.32, 0.28, 0.24, and 0.2 grams of fructose plus 0.04, 0.08, 0.12, 0.16, and 0.2 grams of water respectively each in 15 X 45 mm vials. The calibration curve for the analysis with the autosampler on the GC-BID was produced using solutions of 0.45, 0.4, 0.35, and 0.3 g of fructose plus 0.05, 0.1, 0.15 and 0.2 g of water respectively in individual 22 X 75 mm autosampler vials. Sample sizes for the calibration curves were determined based on the vial size and the sensitivity of the detector. The

standard addition samples were made by adding 0.043, 0.085, and 0.128 g of water to 0.5 g of honey. All samples were made and analyzed in triplicate.

5.2.3.2 Preparation of Samples for Refractive Index

The refractive index samples were heated to 50 °C. Once the samples were heated the honey was added to the measuring cell kept at 20 °C without any air bubbles (Isengard & Schultheiß, 2003). The dry material (DM) was determined by using a formula DM = 78 + 390.7 (RI - 1.4768) (Auerbach & Borries, 1924). The water content was then determined by subtracting the DM from 100 (Auerbach & Borries, 1924). The samples were measured in triplicate and the percent water was calculated from a conversion table.

5.2.3.2 Preparation of Samples for Gravimetric Determination after Drying

The gravimetric determination after drying was performed by first weighing 0.5 grams of honey into a vial and the sample was heated at 70 °C for 24 hours. The sample was then cooled and reweighed. It was then heated again for 2 hours to assure a constant mass was achieved (Herrick, 1995).

5.3 Results and discussion

5.3.1 Gas Chromatographic Conditions

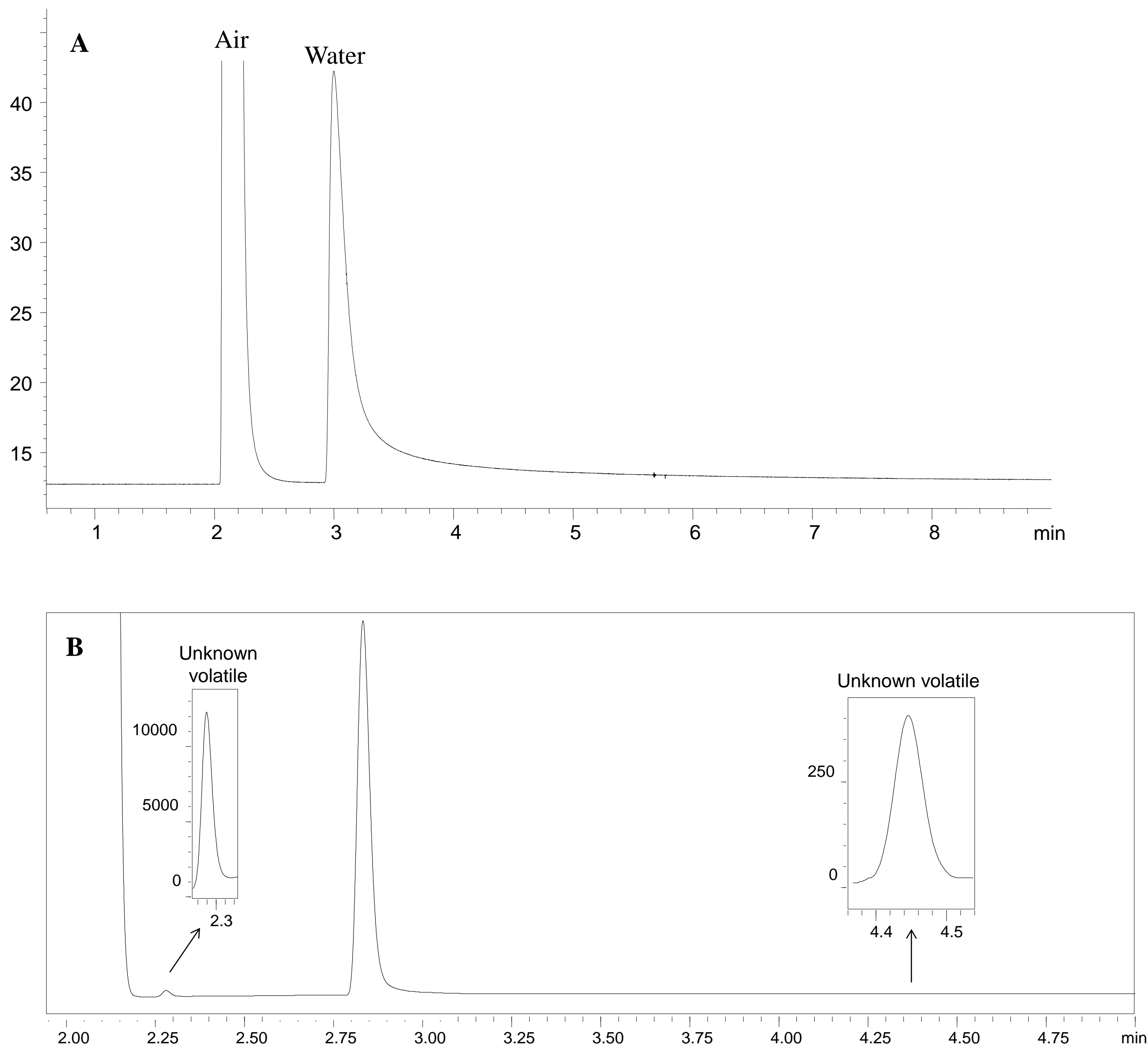


Figure 5-1: Chromatograms of buckwheat honey using a TCD and BID detectors

5-1A: A chromatogram of a typical analysis of water in buckwheat honey using at 110 °C_with a split ratio of 10:1 on the GC-TCD. Figure 5-1B: A chromatogram of a typical analysis of water in buckwheat honey using a GC-BID at 110 °C with a split ratio of 100:1.

The GC conditions were optimized in order to obtain a rapid, robust and efficient method. It was found that the SLB-IL107 capillary GC column produced the most symmetrical water peaks compared to the other commercially available ionic liquid stationary phases (data not shown). In addition, no column degradation or changes were observed over the course of the study. A split ratio of 10:1 and the oven temperature of 110 °C were determined to be optimal for the GC-TCD determination. With the more sensitive GC-BID, a higher split ratio of 100:1 was used, however, the chromatography was still performed at 110 °C. Figure 5-1 gives example chromatograms using both the TCD and BID for the headspace analysis of buckwheat blossom honey (see Experimental). It can be seen that when the more sensitive BID is utilized fragrance peaks can be observed in addition to the water peak. Also the peak shape for water is improved since less sample is loaded onto the column with a 100:1 split ratio.

5.3.2 Optimization of Headspace Gas Chromatographic Conditions

Prior to analysis, the headspace “void” was briefly purged with dry argon to remove atmospheric water while not purging to such an extent that water was removed from the sample itself. An empty vial required an initial purge duration of 2 minutes with dry argon in order to produce the lowest background effect from residual atmospheric water as seen in Figure 5-2.

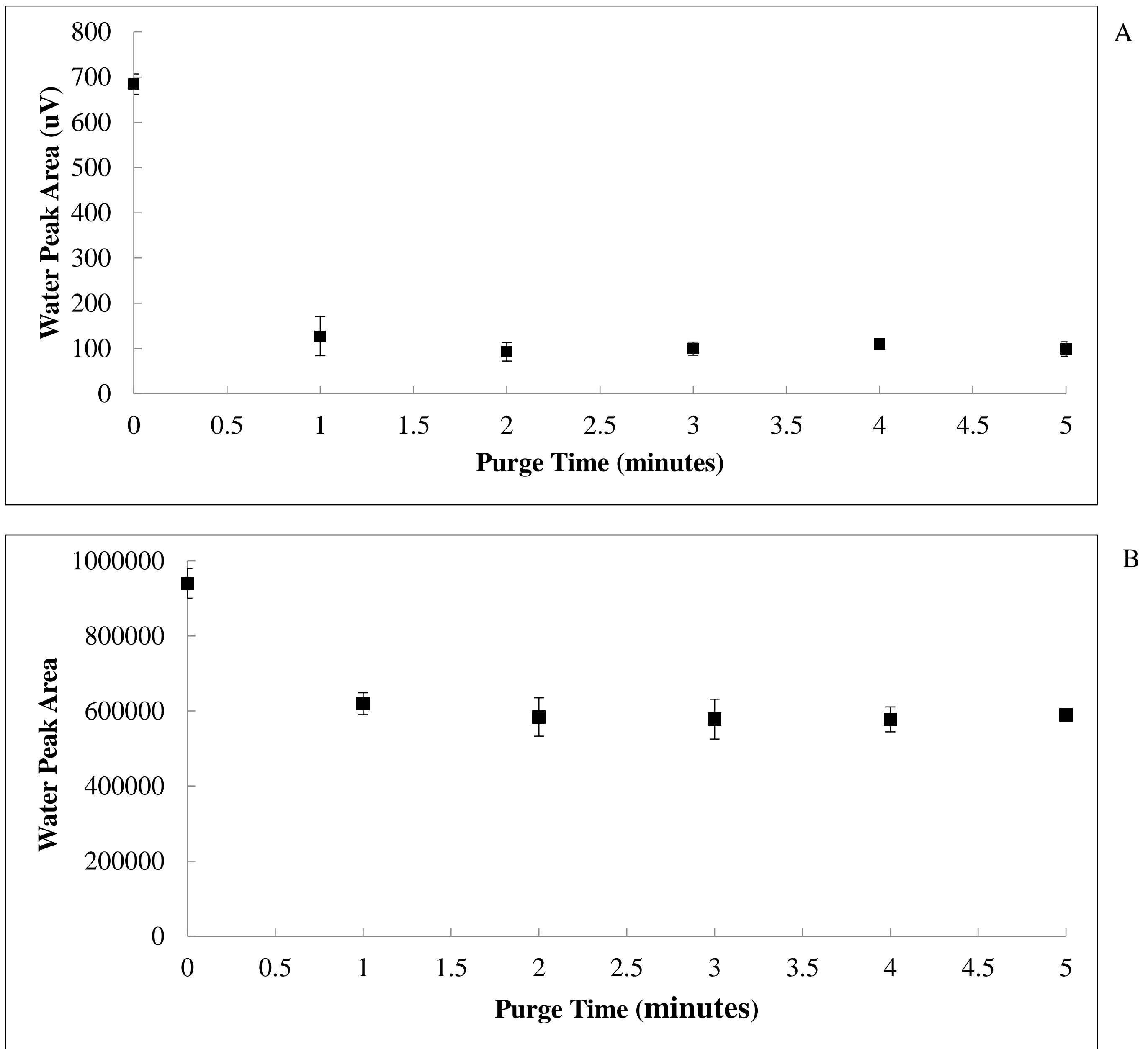


Figure 5-2: The effects of purge time on vials of different size

(5-2A) the initial purge of empty 1.5 dram vials for the manual injection with the GC-TCD. The analysis was at 6 different purge times ranging from not purging with argon (*i.e.,* 0 minutes or atmospheric air) to a 5 minute purge of dry argon. The change in the background water content between not purging and purging the vials is seen. (5-2B) the initial purge at 6 different purge times of the larger empty autosampler vials analyzed with the GC-BID. These larger vials also had minimal affect from atmospheric moisture after 2 minutes of purging with dry argon.

Also shown in Figure 5-2 is that that larger autosampler vials require the same initial purge time of 2 minutes. The initial purge time was then evaluated using a sample of honey to determine at what purge time the water content of the sample was affected. As Figure 5-3 illustrates, no further change in water content was seen after a 2 minute purge. Therefore an initial purge time of 2 minutes was optimal for the vials and vials filled with sample. The thermal equilibrium time was also shown to be optimal at 30 minutes (data not shown).

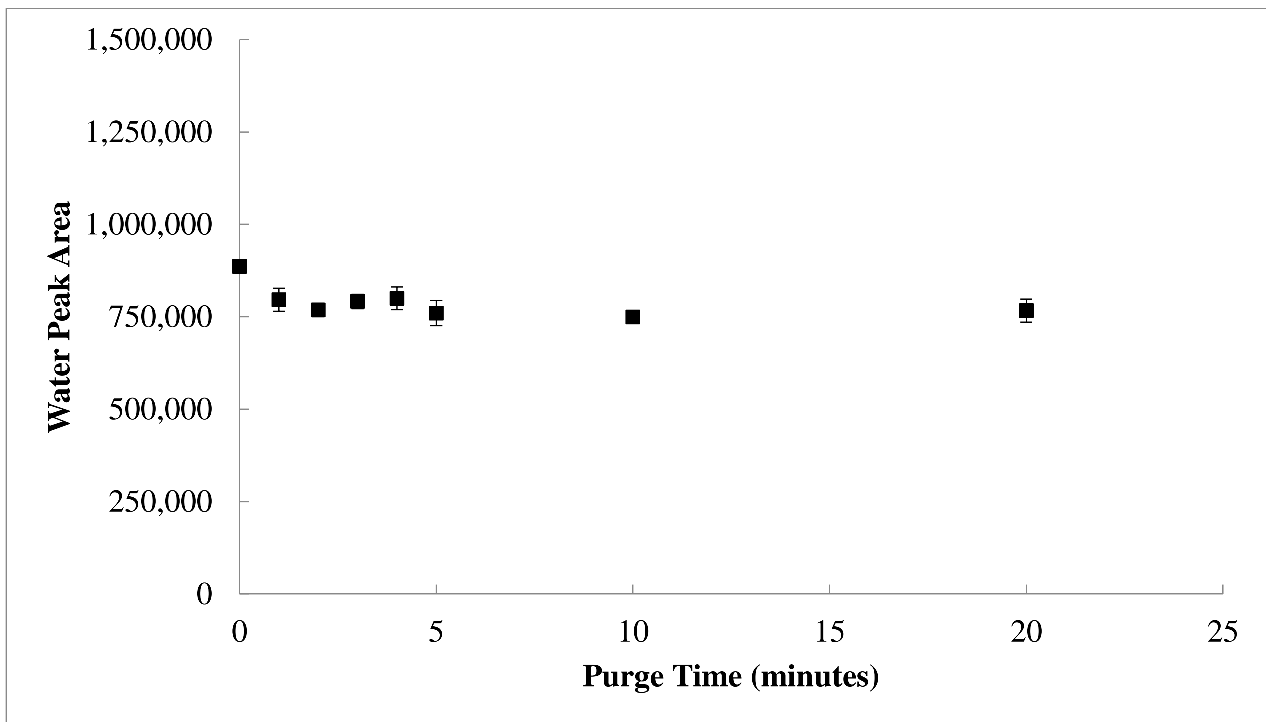


Figure 5-3: The initial purge of a vial with 500 mg of honey ranging from 0-20 minutes. After 2 minutes of purging the vials with dry argon the response plateaus showing the presence of sample does not affect the purge of the vials.

5.3.3 Quantitative Analysis of Residual Water in Samples

Calibration curves were produced for both vial sizes using fructose (see Experimental) in order to quantify the water in the honey samples. A correlation (r2)

equal to 0.994 with a best fit line of y = 35.9x - 1.7 was determined for the linear relationship between the peak area and the percent water for manual injections utilizing the GC-TCD. The HSGC method utilizing the GC-BID produced a r2 equal to 0.98 with an equation for the slope of the line to be y = 24,000 x + 1,090,000. The calibration curve was then used to quantify the water content of five different honey samples. The water content had a range of 14-16% water. For comparison purposes, the water content was also determined using standard loss on drying and RI methods as seen in Table 5-1.

Table 5-1: The table shows the average percent water for the five different honey samples utilizing refractive index, loss on drying and headspace gas chromatography (both the TCD and the BID).

Type of Honey	RI (% Water)	Loss on drying (% Water)	HSGC GC-TCD (% Water)	HSGC GC-BID (% Water)
Buckwheat Blossom Honey	15.7 ± 0.2	12.9 ± 0.9	14.9 ± 0.2	15.1 ± 0.5
Wild Flower Honey	15.5 ± 0.2	15.9 ± 0.3	15.3 ± 0.3	15.3 ± 0.06
Mandarin Blossom Honey	15.8 ± 0.2	12.5 ± 0.2	16.1 ± 0.9	15.9 ± 0.2
Raw Honey	14.2 ± 0.2	9.9 ± 0.1	14.9 ± 0.3	14.9 ± 0.4
Organic White Raw Honey	15.4 ± 0.2	12.6 ± 0.2	15.4 ± 0.7	15.6 ± 0.2

5.3.4 Precision

The relative standard deviation (RSD) of multiple manual injections was measured to determine the precision of the HSGC method. It can be seen from the data in Table 5-1 that the RSDs were all <5%, showing a precise method for all honey samples. The RSDs for the RI method was determined to have an average of 1.3% RSD. When utilizing loss on drying the RSD was higher, with an average RSD of 3.1%.

When compared to RI and loss on drying it can be seen that HSGC is comparable to the RSD of the RI and loss on drying.

5.3.5 Accuracy

The accuracy of the method was examined by comparing HSGC to the leading method, RI. The HSGC method utilizing both the GC-TCD and the GC-BID were shown to be comparable, as seen in Table 5-1, in all cases but one. When the buckwheat honey was analyzed with the refractive index, it indicated higher water content than HSGC method. This sample was a dark brown compared to the other honey samples which were a golden color. In addition, this sample was more granular and contained many suspended particles. The accuracy was also determined by standard addition of water to the wild flower honey (see Experimental). The wild flower honey was determined to have 15.3% water which matches the water content determined directly from the HSGC method. Comparing the HSGC results to the RI method and standard addition approach, shows it is an accurate method. Also it is obvious that the loss on drying method consistently underestimates the amount of water in all samples.

5.4 Conclusions

The HSGC method was shown to be precise and accurate for the determination of water content in honey. This method does not need any heated pretreatment procedure; therefore no water is lost when prepared the samples which improves the accuracy compared to currently used methods. The presence of salts and the composition of the sugars in honey directly affects the RI method's ability to accurately estimate water content. Since the HSGC method is not affected by the composition of the honey it is able give exact water concentrations. Furthermore, this allows it to have

broad applicably for both treated and raw honeys. Quantification of water is simpler with the novel HSGC method since it utilizes an external calibration curve compared to RI which must utilize one of the many conversion tables or equations to convert the RI to "dry material" (see Experimental). The HSGC method has high throughput capability and can be automated leading to a more rapid water analyses. The new HSGC method was shown to work with both the TCD and the more sensitive BID. Due to its higher sensitivity, the BID could also detect, simultaneously, trace levels of other volatile honey compounds. Utilizing HSGC the water content in a wide variety of honeys can be measured rapidly, accurately and the analyses can be automated easily thereby increasing throughput.

5.5 Acknowledgments

This work was also supported by the Robert A. Welch Foundation (Y0026). We would also like to thank the *Shimadzu Center* for Advanced Analytical Chemistry for the use of the Shimadzu Tracera GC-2010 Plus.

Chapter 6

UTILIZING HEADSPACE GAS CHROMATOGRAPHY FOR MEASUREMENT OF WATER IN SUGAR AND SUGAR FREE SWEETENERS AND PRODUCTS

Abstract

An automated method for determination of water in liquid sweeteners was developed utilizing headspace gas chromatography (HSGC) and ionic liquid based capillary GC columns. This method allowed for the rapid determination of water with minimal sample pretreatment. In addition to providing fast analysis time for the samples, the HSGC method was found to be accurate and precise for the measurement of water in sixteen liquid sweeteners. The method does not employ high temperatures lowering the chance of the Maillard reaction which would produce an inaccurate determination of water content. This method was shown to be widely applicable for sugar and sugarless based sweeteners and likely, more accurate than Karl Fischer titration.

6.1 Introduction

Historically, honey was the primary sweetener used to enhance many foods; however with advances in technology, sweeteners with high concentrations of fructose and/or sucrose are now employed.[112, 113] In syrup-form, they provide an economical, easy to handle alternative to honey.[113] Sweeteners can now be found in most foods; carbonated beverages, canned goods, jellies, jams, baked goods, dairy products and many pharmaceutical products.[112-114] These additives improve the humectancy, color, and flavor of food.[112] Sweeteners are found in many foods, and their compositions are monitored and regulated.[115, 116] One important component, water, has to be regulated

as it affects both the physical characteristic of the product and consumer safety.[112, 116] The water content directly affects the viscosity of syrups and sweeteners. When the water content in syrup is low, the sugar may precipitate and the ability to easily handle the product as well as consumer satisfaction will be compromised.[117]

In addition to human consumption, many syrups (*e.g.,* molasses, corn syrup) are used as additives in animal feed.[113, 115, 116] The water content is monitored and if the level exceeds the regulated range, both mold and other microbial growth can occur.[115-117] This can lead to significant problems due to the toxic nature of some molds, spores, and their byproducts.[7-9, 11] If the mold and spores are consumed it leads to a reduction of feed intake, which can cause weakness, weight loss and decreased production in dairy cattle.[11] Furthermore, spores and mold can cause many diseases along with their related symptoms of vomiting, diarrhea, skin lesions, kidney and liver damage, lack of muscle control and nervous system disorders.[7-9, 11] Other effects are an increase in infertility and abortions among exposed cattle.[7-9, 11] Mold is known to causes respiratory distress, coughing, and shortness of breath in both humans and livestock.[7-9, 11] Consequently the measurement of water in many products is often required by regulatory bodies worldwide.

Water content traditionally is measured by refractive index and reported in degrees Brix or by percent by weight of sucrose in water.[47, 116-118] Degrees Brix is used because it is a fast and easy way to measure the moisture in sucrose based sweeteners.[116, 118] While it is fast, many sweeteners are fructose based or have a combination of sucrose and other sugars causing it to be inaccurate and therefore the Brix measurement is actually an “apparent Brix”.[116, 118] In addition to sugars other than

sucrose, salts are known to cause an apparent change in the water content.[116, 118] When salt is present, the measured degrees Brix can indicate 5-10% less water than is actually contained in the sample.[116] Headspace gas chromatography (HSGC) is another method which has been utilized, in a few instances for the determination of water in select foods, solvents, and active pharmaceutical ingredients.[34, 47, 20, 52, 54] Early on, the amount of water in food was measured by the formation of a suspension in methyl glycol and then multiple headspace extractions were utilized.[47] One problem that occurred with early this GC method was that the various supports (*e.g.,* diatomaceous earth and molecular sieves) in packed columns led to a nonideal absorption of water, therefore these columns produced broad tailing peaks with poor peak area reproducibility.[35, 46, 48, 49] In addition, these packed columns tended to have low selectivity and resolution between water and many other common solvents.[34, 46, 48,54] Also, air, water as well as some solvents can degrade common liquid stationary phases at the elevated temperatures required for the analyses.[50] New GC stationary phases composed of ionic liquids (IL), have been developed which allow GC to be utilized for the analysis of water.[34, 50-52, 54, 56] The ILs with trifluoromethylsulfonate (TfO^-) anions improves the water's peak shape and peak area reproducibility lowering the limit of detection.[50, 51, 56, 58] Further, these stationary phases are unchanged when exposed long term to water and oxygen containing samples.

In this work we report a simple, effective and accurate method for the determination of water content in fructose, sucrose and sucralose based syrups. This method, unlike previous methods, is not affected by the sugar composition, the presence of solid particles in the sample, or the presence of salts. Also, the method

does not entail multiple headspace extractions, additional solvents or standards as in some of the previous HSGC methods. This effective approach is easily automated and is possible due to advent of advanced IL stationary phases for GC coupled with a specific GC configured for water analysis and containing devices for the stringent reduction of ambient moisture.

6.2 Method

6.2.1 Materials

The fructose was obtained from Sigma Aldrich (St. Louis, MO, USA). The blue agave nectar was purchased from C&H Sugar (Crockett, CA, USA). The Grandma's Molasses was from B&G foods (Parsippany-Troy Hills, NJ, USA). Karo Light corn syrup was obtained from ACH Food Companies, Inc. (Cordova, TN, USA). The Pancake syrup was purchased from Safeway (Pleasanton, CA, USA). The Hershey's chocolate syrup and caramel topping were purchased Hersheys Company (Derry Township, PA, USA). The Nesquik chocolate syrup and strawberry syrup were from Nestle (Glendale, CA, USA). The strawberry jelly and jam were obtained from Smucker's (Orrville, OH, USA).The Mrs. Butterworth's original syrup and Mrs. Butterworth's sugar free syrup were from Mrs. Butterworth's (Miami, FL, USA). The coffee creamer was from Kahala Franchising, L.L.C. (Scottsdale, AZ, USA). The Sugar Free Butter Flavored Syrup was obtained from Maple Grove Farms (St Johnsbury, VT, USA). The Rose's Grenadine syrup was purchased from Mott's LLP (Plano, TX, USA). The dimethyl sulfoxide was purchased from Sigma Aldrich (St. Louis, Missouri, USA).

The 22 X 75 mm screw-thread vials and the magnetic screw-thread covers for the autosampler were purchased from Restek (Bellefonte, PA, USA).The 30 m × 0.25

mm, df 0.20 µm Watercol™ 1460, and Watercol™ 1900 along with a 60 m × 0.25 mm, df 0.20 Watercol™ 1910 were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

6.2.2 Sample Preparation

6.2.2.1 Headspace Gas Chromatographic Sample Preparation

Samples with < 40% water were prepared by adding 500 mg of sample to a clean vial using a pipette. Samples which contained > 40% water were prepared by adding 0.125 g of sample and 0.375 g of dimethyl sulfoxide (DMSO) to a clean vial. After the sample was prepared it was immediately capped. The vial was pressurized to 200 kPa for two minutes at room temperature utilizing the Shimadzu HS-20 headspace autosampler. The headspace was then loaded or extracted for one minute. After the purging process was complete the vial was heated for five minutes at 100 °C. The sample was pressurized to 100 kPa and the head space vapor was loaded for two minutes into the 0.2 mL sample loop. A half minute injection was then made into the GC. Two external calibration curves were produced one for lower water content (< 40% water) and a second calibration curve for samples with higher water content (> 40%). The first was used for samples with lower water content, by combining 0.4, 0.5, 0.6, 0.8 g water with 2.6, 2.0, 1.9, 1.8 g of fructose respectively. Samples were made in quadruplicate by adding successive aliquots of 500 mg sample to clean vials. Then the vials were purged, heated and analyzed the same as the samples. The second calibration curve for higher water contents was produced by making samples with 5%, 10%, 15%, 20% and 25% water in a DMSO matrix. This was achieved by making combining 0.125 g, 0.250 g, 0.375 g, 0.500 g, and 0.625 g water with 2.375 g, 2.250 g,

2.125 g, 2.000 g, and 1.875 g of DMSO respectively. The solutions are then divided into 500 mg aliquots and treated in the same manner as the samples.

6.2.2.2 Loss on drying Sample Preparation

Loss on drying was performed by weighing four clean empty vials. A sample, ~500 mg, was added to each vial and the new mass was recorded. Samples were heated for 12 hours at 60 °C and then cooled and weighted. A second 12 hour evaporation step is performed at 60 °C. The process is repeated until a constant mass is obtained.

6.2.2.3 Karl Fischer Titration Sample Preparation

The KFT analyses were performed by Robertson Microlit Laboratories. The atmospheric moisture was measured by adding 3-10 mg of sulfosalicylic acid dehydrate to the Hydranal Coulomat AG in the titration cell. The standard was titrated coulometrically to the electrometic endpoint and used to determine the response of residual moisture. The sample, 10 mg, was then added to the titration cell and titrated. The atmospheric moisture was subtracted for the reported value to obtain the moisture in the sweetener sample.

6.2.3 Apparatus and Conditions

The analyses were performed using a Tracera 2010 equipped with a barrier discharge ionization detector (BID) and thermal conductivity detector (TCD), Shimadzu Scientific Instruments (Kyoto, Japan). Labsolutions 5.82 was used for all peak integration. A Shimadzu HS-20 headspace autosampler was employed to purge, heat and inject all samples. The transfer line and sample line were kept at 170 °C. The oven in the HS-20 was kept at room temperature (25 °C) when purging the vials and at 100

°C for all analyzes. A 60 m x 0.25 mm ID x 0.2 um film coat thickness Watercol™ 1910 fused silica capillary column coated with IL synthesized as previously reported or commercially acquired from Supelco/Sigma-Aldrich.[51] The GC oven temperature was held isothermally at 170 °C with a run time of 5 minutes. The carrier gas for all runs was helium at 1.5 mL/min (26 cm/sec) dried with a High Capacity Gas Purifier and an OMI® Purifier Tube (Supelco Bellefonte, PA, USA). The injection port was set at 280 °C and the TCD was set at 200 °C with a current of 80 mA. A split ratio of 100:1 was utilized for all analyses of the sweeteners. Selected analyses were performed using a 6890N gas chromatograph with TCD Agilent Technologies Inc. (Wilmington, Delaware, USA), and equipped with Chemstation plus software (Rev.B.01.03). A 1 mL Gastight syringe (Hamilton, Reno, Nevada, USA) was used for all injections.

6.3 Results and Discussion

6.3.1 Optimization of Separation

The GC oven temperature, split ratio, and GC column were evaluated and the optimized conditions are specified in the Experimental section. It was determined that a temperature of 170 °C and a split ration of 100:1 was optimal for these analyses. The Watercol™ 1910 GC column gave the best peak symmetry for water compared to Watercol™ 1460 and Watercol™ 1900 (see Experimental). The Watercol™ 1900 gave the lowest retention time, however the peak shape was slightly less symmetrical the Watercol™ 1910. The improved peak shape of the water when analyzed on the Watercol™ 1910 in turn, provided for more precise water determinations (*vide infra*). It was found that the water concentrations were not in the linear range of the sensitive BID, however, they were well within the linear range of the TCD. One of the virtues of a

GC specifically configured for water analysis is that it has both of these detectors and therefore the flexibility to handle samples containing trace levels to higher levels of water. In the case of these sixteen sweeteners the higher water content in the samples allowed the TCD to give a response $4x10^4$-$8x10^4$ times higher than the blank.

6.3.2 Optimization of Headspace Conditions

The headspace analysis of samples for water requires optimization of a few parameters. These include the purging conditions, sample loop size, and the length of time the sealed samples are heated (*e.g.,* equilibrium time). It should be noted that the Shimadzu HS-20 headspace autosampler has a unique configuration that is advantageous for water analysis.[16] The equilibrium temperature was set at 100 °C in order to reduce side reactions (*e.g.* Maillard reaction) which produce water as a byproduct.

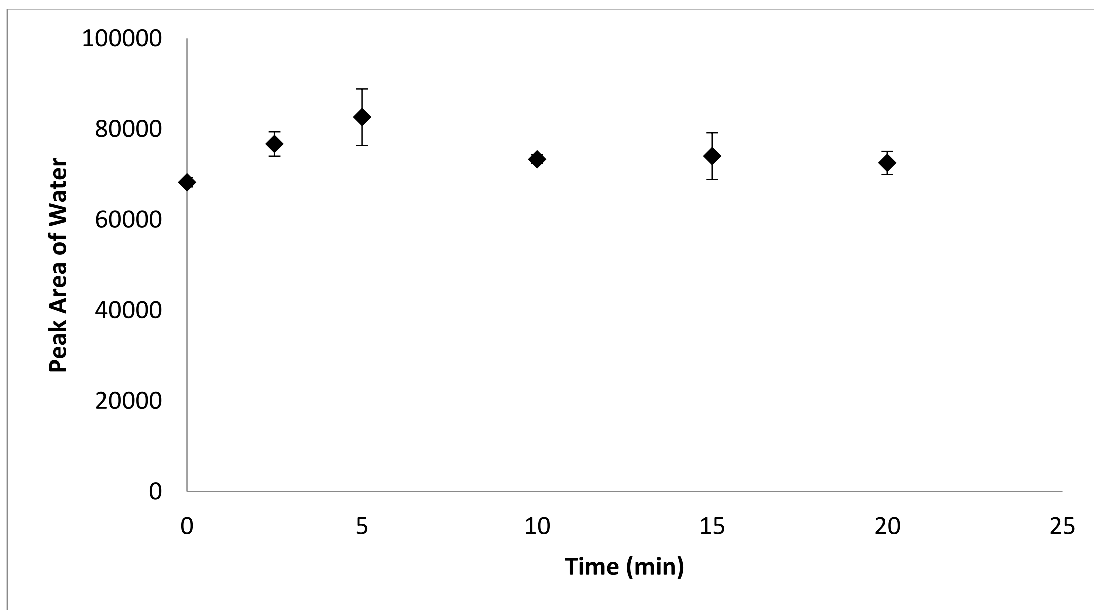


Figure 6-1 The equilibrium time when using the HS-20 autosampler

The amount of water in the headspace of sealed samples at 100 ºC was evaluated every 5 minutes for 20 minutes. It can be seen the maximum response is at 5 minutes and after that point the response plateaus

The equilibrium time was also optimized as seen in Figure 6-1. The sample required 5 minutes at 100 ^{0}C in order to reach equilibrium. Various sample purge conditions were evaluated using the HS-20 autosampler. For example, the vials were pressurized in a range of 25-200 kPa and the headspace was then removed for 6-120 seconds. It was found that a pressure of 200 kPa and then extracting 30 seconds of headspace was optimal and provided the lowest residual moisture in the vials. Two different sample loop sizes, 0.2 and 1.0 mL, were compared. When the larger sample loop was used with samples that contained high water amounts, the GC column become overloaded, and the peaks become asymmetrical as increased tailing was observed.

6.3.3 Quantitative Analysis of Residual Water in Samples

Two calibration curves for fructose were developed in order to quantify the water content of sixteen syrup samples (see Experimental).

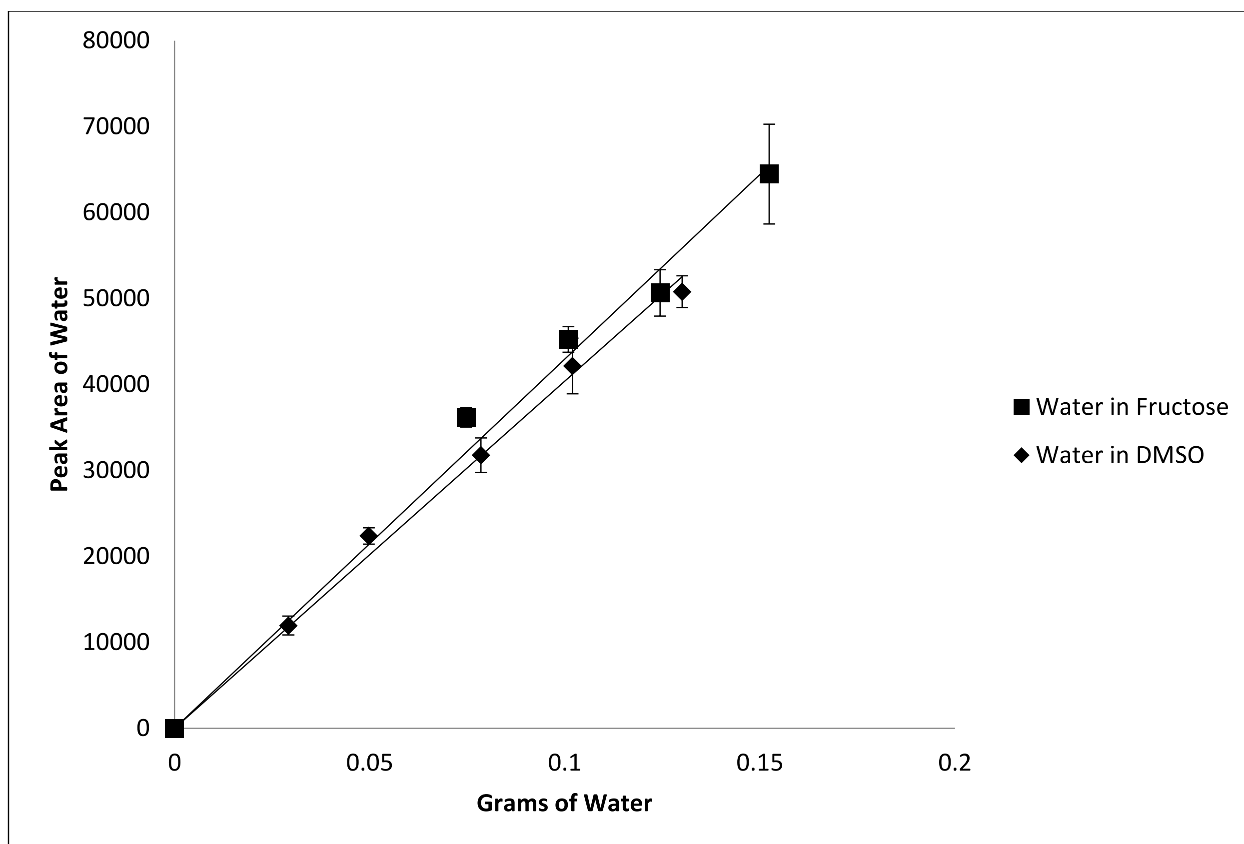


Figure 6-2 The calibration curve for water in fructose and water in DMSO using a TCD detector. The linear relationship between the TCD detector response and the water content in fructose-water solutions and DMSO-water solutions ware indicated, both had a correlation of 0.99. The equation for the line produced by the fructose-water solutions is y = 429,000x and the equation of the line produced by the DMSO-water solutions was 403,000x.

Figure 6-2, illustrates the linear relationship between the peak area and the percent water for different concentrations of water in fructose. The correlation (r^2) was found to be 0.99. A second calibration curve produced in DMSO was also found to have correlation of 0.99. The water in sixteen syrups and sweeteners was analyzed and the percent water therein is presented in Table 6-1 (they ranged from ~20-90%). The water content was also analyzed with loss on drying which gave comparable values but required much longer analysis times (Table 6-1) and by Karl Fischer titration (KFT).

Table 6-1: The table compares the water content, standard deviation and relative standard deviation (RSD) for sixteen syrup samples utilizing loss on drying, headspace gas chromatography (HSGC) and Karl Fischer titration (KFT).

Products [a]	Loss on drying		HSGC		KFT	
	Average	RSD	Average	RSD	Average	RSD
Mrs. Butterworth's Original Syrup	27.0 ± 0.1	0.4	28.7 ± 1.6	5.7	33.0 ± 0.7	2.1
Mrs. Butterworth's Sugar Free Syrup	85.5 ± 0.03	0.04	84.9 ± 2.7	3.2	91.9 ± 4.4	4.8
Sugar Free Butter Flavored Syrup	89.1 ± 0.1	0.1	85.7 ± 3.4	4.0	88.3 ± 0.8	0.9
Nesquik Chocolate Syrup	18.3 ± 1.6 [b]	8.5	29.0 ± 0.5	1.8	32.6 ± 1.2	3.7
Nesquik Strawberry Syrup	24.0 ± 0.2	1.0	28.1 ± 1.5	5.5	31.2 ± 0.6	2.1
Hershey's Chocolate Syrup	27.2 ± 1.3	4.8	25.5 ± 0.9	3.6	34.6 ± 1.0	2.9
Hershey's Carmel Topping	19.4 ± 1.6 [b]	8.3	29.0 ± 1.4	4.7	24.9 ± 0.3	1.3
Rose's Grenadine Syrup	40.5 ± 0.6	1.5	46.6 ± 2.0	4.2	48.6 ± 2.7	5.5
Smucker's Strawberry jelly	29.3 ± 0.5	1.7	30.1 ± 0.9	3.0	36.7 ± 0.7	2.0
Smucker's Strawberry jam	31.4 ± 0.3	0.9	30.4 ± 1.1	3.6	35.4 ± 0.8	2.3
Pure Maple Syrup	26.7 ± 1.7	6.4	27.5 ± 0.5	1.9	33.7 ± 0.3	1.0
Blue Agave Nectar	22.8 ± 1.4	6.1	23.6 ± 1.0	4.1	22.4 ± 1.0	4.3
French Vanilla Coffee Creamer	36.1 ± 1.8	5.0	33.6 ± 1.9	5.6	46.1 ± 1.9	4.1
Pancake syrup	26.9 ± 0.5	1.8	27.9 ± 1.5	5.3	31.6 ± 0.7	2.2
Molasses	24.4 ± 0.03	0.1	25.4 ± 0.5	2.0	22.1 ± 0.8	3.7
Corn Syrup	22.9 ± 1.4	6.1	22.4 ± 0.8	3.6	23.9 ± 0.8	3.4

[a] See Experimental section for sample details.
[b] After 7 days the mass had not stabilized, increase analysis temperature lead to degradation.

6.3.4 Precision

The precision of the HSGC method was evaluated by analyzing all of the samples in quadruplicate. The relative standard deviation (RSD) for loss on drying,

HSGC and KFT were similar in most cases. When the average precision of the HSGC method was compared to the average RSD produced by loss on drying, there were a few values with over 8% RSD for the latter approach (*i.e.,* Nesquik chocolate syrup and Hershey's caramel topping). It should be noted that while loss on drying usually produced similar RSDs, the procedure took 4-7 days to complete whereas the HSGC method took 10 minutes. As has been noted previously KFT often provides good precision while producing inaccurate results (16). This will be discussed in the following section.

6.3.5 Accuracy

The National Institute of Standards and Technology (NIST) does not currently provide standard reference materials for moisture in sugar solutions, therefore, accuracy of the HSGC method was estimated by comparing it to results obtained by two other methods, KFT and loss on drying. The three methods gave similar results however it was determined that KFT often appeared to overestimate the water content compared to the other two methods. When KFT and HSGC were compared with a T-test it was found that they were only similar in the case of 5 samples, and KFT was similar to 3 of the loss on drying samples. Whereas when loss on drying and HSGC were compared it was found that most of the samples were similar. When the French Vanilla Coffee Creamer was analyzed with HSGC and loss on drying, a similar result of ~30% water was found. In contrast KFT gave a significantly higher water content (46% water). On average, KFT produced ~5% higher water contents than the other two methods. It appears likely that the KFT reagent reacted with some of the nonaqueous components/constituents of many of the samples. Results with high bias have been

previously noted for KFT for samples that contain large amounts of sugar (24). Loss on drying usually had the lowest measured water content of the three methods, however it has been known to underestimate water when the viscosity of the sample increased substantially after heating which, in turn, decreases the diffusion rate of water. (25) This would also apply to a few of the samples (*i.e.,* Nesquik Chocolate Syrup and Hershey's Carmel Topping) which still had small decreases in mass after being heated for seven days. In addition, when the incubation temperature was further increased for a few hours it led to sample degradation. Since the mass did not completely stabilize it can be assumed that there was still some moisture present.

6.3.6 Instrumental Variations

The effect of different instrumentation can affect the GC conditions used as well as, peak shape, split ratios and resolution. This is particularly true when comparing new state of the art instruments with analogous types that are 10 or more years old. It was found that the older TCDs had poorer sensitivity and therefore require a lower split ratio, 5:1. As seen in Figure 6-3 the decrease in split ratio caused the water peak to loose symmetry via increased tailing. The lower split ratio also led to broader peaks and therefore a lower resolution between the air and water peaks. In addition when samples were analyzed with the older, less sensitive TCDs the analysis temperature had to be kept slightly lower, 150 ^oC versus 170 ^oC to allow for baseline separation between the water and air due to broadening of the peaks and increased tailing.

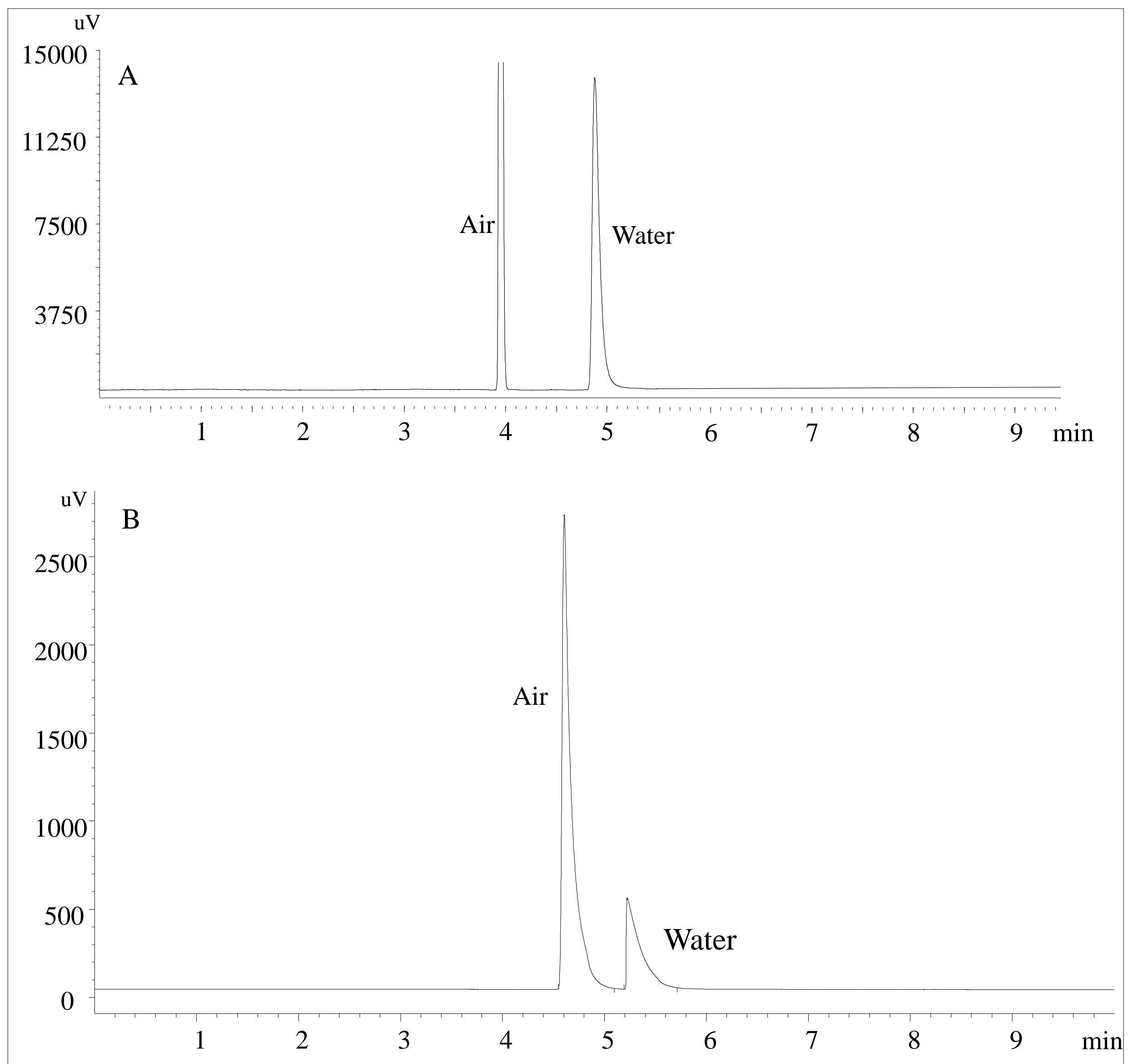


Figure 6-3 The chromatogram of using a new TCD detector compared to using a less sensitive old TCD detector

6-3A: A typical chromatogram for the analysis water in agave nectar when analyzed with the Shimadzu Tracera 2010 TCD at 170 °C with a split ratio of 100:1 on a 60 m Watercol™ 1910. 6-3B: A typical chromatogram produced by an older, less sensitive TCD at 150 °C a split ratio of 5:1 on a 60 m Watercol™ 1910.

6.4 Conclusions

The water content of 13 liquid sweeteners were determined using HSGC. This method was rapid, accurate and precise. It was shown to be broadly applicable to a variety of sugar and sugarless sweeteners. The method does not require long heating periods (4-7 days) in an oven as with the loss on drying method, thus, the water content can be rapidly determined in the syrups. KFT, while faster was shown to overestimate the water content in most of the samples. The ease, accuracy and robustness of the HSGC analyses, are greatly enhanced when using an ionic liquid-based column and a GC instrument that is specifically designed and configured for the analysis of water.

Chapter 7

DETERMINATION OF TRACE WATER CONTENT IN PETROLEUM AND PETROLEUM PRODUCTS

Abstract

The measurement of water in petroleum and petroleum based products is of industrial and economic importance, however, the varied and complex matrices make the analyses difficult. These samples tend to have low amounts of water and contain many compounds which react with iodine causing Karl Fischer titration (KFT) to give inaccurate, typically higher, results. A simple, rapid, automated headspace gas chromatography (HSGC) method which requires modified instrumentation and ionic liquid stationary phases is developed. The measurement of water in twelve petroleum products along with three NIST reference materials was performed with the developed method. The range of water found in these samples was ~12-3300 ppm. This approach appeared to be unaffected by complicated matrices. The solvent-free nature of the HSGC method also negates the solubility limitations which is common with KFT.

7.1 Introduction

The quantification of water is one of the most ubiquitous and recurrent of analytical measurements worldwide. It is required for the understanding of most natural systems, controlling manufacturing and synthetic processes, and is required by regulatory agencies for pharmaceutical and many consumer products.[10] More specifically, the measurement of water in petroleum and petroleum based products poses a variety of problems and complications. This stems from the varied and complex nature of crude oil itself as well as the plethora of different products produced from it.

Given the hydrophobic nature of these mixtures and the presence of interfering substances, very low levels of water are not uncommon and can be difficult to measure accurately. Also some petroleum based products have additives which support the presence of more moderate levels of water. The measurement of water is necessary for raw materials (various crude oils), intermediate refinery products and the wide variety of finished products. Water levels affect the economics of transport, storage, refining, formulating, safety, and the proper performance of end products.[10, 23, 25, 119-124] Because of the difficulty in obtaining accurate measurements in the variety of different hydrophobic and complex matrices, there is a need for accurate reproducible standards. Thus it is not surprising that the National Institute of Standards and Technology (NIST) has produced different reference materials to encompass different types of petroleum and petroleum products. Indeed, there has been some controversy and debate over the procedures used to produce some of the reference values even within this journal.[23, 20, 22, 24, 27–29]

In one case the certified water levels listed on the Certificate of Analysis differ by almost an order or magnitude depending on whether the NIST or American Society for Testing and Materials (ASTM) method was used.[23, 22, 125] This was believed to be due to the fact that significant amounts of interfering compounds to Karl Fisher titration reagent were present in this standard (light sour crude oil) and that additional experimentation was done to minimize these effects with the NIST method but not with the ASTM one.[23, 22, 125] That notwithstanding, the NIST value still has a deviation > ± 13%. In another NIST reference material (transformer oil) four different reference values are provided one of which is the consensus result from a 14 inter-laboratory study from which some

results were excluded.[23, 126] The consensus result (21.2 mg/kg ± 1.7 water) differed from the NIST result minus interferences (12.1 mg/kg ± 1.9 water) and from the NIST volumetric/ASTM method (34.5 mg/kg ± 2.2 water).[23, 126] The point here is not to cast dispersions on such standards which we believe are the best available and were produced with high integrity by the best available methods and in which the variations and their possible causes are clearly indicated.[125-127] Rather any problems are with the limited methodologies that are available to measure water in these (and other) samples.

The primary method used to determine water in petroleum and petroleum products is the Karl Fisher titration (KFT).[10, 20, 27, 120, 121, 128] KFT has a wide dynamic range but can be problematic for samples having very low amounts of water.[19, 20, 25, 27, 120-124] Under rigidly controlled conditions KFT can have good precision but the accuracy of problematic samples is open to question.[120] The main problems of KFT are well known as indicated in Table 7-1. In general, samples are often affected by at least one or more of the biases listed here (Table 7-1). Sometimes these can be mitigated by modifying the procedure.[20, 24] However, in the case of petroleum and its products, many/most of the possible bias/problems are operative. In addition to having low concentrations of water, sample insolubility, reactive impurities, and other matrix effects; there are inaccuracies due to the extensive sample handling procedures needed as well as other instrumental effects (Table 7-1).[10, 19, 20, 23-25, 120, 121, 128-131] Indeed the dominant KFT response in some crude oils is from other compounds in the sample.[22, 23] Thus different dual titrations are needed and used to subtract the “unwanted” from the “wanted” responses.[22, 23] Other KFT alternatives include removing the water from the sample (e.g., stripping oven KFT or by azeotropic distillation).[20, 24] In either case,

contamination by atmospheric moisture or the residual water in the azeotropic distillation solvent further complicate these procedures and greatly extend analysis time as well as hinder automation.[25, 26]

Table 7-1: Summary of the main biases associated with Karl Fischer Titration (KFT) and how they influence the ability of KFT to analyze samples.

Bias	*Influence from Bias*	*References*
Incomplete solubility of petroleum in KFT solvents	Negative	20, 121, 129-131
Interfering compounds and side reactions	Positive	10, 20, 23-25, 131
Matrix effects	Positive or negative	20, 129
Dilution effects caused by large sample sizes	Positive or negative	20
Selection of reagents, solvents, and additives	Positive or negative	10, 19, 24, 25, 129 - 131
Reagent instability	Positive	25
Sample handling (accuracy of syringes, introduction of sample to titration cell)	Positive or negative	10, 25
Design of the titration cell (1 or 2 cells)	Positive or negative	130
Permeability of the titration cell to moisture	positive	10
Residual moisture in the titration cell	positive	10, 25
Interaction of relative humidity with the sample	Positive	128
Accurate determination of the end point	Positive or negative	19, 130
Instrumental bias	Positive or negative	20, 25, 131
Instrumental settings (end point potential, drift limits, time delay)	Positive or negative	10, 24, 130
The magnitude of the background current	Positive or negative	10
Adsorption of compounds by the electrode	Negative	123
Calibration of the instrument with proper calibration standards	Positive or negative	19, 121, 130

A few alternatives to KFTs have been attempted for petroleum products, each having limited applicability and/or other analytical issues. For example, headspace GC (HSGC) has been attempted for the determination of water[27-31, 52, 132] even though most stationary phases are not compatible with long term exposure to water and/or provide poor peak shapes.[32] With direct injection, volatile compounds often interfere with the water peak while the nonvolatile compounds degrade in the injection port producing

unpredictable slowly eluting peaks and column deterioration.[27, 32, 33] In HSGC, as in all trace water methods, atmospheric moisture is a significant problem.[27] Indeed this has given rise to a variety of attempts to reduce residual moisture by a variety of heating and "dry gas" purging procedures even including placement of the entire GC apparatus and sample preparation area in a glove box.[27, 28, 34] What is rarely discussed is that the "dry" purge gasses as well as the GC carrier gases still may not be sufficiently dry to produce accurate and reproducible results for trace water analyses.[133]

There are at least four additional problems in the analysis of trace water in samples by GC. First it is well known that conventional columns produce poorly shaped asymmetric water peaks making their accurate integration difficult.[35, 46, 47, 49, 50] Even in more recent literature, packed columns which were developed decades earlier are still utilized.[48, 134] These columns do not strongly retain water, produce tailing and often require flow rates of ~20 mL/min.[48. 134] Second, in analyzing many petroleum products, even by headspace, there can be a variety of closely eluting and overlapping peaks. Third, conventional stationary phases tend to be degraded by repeated high temperature exposure to water.[50] Finally, while thermal conductivity detection (TCD) may be adequate for samples with higher water levels, a more sensitive and robust detector is needed for trace water detection.

In this work we report an automated system that is not only broadly applicable for most water analyses, but is particularly well suited for trace water determinations in petroleum and its plethora of products. The aforementioned problems and deficiencies with headspace GC have been eliminated or greatly minimized. As will be discussed, this was accomplished by using a combination of a specific catalytic gas drying

system,[35] new high polarity ionic liquid based GC columns,[34, 50-52, 56-58, 60, 61, 73, 89] a highly sensitive barrier discharge ionization detector [63, 64] and a new pressurized loop headspace injection system.[133] This technique is not affected by side reactions, other volatile constituents/contaminants and is significantly faster, more accurate and precise than other current methods.

7.2 Experimental

7.2.1 Apparatus and Conditions

A Tracera GC-2010 Plus (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a barrier ion discharge detector (BID), and a thermal conductivity detector (TCD). The Shimadzu HS-20 autosampler was used to purge the samples, heat the vials and inject the headspace in all analyses. Labsolutions software (version 5.82) was used for all integrations. The helium gas utilized for purging the vials and as a carrier gas was dried with a High Capacity Gas Purifier, the OMI Purifier from Supelco (Bellefonte, PA) and a Heated Helium Purifier from Valco Instruments Co. Inc. (Houston, TX). The samples were heated at 150 °C for 10 minutes then the vials were pressurized to 100 kPa for 1 minute. The 1 mL sample loop was filled with headspace for 2 minutes and an injection of 0.5 minutes was made. The analyses were performed on a 60 m x 0.25 mm ID x 0.2 um film coat thickness Watercol™ 1910 (1, 11-di(3-hydroxyethylmidazolium) 3,6,9-trioxaundecane trifluoromethanesulfonate) fused silica capillary column, a 30 m x 0.25 mm ID x 0.2 um film coat thickness Watercol™ 1460 (tri(tripropylphosphoniumhexanamido) trimethylamine trifluoromethanesulfonate) coated fuse silica capillary column or a 30 m x 0.25 mm ID x 0.2 um film coat thickness Watercol™ 1900 (1, 11 di(3-methylimidazolium) 3, 6, 9 trioxaundecane

trifluoromethanesulfonate) fused silica capillary column from Supelco (Bellefonte, PA). All of these ionic liquid stationary phases were originally synthesized in our laboratiory.[51] The oven temperature was held isothermally as indicated in Supplemental Information S-1. The carrier gas for all runs was helium at 1.5 mL/min (26.3 cm/sec) with a split ratio of 100:1. The detector was set to a temperature of 250 °C.

7.2.2 Materials

M-Pro7 was obtained from M-Pro7 Weapon Care Products (Phoenix, AZ). The Synthetic gun oil with PTFE lubrication was purchased from Birchwood Casey (Eden Prairie, MN). The Remington Moistureguard Rem Oil and Remington Rem Oil were obtained from Interstate Arms Corp (Billerica, MA). The Transmax Mercon V transmission fluid was obtained from Castrol (Lewiston, NY). Prestone power steering fluid was purchased from UCI-FRAM AutoBrands (Lake Forest, IL). The Coastal SAE 85V-140 API Service GL-5 gear oil was purchased from Petroleum Service Company (Coralville, IA). 3-in-One multi-purpose oil and WD-40 were obtained from WD-40 Company (San Diego, CA). The CLP gun oil was obtained from the Safariland Group (Jacksonville, FL). The conventional 10W-30 motor oil and 40:1 stroke engine oil were purchased from Autozone (Memphis, TN). The light sour crude oil (SRM 2721), heavy sweet crude oil (SRM 2722) and transformer oil (RM 8506a) were purchased from National Institute of Standards and Technology (Gaithersburg, MD, USA). The 1-hexyl-3-methylimidazolium tris(pentafluoroethyl) trifluorophosphate (HMIM FAP) was purchased from Merck KGaA (Darmstadt, Germany).The 22 X 75 mm screw-thread vials and the magnetic screw-thread covers for the autosampler were purchased from Restek (Bellefonte, PA, USA). The 30 m x 0.32 mm x 0.25 µm HP-1 capillary column

was purchased from Agilent Technology (Santa Clara, CA, USA). The 30 m x 0.2 mm x 0.25 μm SP-2331, Supelcowax 10, Watercol™ 1460, Watercol™ 1900, and Watercol™ 1910, along with the 60 m x 0.2 mm x 0.25 μm Watercol™ 1910 capillary columns were obtained from Supelco (Bellefonte, PA, USA) or as previously synthesized.[51]

7.2.3 Sample Preparations

First the samples were evaluated by adding 3 μL of water to 2 g of sample and were divided into two groups, those which could dissolve water and those which were unable to dissolve water. When water could be dissolved into the sample, standard addition was performed by adding 0.4, 1.2, 1.6 and 2.4 μL of water to 2 g of sample. The samples were mixed and divided into four 0.5 g aliquots. The cover was immediately placed on the vial. The headspace was purged utilizing the Shimadzu HS-20 autosampler. The autosampler was kept at room temperature (22 °C) and the vial was pressurized to 200 kPa for 1 minute. After the vial was pressurized the headspace was extracted/loaded into the sample loop for 0.5 minutes. The sample was heated and analyzed. When the samples were not able to dissolve water, an external calibration curve was produced by adding 0.375, 1, 2, 3, and 4 μL of water to 2.5 g of HMIM FAP. Then the solutions were divided into five 500 mg aliquots. The vials were covered, purged, and analyzed in the same manner as the samples. Samples are prepared by adding 500 mg of sample to an empty 10 mL vials. The samples were purged with dry helium, heated and finally analyzed. In order to perform standard addition in hydrophobic samples, 120 mg of dioctyl sulfosuccinate sodium salt was dissolved into 12.5 g of water and 0 mg, 0.4 mg, 1.0 mg, 1.5 mg, 2.0 mg were added to 2 g of sample.

After the samples are mixed, 500 mg of the sample were added to a clean 10 mL vials and the vials were analyzed.

7.2.4 Minimizing Background Noise

Purging vials to remove residual water is important since the petroleum samples all contain under 3300 ppm water with one sample having only ~12 ppm water. The ability of the recently developed autosampler (HS-20, see Supplemental Information 2) to pressurize the covered vial by adding dry helium thus diluting the residual moisture in the headspace of the empty vials and then removing the pressurized gas thereby decreasing the residual moisture in the vials is essential. The capability of the autosampler to purge the empty vials was analyzed for a range of pressures and times, 25-200 kPa and 1-2 minutes respectively. In addition, the length of extraction or load time was analyzed for 0.1-2 minutes. It was found that a pressure of 200 kPa for 1 minute followed by an extraction of the vial for 2 minutes reduced residual water to the lowest level (see Table 7-2). Method 8 was equivalent within experimental error to method 10, however it took half the time. Therefore Method 8 (Table 7-2) was used in the analysis of all petroleum products.

Table 7-2: Ten different conditions were evaluated for purging atmospheric moisture from empty vials utilizing the HS-20 autosampler.

Purge Method	Pressure (kPa)	Time to Pressurize (min)	Load Time (min)	µg water/ mL
1	100	1	1	14.1 ± 0.7
2	50	1	1	17.1 ± 0.2
3	25	1	1	17.9 ± 0.1
4	100	1	0.5	15.8 ± 0.5
5	100	1	0.1	14.9 ± 2.3
6	50	1	0.5	19.0 ± 0.3
7	200	1	1	13.0 ± 0.2

8	200	1	0.5	7.7 ± 0.01
9	200	2	1	13.3 ± 0.3
10	200	1	2	6.5 ± 0.2
Air	NA	NA	NA	43.8 ± 0.3

7.3 Results and Discussion

7.3.1 Headspace Gas Chromatographic Conditions

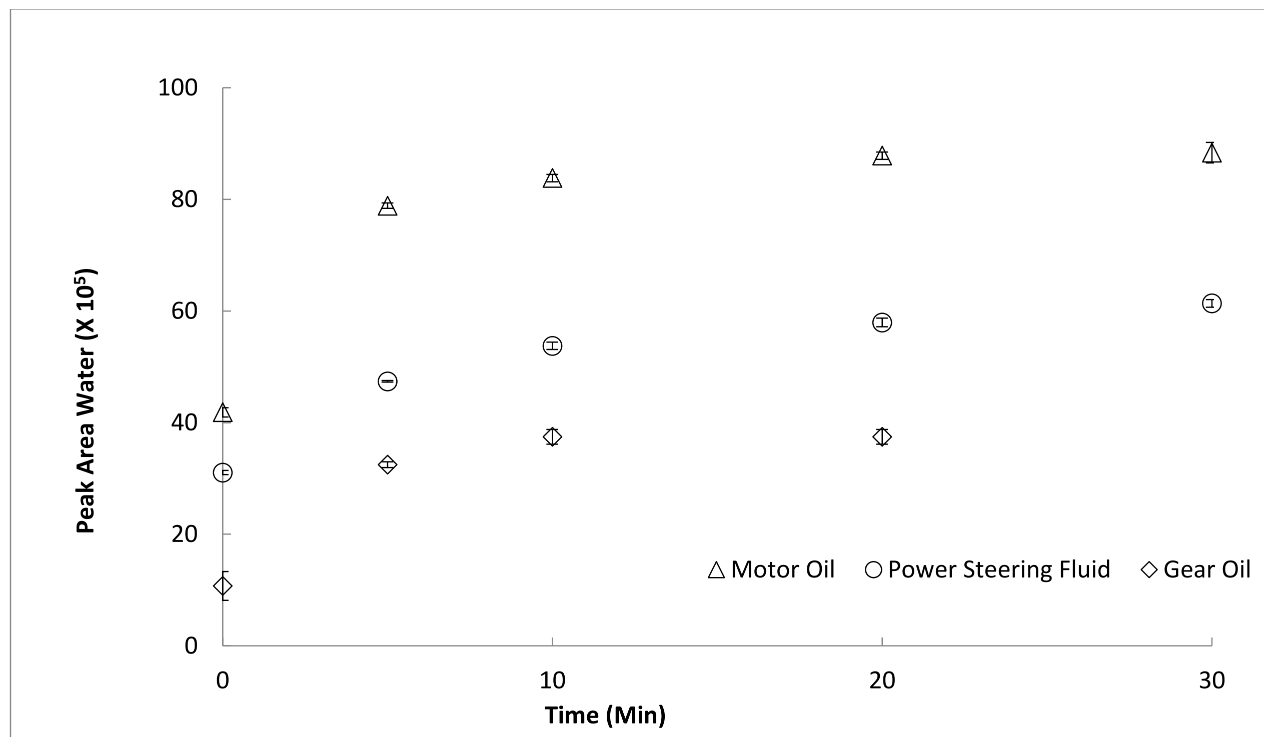


Figure 7-1: The evaluation of equilibrium time for petroleum products

The equilibrium time was evaluated by heating motor oil, power steering fluid, and gear oil (see Experimental) at 125 °C for increasing amounts of time, up to 30 minutes and measuring the water content present in the headspace.

The optimal HSGC conditions (*i.e.* equilibrium time, equilibrium temperature) were determined for three different viscosity petroleum product samples (see Figure 7-1 and Experimental). The signal response from a change in equilibrium time leveled after 10 minutes and this equilibration time was used in all subsequent experiments. The

optimal equilibrium temperature was found to be 150 °C. This was the temperature that provided the maximum response for water uncontaminated with other matrix components (see Figure 7-2). Obviously the presence of any overlapping interfering compounds impeded proper quantification of the water peak.

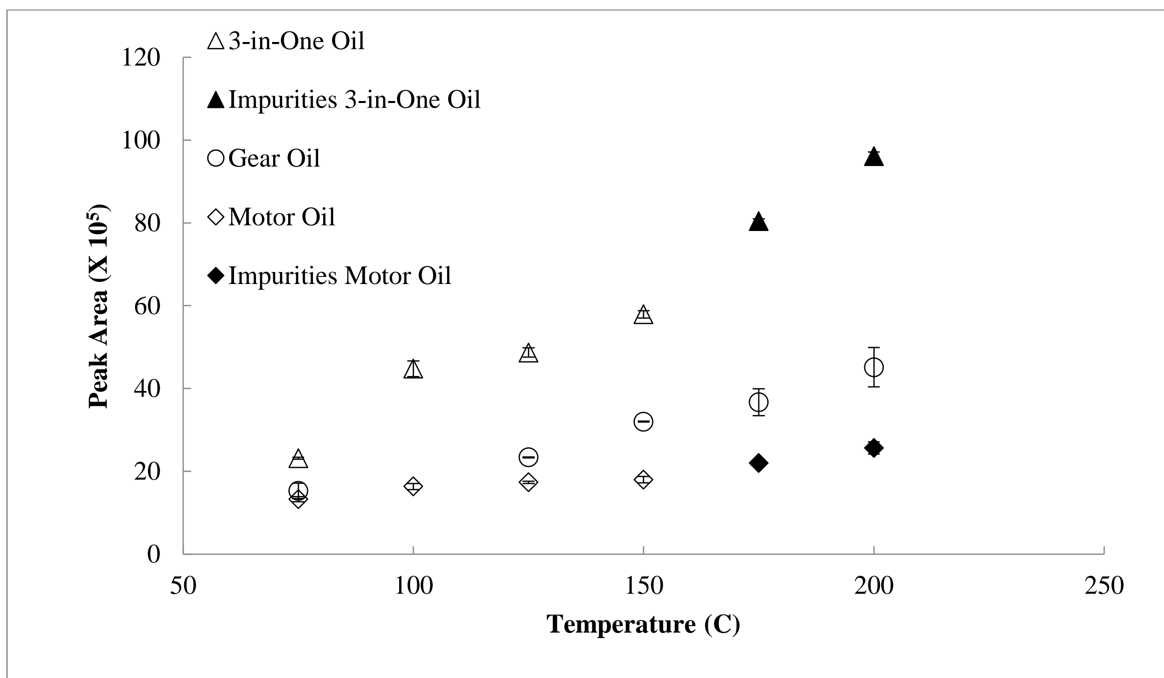


Figure 7-2: The effect of equilibrium temperature on petroleum samples.

The effect of equilibrium temperature on a sample of 3-in-One oil, motor oil, and gear oil (see Experimental) utilizing HSGC-BID. In the 3-in-One oil and motor oil, after 150 °C, other less volatile compounds were volatilized and coeluted with the water peak.

7.3.2 Evaluation of Stationary Phases

The evaluation of six GC stationary phases (see Experimental and in Supplemental Information 1), was done to discern stability, reproducibility and the ability to separate water from the other volatile components in petroleum and petroleum products. In addition, the sharpness, the width and the symmetry of the water peak were assessed. As shown in the Supplemental Information S-3A and S-3B that water

was not sufficiently retained at the analysis temperature on nonpolar stationary phases and would coelute with the air peak. More polar columns were able to retain the water, however most were unable to separate the water from the other various volatile petroleum-based matrix components. Also many columns tend to produce poor water peak shapes (see Supplemental Information S-3).

The three ionic liquid columns were analyzed in greater detail since they were optimal for the analysis of water in these matrices. The Watercol™ 1460 was found to have the most tailing of the three columns however significantly less than other polar stationary phases (see Appendix 1 Figure 7-1).

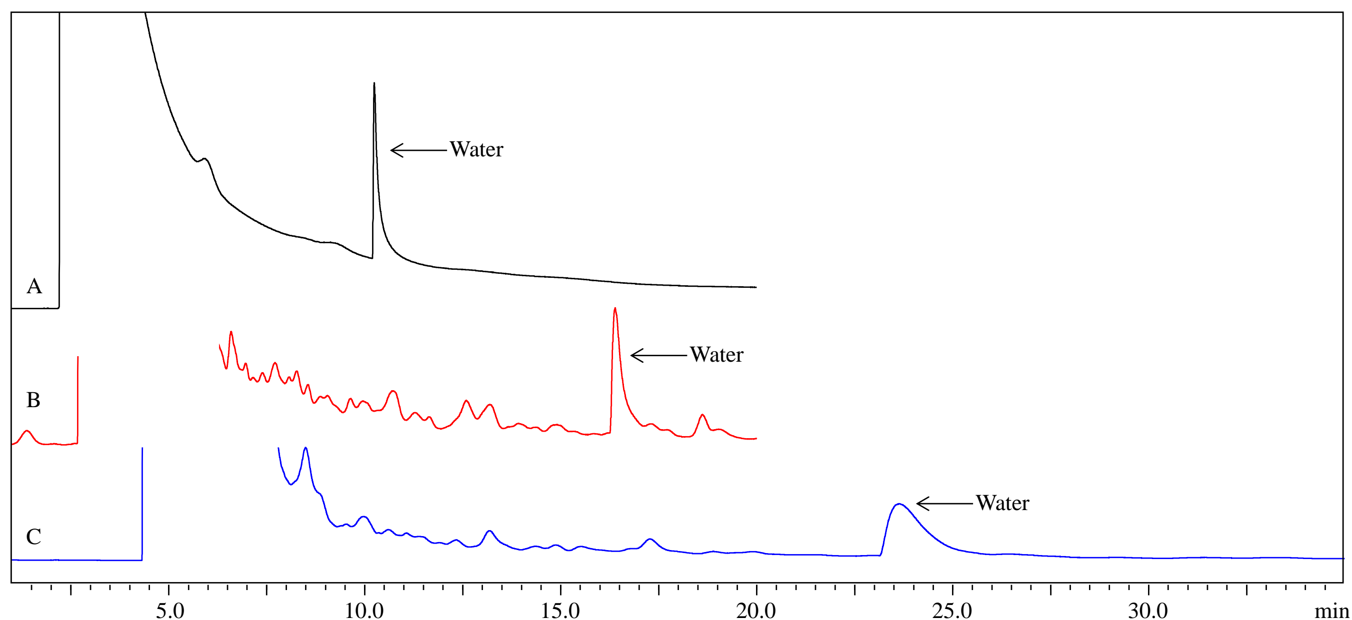


Figure 7-3: The analysis of heavy sweet crude oil (SRM 2722) on three ionic liquid stationary phases

The analysis of heavy sweet crude oil (SRM 2722) (see Experimental) on the Watercol 1460 at 70 ºC, Watercol 1900 at 50 ºC and the Watercol 1910 at 60 ºC are shown in 7-3A, 7-3B and 7-3C respectively.

The lower polarity of the Watercol™ 1460 results in a smaller separation window between water and the other volatile compounds in the oil samples. The other petroleum-based volatiles elute later and in a broader peak envelope with increased

tailing which can interfere with the water peak as illustrated in heavy sweet crude oil (SRM 2722) and WD-40, Figure 7-3A and Supplemental Information S-12 respectively. At higher analysis temperatures the Watercol^{TM} 1910 produced symmetrical peaks however when the column temperature was decreased below 100 °C the peak broadens and loses some symmetry. To allow for higher analysis temperatures the length of the Watercol^{TM} 1910 was increased from 30m to 60m. The Watercol^{TM} 1910 produced the longest retention times of the three columns however this was expected since it is twice the length of the other two columns. The longer retention leads to some broadening of the water peaks, but peak symmetry was sufficient. The Watercol^{TM} 1900 produced narrower water peaks with shorter retention times than the Watercol^{TM} 1910, and the peak shape was improved when compared to the Watercol^{TM} 1460. As will be discussed, there is a benefit to having a choice of three somewhat different ionic liquid columns for water analysis, in that some samples are easier to analyze on one column vs another (*vidae infra*).

The three ionic liquid based columns were found in some cases to produces similar optimized retention times and comparable peak shapes as seen with CLP oil in Figure 7-4 and with motor oil in Supplemental Information S-4. In many of the petroleum and petroleum based products any of the three columns could be used to study the water content, however one may be superior to the others for specific samples.

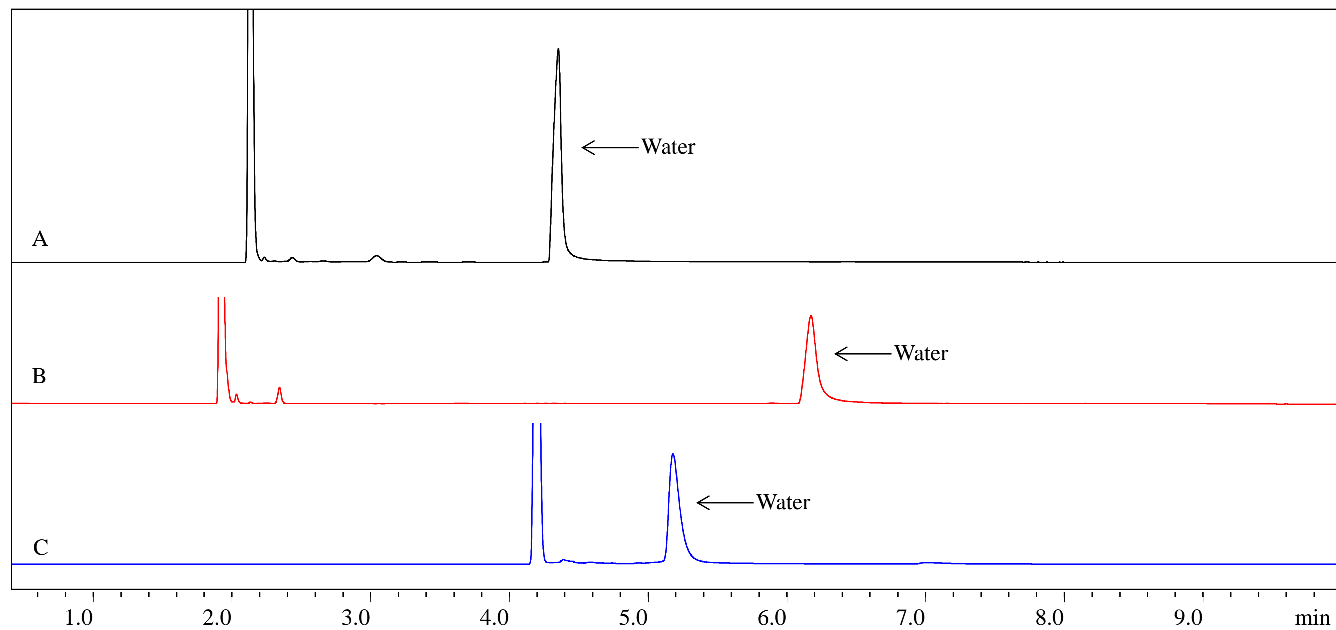


Figure 7-4: The chromatograms of CLP oil on the Watercol series of stationary phases

7-4A shows the analysis of CLP oil (see Experimental) on the Watercol™ 1460 at 70 ºC. 7-4B shows the chromatogram of Remington oil on the Watercol™ 1900 at 70 ºC. The final chromatogram in 7-4C is the examination of Remington oil at 90 ºC on the Watercol™ 1910.

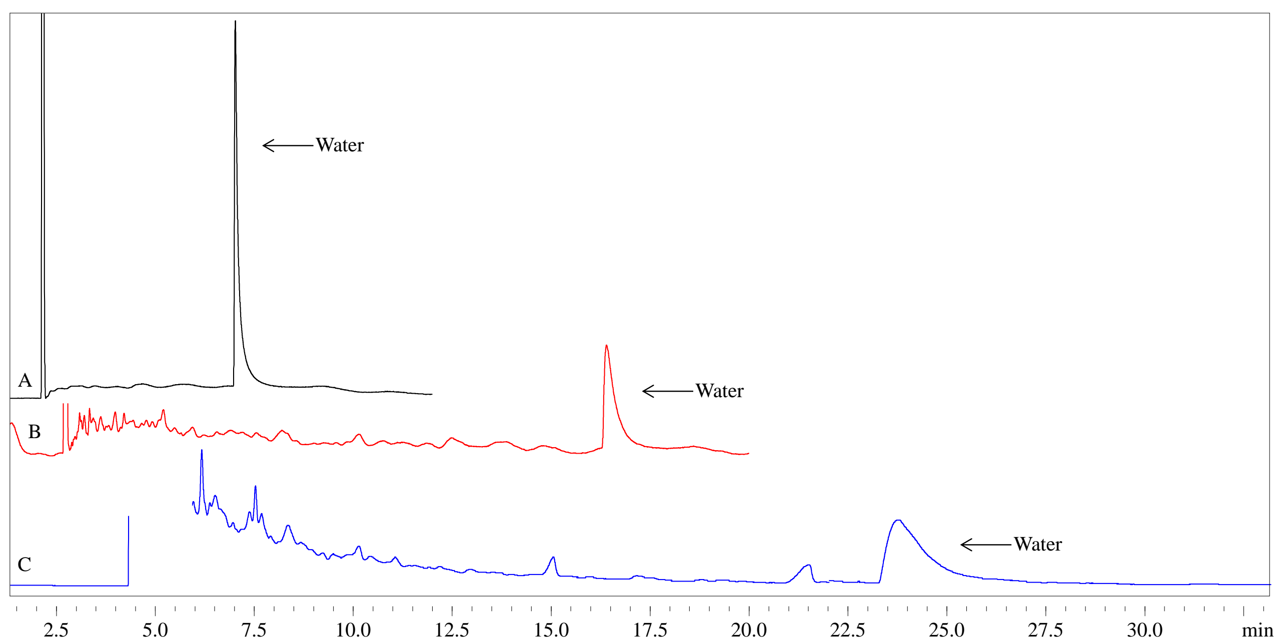


Figure 7-5: The chromatograms for the analysis of transformer oil (RM 8506a) (see Experimental) at 50 ºC on the WatercolTM 1460, WatercolTM 1900, and WatercolTM 1910 in 7-5A, 7-5B, and 7-5C respectively.

When transformer oil (RM 8506a), Figure 7-5, was analyzed with the three ionic liquid columns all three were able to separate water from the other components in the headspace, however the WatercolTM 1460 took only 7.5 minutes to complete the analysis whereas the WatercolTM 1900 and WatercolTM 1910 took 17.5 and 25 minutes respectively. The WatercolTM 1460 is a less polar tricationic based stationary phase whereas the WatercolTM 1900 and 1910 are more polar germinal dicationic based stationary phases (see Experimental). Consequently, some of the volatile sample matrix components interact differently. The most notable example, engine oil, is shown in Figure 7-6.

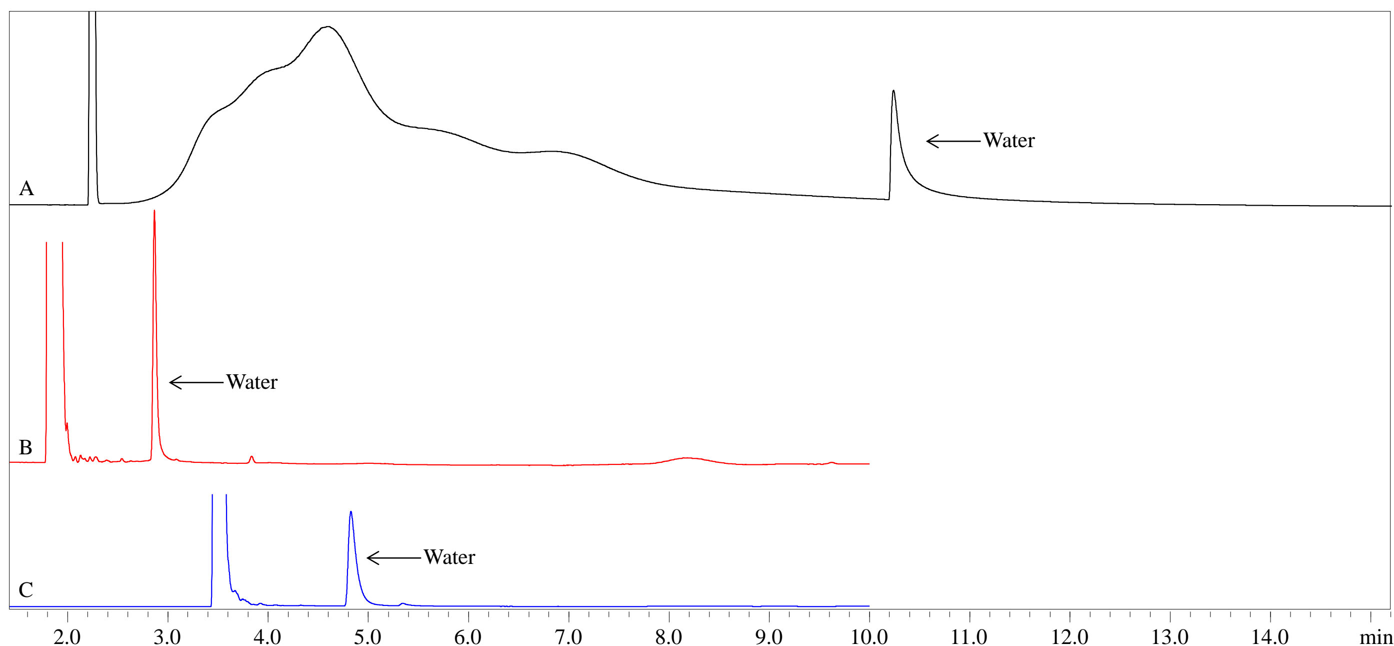


Figure 7-6: The analysis of engine oil on three stationary phases

7-6A shows the analysis of engine oilI (see Experimental) on the Watercol™ 1460 at 40 ºC. 7-6B shows the chromatogram of engine oilIon the Watercol™ 1900 at 110 ºC. The final chromatogram in 7-6C is the examination engine oilIat 130 ºC on the Watercol™ 1910.

When the headspace was analyzed with the Watercol™ 1900 and 1910 a distinctive and separated water peak was observed whereas the Watercol™ 1460 showed multiple large overlapping matrix peaks between the air and water peaks. Some petroleum samples were adequately analyzed with only one or two of the three columns. The water in the two NIST crude oil samples was only able to be quantified when the 60m Watercol™ 1910 was employed at lower temperatures (Figure 7-3 and Supplemental Figure S-13). When the other two ionic liquid columns were used, the water peaks overlapped with coeluting matrix peaks or was in the tail of the matrix components making quantification difficult or resulting in the overestimation of the water concentration.

7.3.3 Evaluation of Detectors

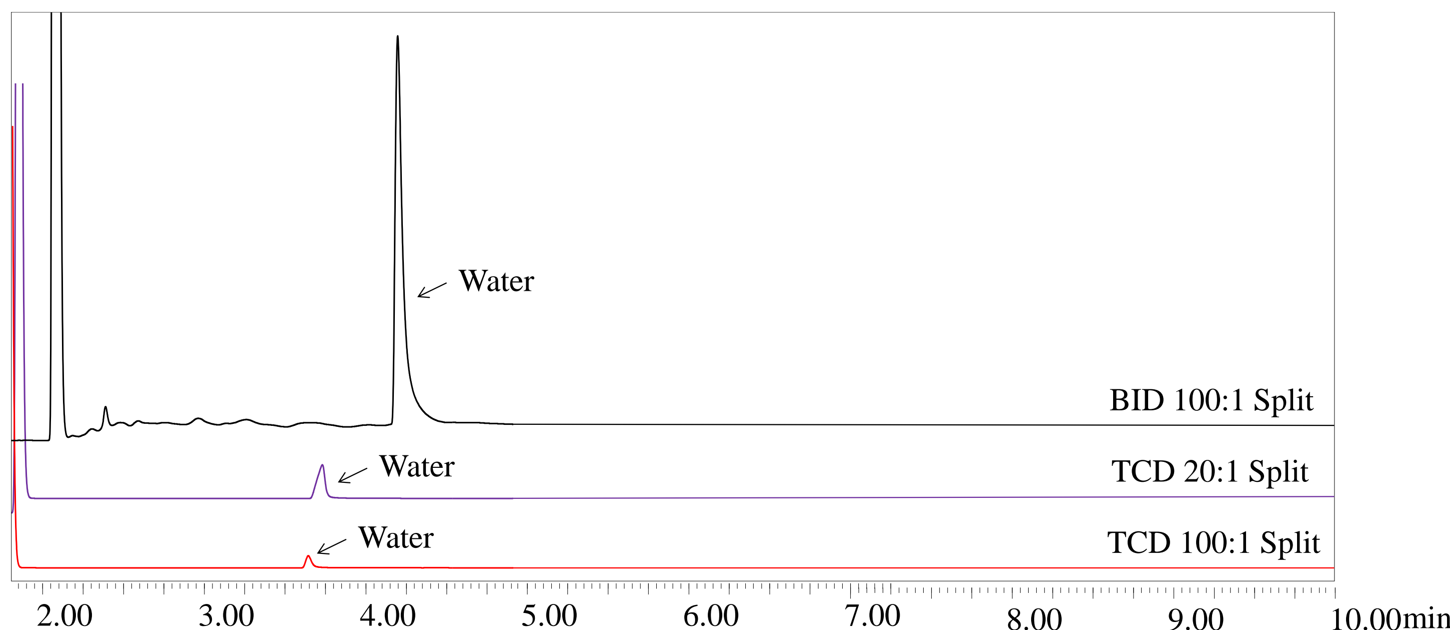


Figure 7-7: The chromatogram of trace water analyzed with a BID and TCD detectors

Illustrates the chromatograms for the analysis of transformer oil on the Watercol™ 1460 at 70ºC. Chromatogram A the sample uses a split ratio of 100:1anayized with the BID, the water peak is larger and sharper due to the high sensitivity of the detector. For chromatograms B and C the detector utilized is the TCD, in chromatogram B the split ratio is 20:1 where as a higher split ratio, 100:1 is used.

Two detectors were compared, the thermal conductivity detector (TCD) and the barrier discharge ionization detector (BID). Figure 7-7 provides chromatograms produced from the headspace analysis of transformer oil utilizing the BID and the TCD. When the TCD is used at the same split ratio as the BID, 100:1, the water peak is very small and the difference between the sample and the blank is not sufficient for accurate determinations. A lower split ratio of 20:1 required to measure water in the petroleum sample utilizing a TCD caused the water to oversaturate the column and the peak produced becomes quite unsymmetrical (Figure 7-7). It can be seen that the high sensitivity of the BID detector allows for a higher split ratio and much improved peak shape.

7.3.3 Quantification of Petroleum Based Samples

The quantification of water in fifteen diverse petroleum-based samples, ranging from crude oils to final products, was determined by utilization of an external calibration curve and/or standard addition (see Experimental and Table 7-3). An ionic liquid calibration curve with an equation of $\mathbf{Y} = 9.764\times10^{9}\mathbf{X}$ and a correlation (r^2) between the peak area and water content equal to 0.996 was utilized to quantify the water in 12 of the 15 samples (see Experimental). The absolute amount of water measured in twelve samples ranged from 6.1 pg – 1630 pg of water which correlates to samples having a concentration of water of 12 ppm – 3258 ppm (see Table 7-3). In addition it was determined that the calibration curve produced with the HMIM FAP ionic liquid did not

adequately simulate the matrix of the three samples which could dissolve water (*i.e.* transmission fluid, power steering fluid, and M-Pro7 LPX gun oil) and therefore this approach could not be utilized. These three samples were analyzed with standard addition and the water content was found to have a range of 245-1633 ppm of water (Table 7-3).

Table 7-3: Detection of water in 15 compounds using HSGC with the BID for detection

Product [a]	Analysis Temperature (ºC)			HSGC	Standard Addition
	Watercol™ 1460	Watercol™ 1910	Watercol™ 1900	ppm water	ppm water
Motor Oil	70	70	110	770 ± 2.9	770 ± 1.1 [c]
Transmission fluid	110	90	90	[b]	642 ± 0.4
Engine Oil	40	110	130	261 ± 5.8	269 ± 1.7 [c]
Gear Oil	70	90	130	207 ± 6.9	[d]
Power Steering Fluid	150	150	90	[b]	160 ± 2.4
3 in One Oil	50	90	90	445 ± 21.0	[d]
M-Pro7 LPX Gun Oil	150	100	90	[b]	1540 ± 1.8
CLP Gun Oil	70	70	90	3260 ± 87.4	[d]
Synthetic Gun Oil	40	40	50	234 ± 3.4	[d]
Remington Moistureguard Rem Oil	70	70	90	330 ± 8.1	302 ± 0.9 [c]
Remington Rem Oil	70	70	90	116 ± 4.5	102 ± 5.8 [c]
WD-40	40	70	90	728 ± 5.0	[d]
Transformer Oil NIST (RM 8506a)	50	50	50	12 ± 0.8	[d]
Light Sour Crude Oil NIST (SRM 2721)	50	50	50	146 ± 7.6	[d]
Heavy Sweet Crude Oil NIST (SRM 2722)	70	50	60	102 ± 1.7	[d]

[a] See Experimental section for a complete description of all commercial products, and procedures
[b] See Results and Discussion
[c] Standard addition with dioctyl sulfosuccinate sodium salt dissolved in water (see Experimental)
[d] Standard addition is not feasible due to the samples high viscosity, complexity of and/or immiscibility with the added water standard

7.3.5 Precision

The method's precision was determined by analyzing samples in quadruplicate and determining standard deviation and relative standard deviation (RSD) (Table 7-3). It was found that the average of all RSDs was 2.9 %. The RSD was found to be under 5% in all cases but one. The transformer oil was found to contain 12 ppm of water and had a standard deviation of 0.8 ppm. This sample, by far, had the lowest concentration of water. The RSD for two NIST standard reference materials, SRM 2721, and SRM 2722 were compared to the RSD determined by NIST. It was found that when HSGC was utilized, the RSDs were 5.1% for SRM 2721 and 1.7% for SRM 2722. When NIST evaluated SRM 2721 and SRM 2722 utilizing KFT, the reported RSDs were 13.4% and 6.1% respectively. [125-127]

7.3.6 Accuracy

The accuracy of the present method was compared to three NIST standard reference materials (RM 8506a, SRM 2721, and SRM 2722) as seen in Tables 7-4 and 7-5. In the case of all three NIST standards the values determined in the current study with HSGC were equivalent to the "NIST minus interferences coulometric method". The HSGC method developed measured 101.6 ± 1.7 ppm water in heavy sweet crude oil (SRM 2722) which was comparable to the ASTM method and the NIST method, as seen in Table 7-4.

Table 7-4: Summary of results of water in light sour crude oil, NIST SRM 2721[125] and heavy sweet crude oil, NIST SRM 2722[127] utilizing HSGC and KFT.

NIST Sample	This work	NIST	ASTM- Method

Light sour crude oil (SRM 2721)	146 ± 7.6	134 ± 17.6	941 ± 16.1
Heavy sweet crude oil (SRM 2722)	101 ± 1.7	99 ± 5.8	104 ± 5.8

Table 7-4 also indicates that the ionic liquid HSGC provides the same values as the corrected KFT method for SRM2721. When direct coulometric KFT is utilized to analyze the RM 8506a there an slight over estimation of water content due to the presence of some interfering compounds (Table 7-5).[23] The method developed by NIST which utilizes two titrations or indirect analysis of water with azeotropic distillation or stripping KFT, produced lower water values of 13-15 ppm water which is comparable to the values obtained in this work.

Table 7-5: Summary of water content in Transformer oil, NIST standard RM 8506a, utilizing different techniques.

Publication	Method	Water Content
This work	HSGC	12.1 ± 0.8
Cedergren & Nordmark[129]	Direct coulometric KFT	23.1 ± 0.6
	Stripping KFT	14.1 ± 0.1
Jalbert *et al.*[20]	Direct coulometric KFT	13.0 ± 0.8
	Azeotropic Distillation KFT	14.8 ± 0.6
	HSGC	13.0 ± 0.4
Margolis[130]	Volumetric KFT	34.5 ± 2.2
Margolis Hagwood[23]	ASTM	21.1 ± 1.9
NIST[49]	NIST coulometric mass concentration of water, ASTM method minus interferences	12.1 ± 1.9
	NIST coulometric mass concentration of water, ASTM method	18.3 ± 1.9

Standard addition was also employed to determine the water in 7 samples. In most cases, water was insoluble in the sample and therefore the direct addition of water

was not possible. In these cases dioctyl sulfosuccinate sodium salt, a surfactant, was dissolved in water and the standard solution was then added to the petroleum samples (see Experimental). It was found that standard addition with the water and dioctyl sulfosuccinate sodium salt solution produced similar values to those determined using the external calibration curve for samples which could not dissolve water, as seen in Table 7-3.

7.4 Conclusions

A rapid, accurate, easy and fully automated method for the determination and quantification water in petroleum and petroleum-based products was developed. Three drying apparatus were employed to decrease the residual moisture and allow for trace water to be analyzed. The sensitivity of the BID was beneficial for the analysis of trace moisture. This method has minimum sample preparation and high throughput. The 60 m Watercol^{TM} 1910 column, the 30 m Watercol^{TM} 1460 column and the Watercol^{TM} 1900 were able to effectively separate water from other volatile components in petroleum and petroleum based products. The other volatile compounds found in various petroleum products determined which of the columns was best or if they could all be used. This method wasn't affected by side reactions as are some other methods for determining water. Either of two developed approaches could be used to analyze the water content, *i.e.* standard addition or use of an ionic liquid external calibration curve. The developed HSGC method was found to have better accuracy and precision than the KFT based and ASTM methods for the evaluation of water in crude oil.

7.5 Acknowledgments

This work was supported by the Robert A. Welch Foundation (Y0026). In addition we would like to thank Shimadzu Scientific Instruments for the use of the Tracera GC-2010 Plus.

Chapter 8 General Summary

Part one (Chapters 2-4)

A headspace method was developed for the measurement of water in twenty two active pharmaceutical ingredients (APIs), twelve solid finalized drug products (APIs and excipients), and ten liquid medicines and exiles. Solid samples were dissolved in 1-ethyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate (EMIM FAP) and the liquid samples were diluted in N, N-dimethylacetamide (DMA). The water content in the solid samples was determined using both a thermal conductivity detector (TCD) and barrier discharge ionization detector (BID) detectors. The higher water content in liquid samples was only able to be measured with the TCD. The low concentrations of ethanol along with the low sensitively of the TCD made quantification of ethanol difficult. However when the highly sensitive BID was employed the ethanol in six samples was determined.

Loss on drying and Karl Fischer titration (KFT) were compared to the developed headspace method. It was found that loss on drying was not applicable for liquid medicines and about half of the APIs. However, when it could be utilized, the values produced were comparable to the headspace gas chromatography (HSGC) method. Loss on drying was found to have lower precision and throughput. KFT was found to produce comparable responses and precisions to HSGC when studying the liquid medications and samples with high water content. However, this was not the case when analyzing solid drug products. KFT underestimated the water content in half the samples and overestimated the water content in a quarter of the samples. It was found

that the HSGC approach required far less time, had the highest precision and was not limited by the type of sample which was analyzed.

Part two (Chapters 5-6)

The water content in honeys, sugar based sweeteners and sugar free sweeteners was determined. The higher water content samples (>40% water) were dissolved in dimethyl sulfoxide and then analyzed using HSGC. It was found that the honey samples, which contained lower amounts of water (~15% water) were able to be analyzed using either the TCD or BID detector. It was that the sensitive BID not only detected the water but also a few other volatile components which were not able to be detected with the less sensitive TCD. The water content of samples which had higher water amounts, especially the sugar free sweeteners, were most effectively evaluated with the TCD. The HSGC procedure could be fully automated and had limited sample preparation and was able to analyze the water content in the range of 15-90% water.

Loss on drying, KFT, and HSGC were compared for a variety of foods. It was found that loss on drying only worked when the samples were highly processed and did not form highly viscous solutions when heated. In addition, it was found to have very low throughput, as samples took days to be evaluated. KFT was found to have high precision and moderate accuracy, however the high sugar content of the samples lead to a modest overestimation of the water content. The use of a classic refractive index measurement was able to be used to accurately and precisely measure the water content in a few of the samples, however it becomes less accurate with more complex samples. The HSGC method was determined to have comparable precision and

accuracy to refractive index and KFT. In addition, the HSGC method had a high throughput.

Part 3 (Chapter 7)

The measurement of water in petrochemical products is important; however, due to the complexity of the matrix, current methods for measuring water, *e.g.,* KFT, are not ideal. In this chapter a HSGC method was designed to measure 10-3500 ppm water in hydrophobic matrices.

Separations between water and the other volatile petroleum components were developed on three ionic liquid stationary phases. It was found that in most cases any of the three stationary phases were able to separate the water from the other compounds; however, the analysis time and peak shape could vary. When “3 in one oil” was studied, all three columns were able to separate the water from the envelope of less polar volatile components that eluted in a range of 7 to 11 minutes. However, the Watercol 1900 produced the sharpest water peak and the shortest analysis time. In other cases one type of ionic liquid column was superior to the other two. For example, the Watercol 1460 column method has an analysis time <4 minutes for transmission fluid compared to 11 minutes when using the Watercol 1910.

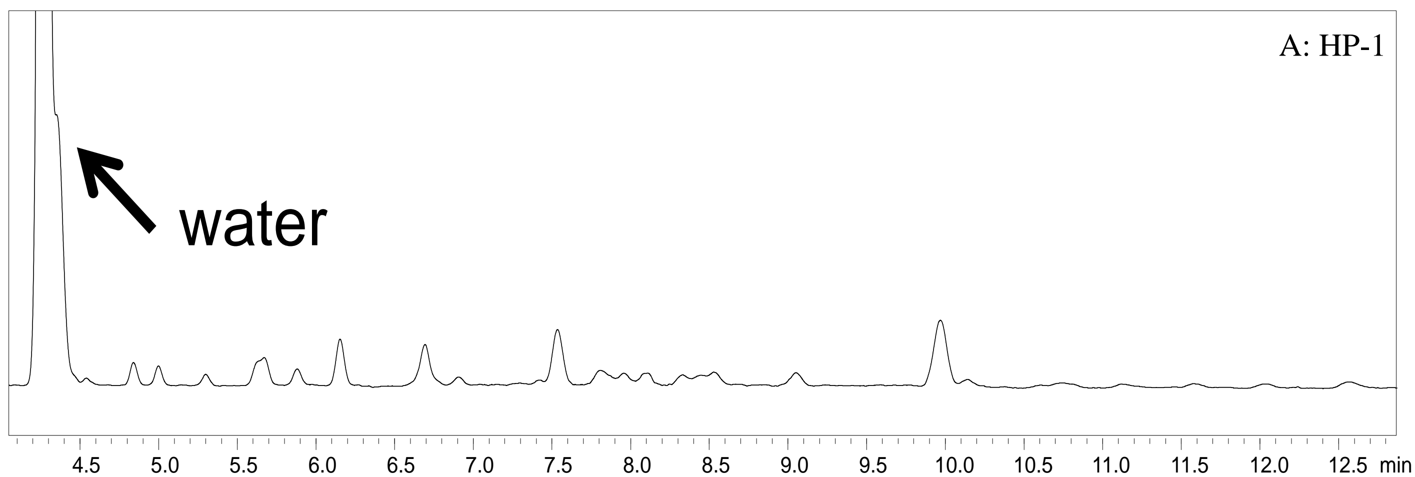
The HSGC method was validated by performing standard addition. In addition, three National Institute of Standards and Technology (NIST) standards were evaluated. The water content in the two crude oils (light sour crude oil and heavy sweet crude oil) were found to be comparable to the reported NIST values. The American Society for Testing and Materials (ASTM) values were found to be high in all cases since the KFT method used by ASTM did not take into account the matrix interferences. In addition to

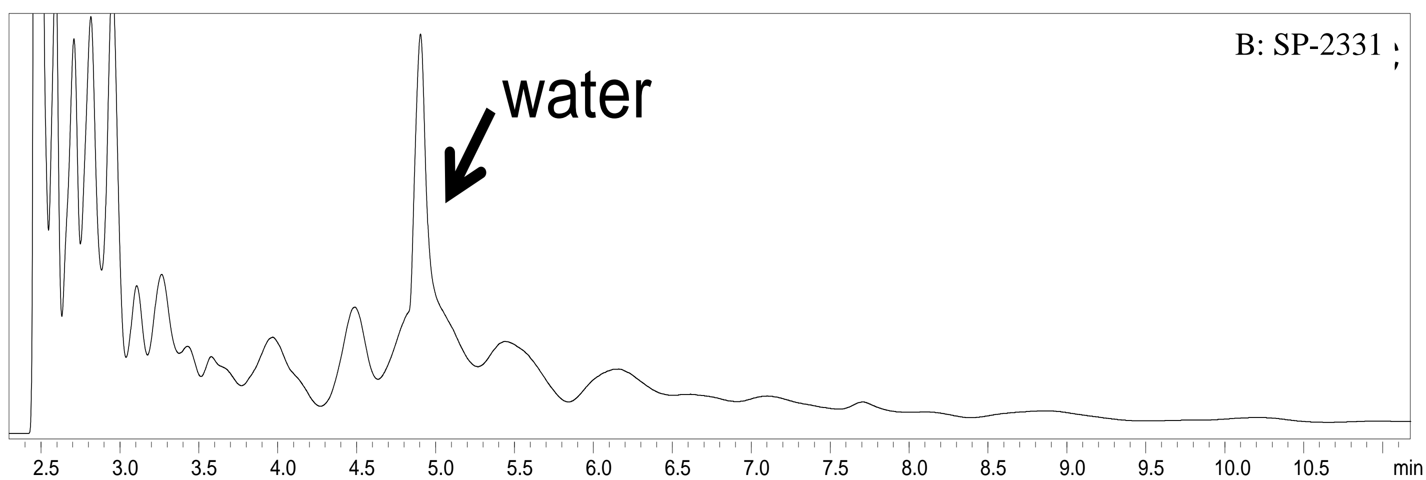
the HSGC method being accurate, it was found that the precision for these complex samples also was improved. The deviation with HSGC technique developed and reported herein was a third to half the deviation of the NIST reported values.

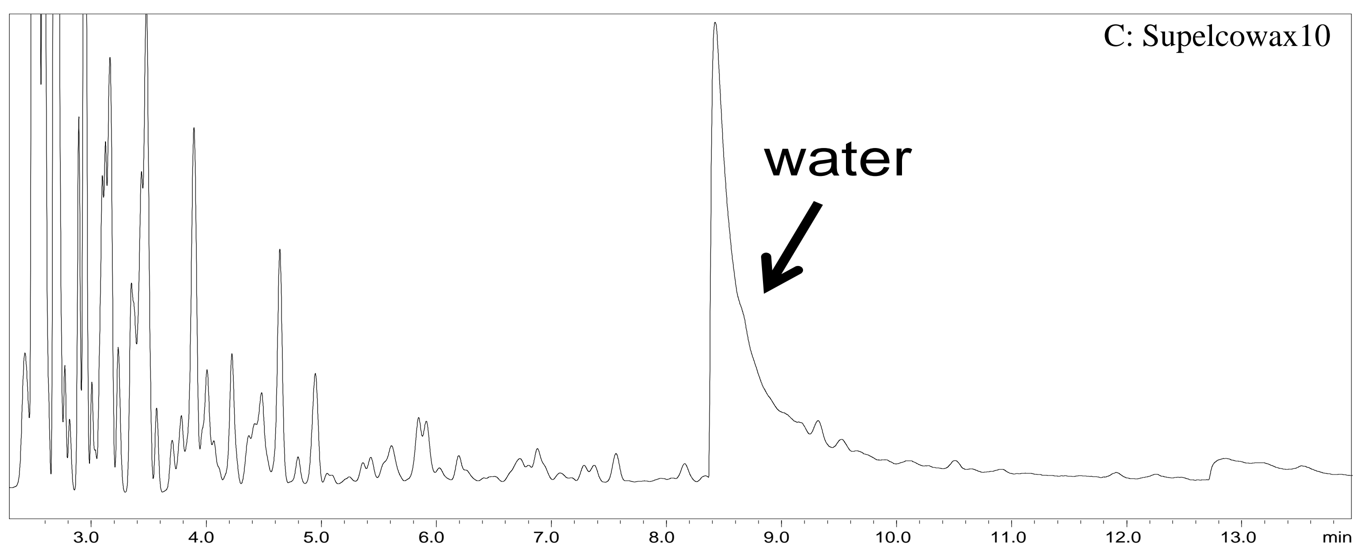
Future Work

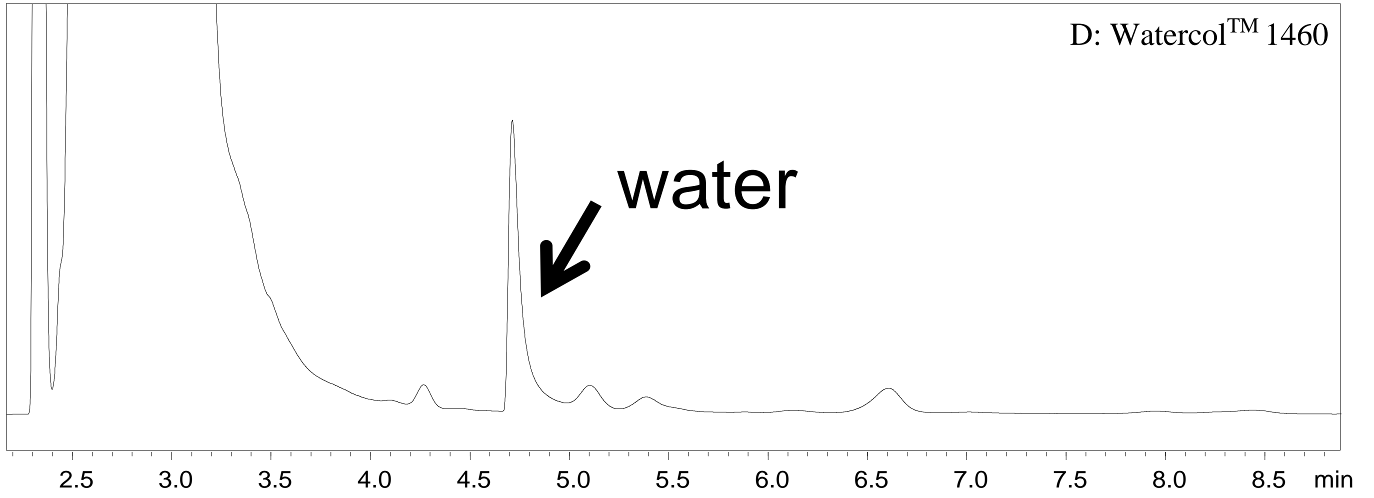
The research which was performed during this study provided proof of concept and a foundation of using headspace gas chromatography with ionic liquid stationary phases to determine water content in diverse samples. While the groundwork has been preformed there is still the need for future research. The next step in water analysis is to use a GCxGC system to allow for more complex samples to be analyzed. Residual solvents in pharmaceutical samples along with water content could simultaneously be analyzed by using a GCxGC equipped with a barrier discharge ionization detector and mass spectrometer. This would allow for smaller sample sizes, faster analysis time and fewer instrumentation requirements to perform the analyses. In addition, GCxGC for the analysis of compounds of interest and water in fragrances and essential oils would be ideal. The first dimension would allow the compounds to be separated and the second dimension would allow for chiral separation of the fragrances.

Appendix 1





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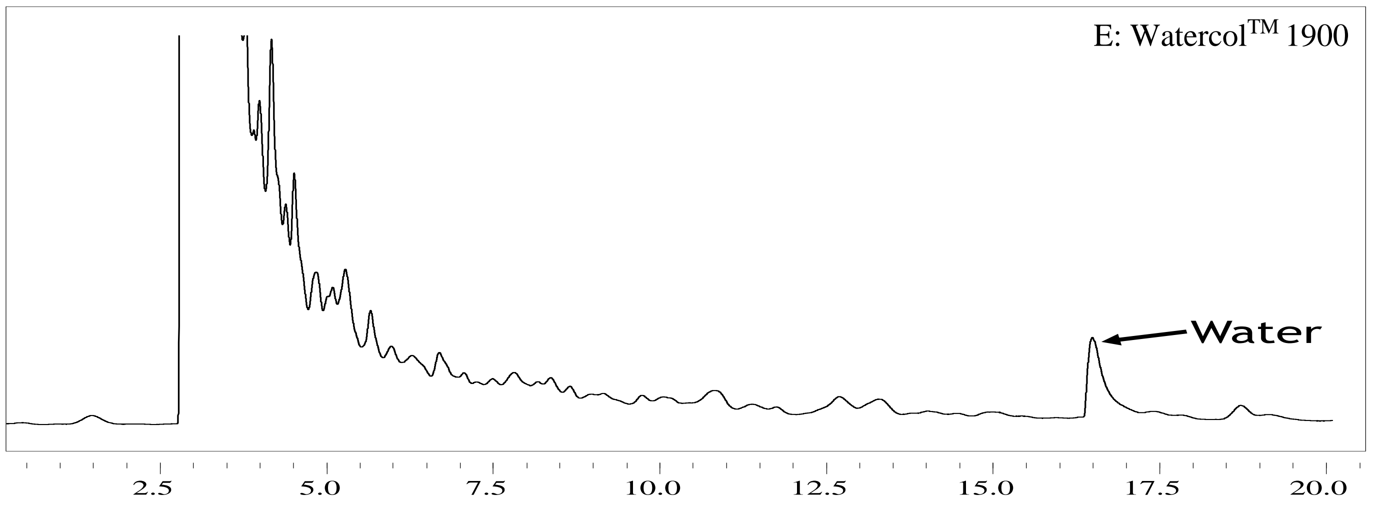
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20.0

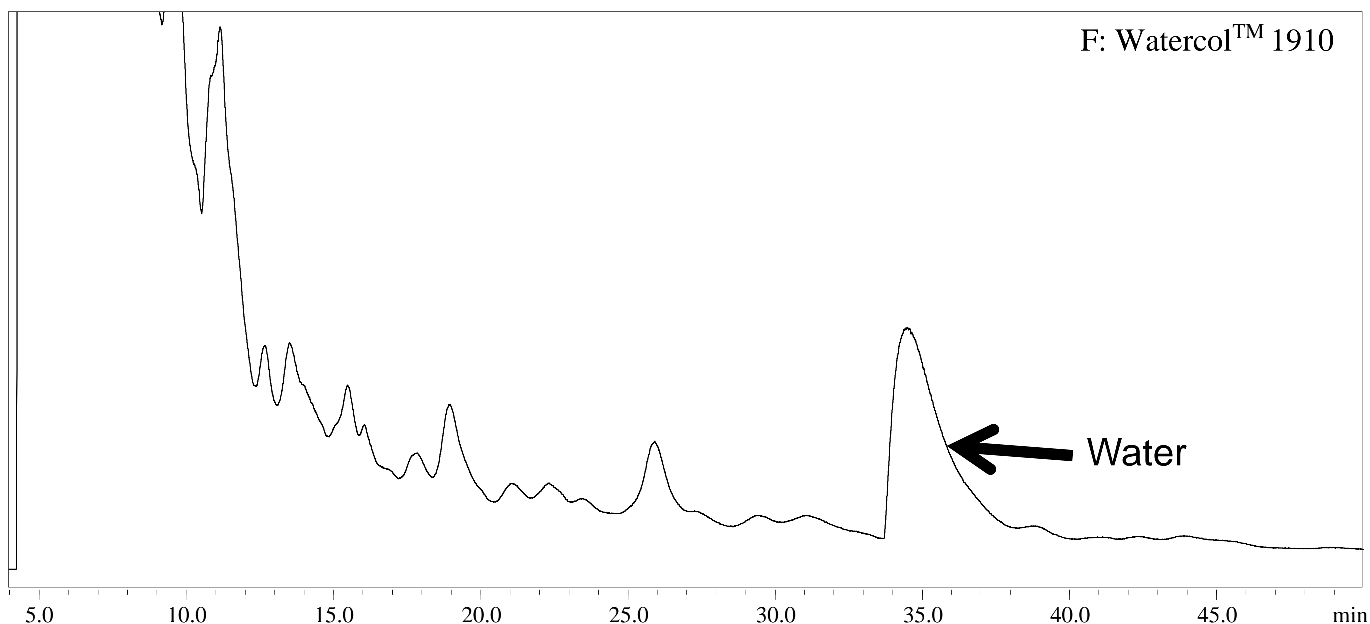

Figure A-1 is the optimized conditions for separation of water from crude oil on six different columns. 1A is the chromatogram of crude oil on a HP-1 (dimethyl polysiloxane) 30m x 0.320mm x 0.25μm column analyzed at 40°C. 1B utilized a SP-2331 (90% biscyanopropyl, 0% cyanopropyl phenyl polysiloxane) 30m x 0.25mm x 0.2μm column to analyze water at 40°C. 1C a 30m x 0.320mm x 0.25μm Supelcowax 10 (poly(ethylene glycol)) was utilized at 40°C to analyze crude oil. 1D shows the chromatogram of crude oil analyzed with a Watercol™ 1460 (Tri(tripropylphosphoniumhexamido)trimethylamine trifluoromethanesulfonate) column with the dimensions of 30m x 0.25mm x 0.2μm at 70°C. 1E is a chromatogram for crude oil on the Watercol™ 1900 (1, 11 Di(3-methylimidazolium) 3, 6, 9, trioxaundecane trifluoromethanesulfonate) 30m x 0.25mm x 0.2μm column analyzed at 50 °C. 1F utilizes a Watercol™ 1910 (1, 11-Di(3-hydroxyethylmidazolium)3, 6, 9-troxaundecane trifluoromethanesulfonate) 60m x 0.25mm x 0.2μm column to analyze crude oil at 50°C.

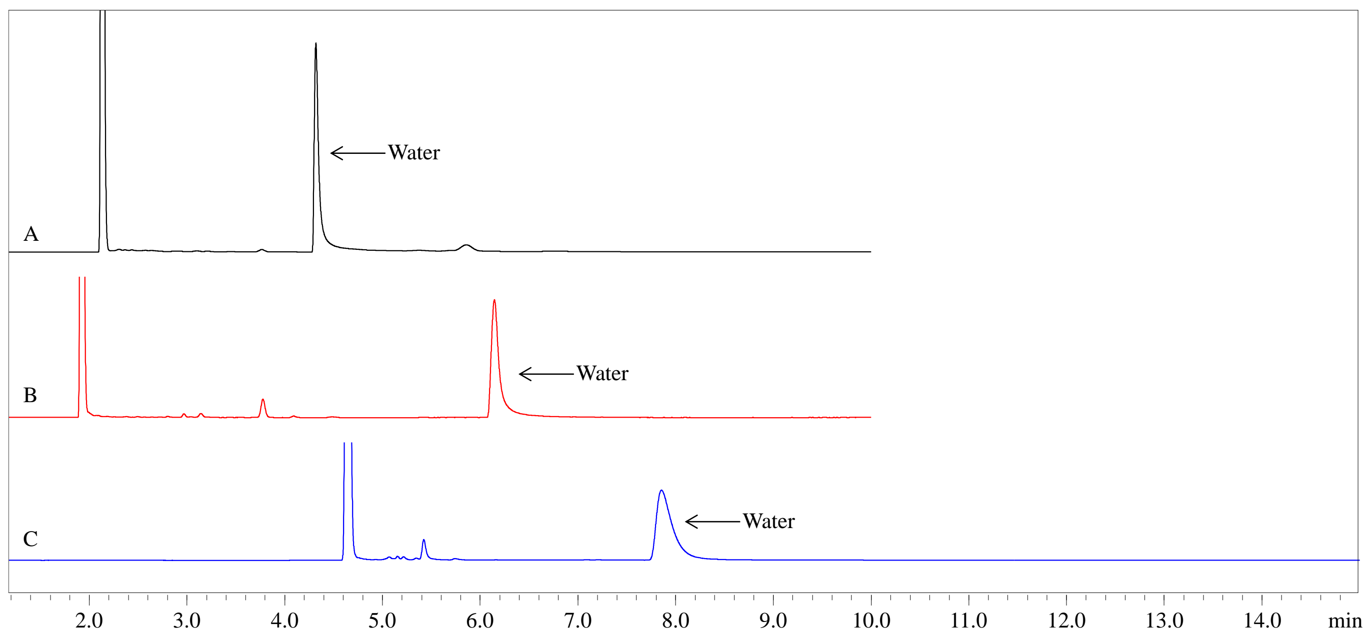

Figure A-2: The chromatograms for the analysis of motor oil (see Experimental), 2A is on the Watercol™ 1460 at70 ºC, 2B is on the Watercol™ 1900 at 70 ºC and 2C is on the Watercol™ 1910 at 110 ºC.

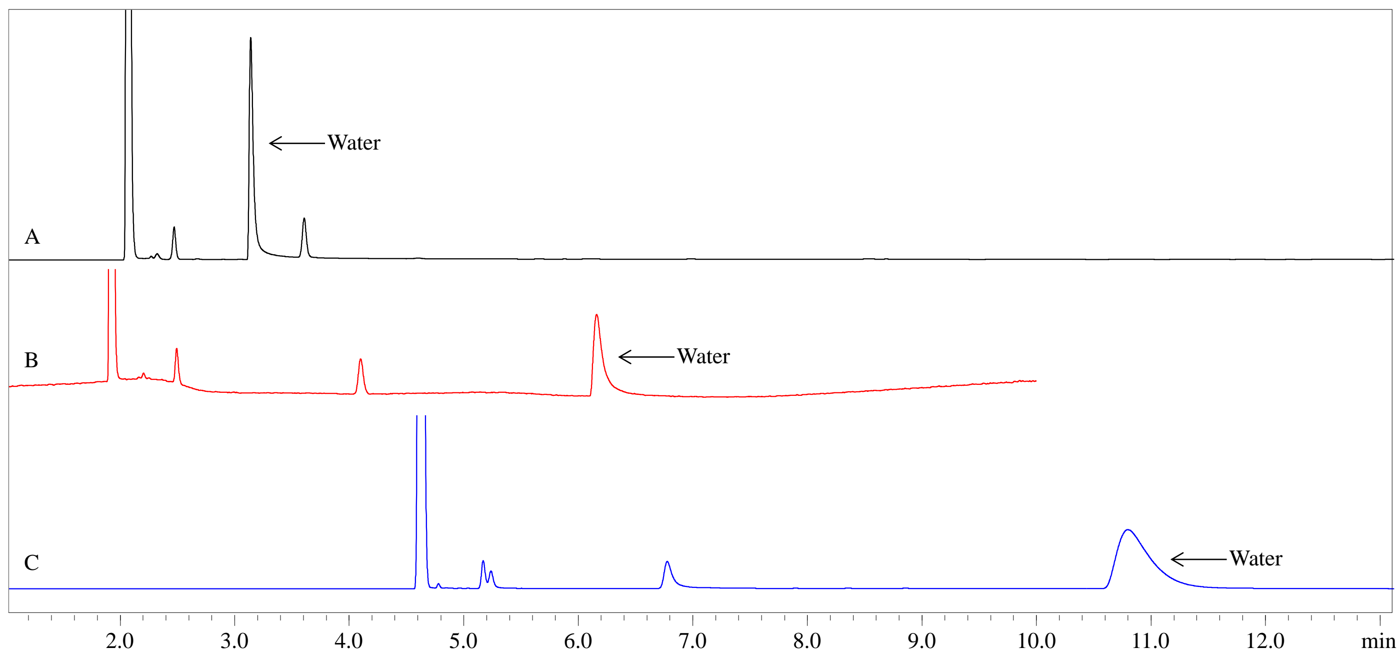

Figure A-3: The analysis of transmission fluid (see Experimental) on three Watercol™, 1460, 1900 and 1910. The chromatogram in 3A was analyzed at 110 ºC on the 1460, in 3B at 90 ºC on the 1900 and in 3C it was studied at 90ºC on the 1910.

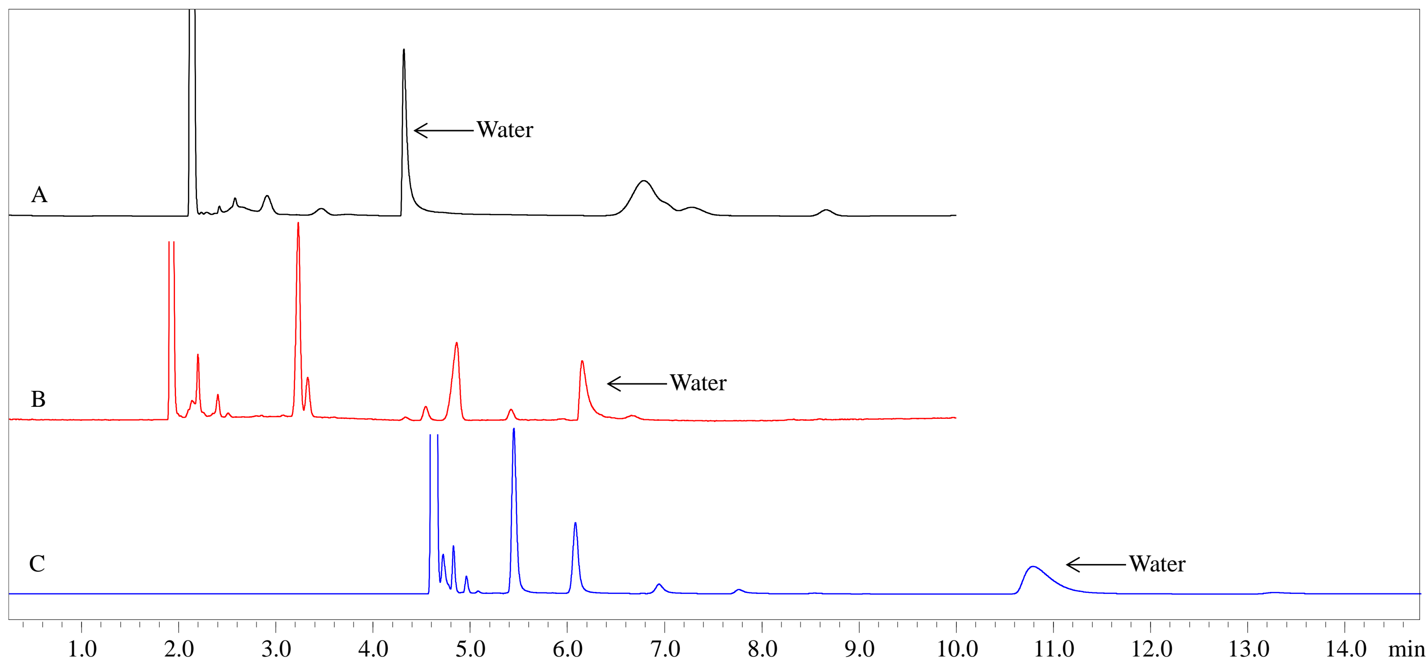

Figure A-4: The chromatograms for the analysis of gear oil (see Experimental), 4A is on the Watercol™ 1460 at70 ºC, 4B is on the Watercol™ 1900 at 90 ºC and 4C is on the Watercol™ 1910 at 130 ºC.

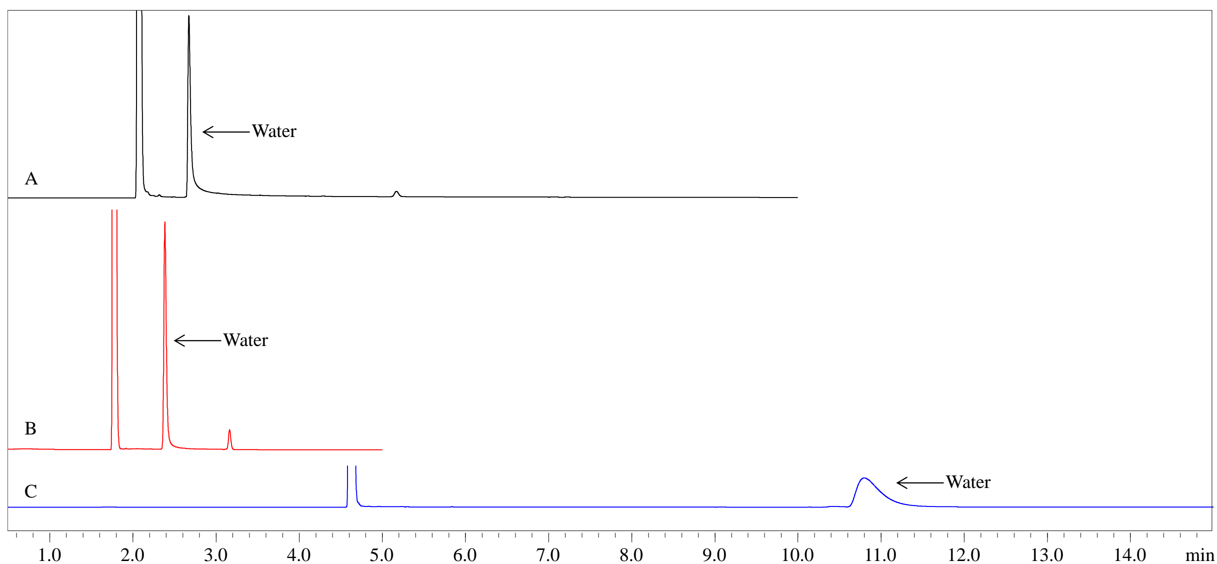

Figure A-5: 5A shows the analysis of power steering fluid (see Experimental) on the Watercol™ 1460 at 150 ºC. 5B shows the chromatogram of power steering fluid on the Watercol™ 1900 at 150 ºC. The final chromatogram in 5C is the examination of power steering fluid at 90 ºC on the Watercol™ 1910.

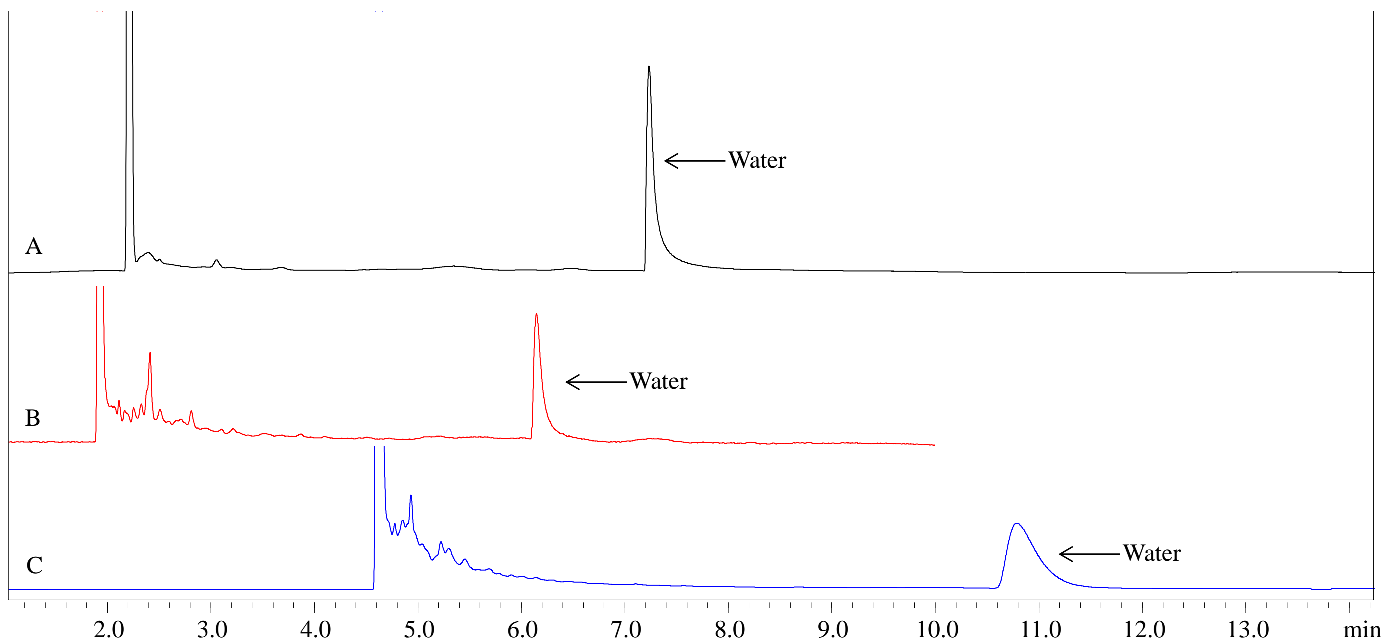

Figure A-6: The analysis of 3 in one oil (see Experimental) on three Watercol™, 1460, 1900 and 1910. The chromatogram in 6A was analyzed at 50 °C on the 1460, in 6B at 90 °C on the 1900 and in 6C it was studied at 90 °C on the 1910.

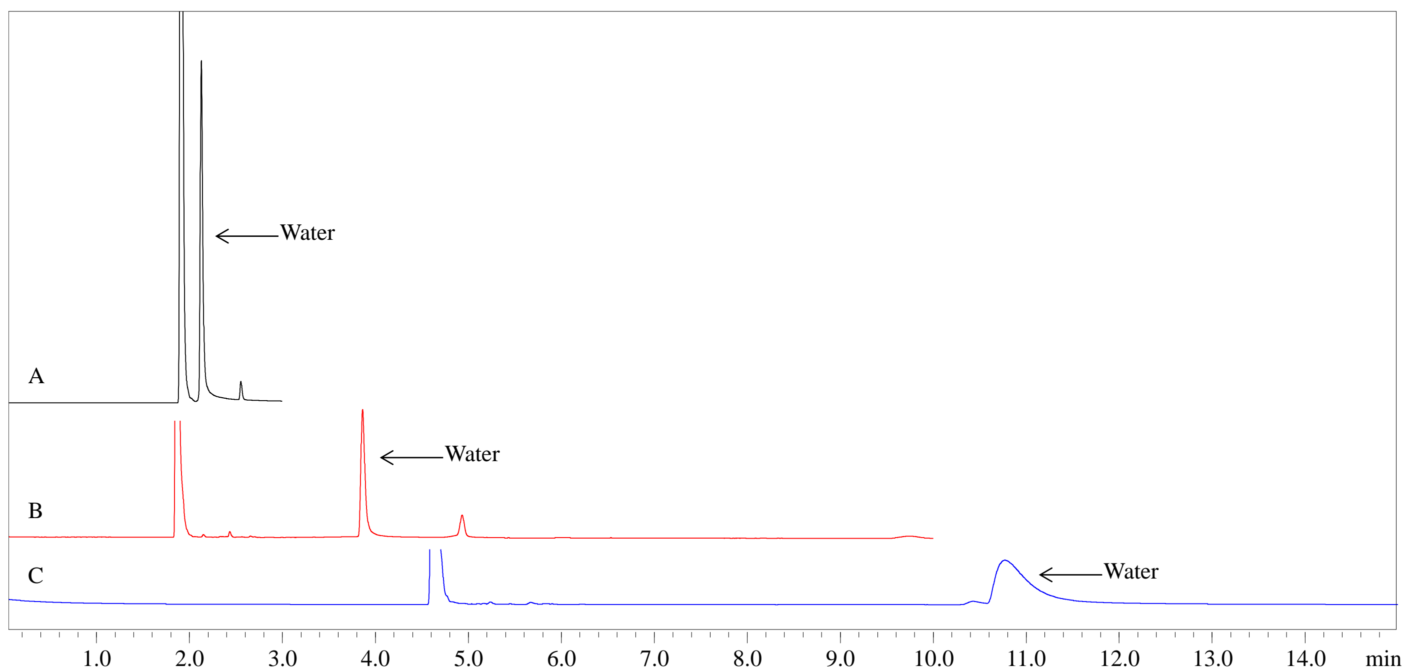

Figure A-7: The chromatograms for the analysis of M-Pro7 LPX gun oil (see Experimental) 7A is on the Watercol™ 1460 at 150 °C, 7B is on the Watercol™ 1900 at 100 °C and 7C is on the Watercol™ 1910 at 90 °C.

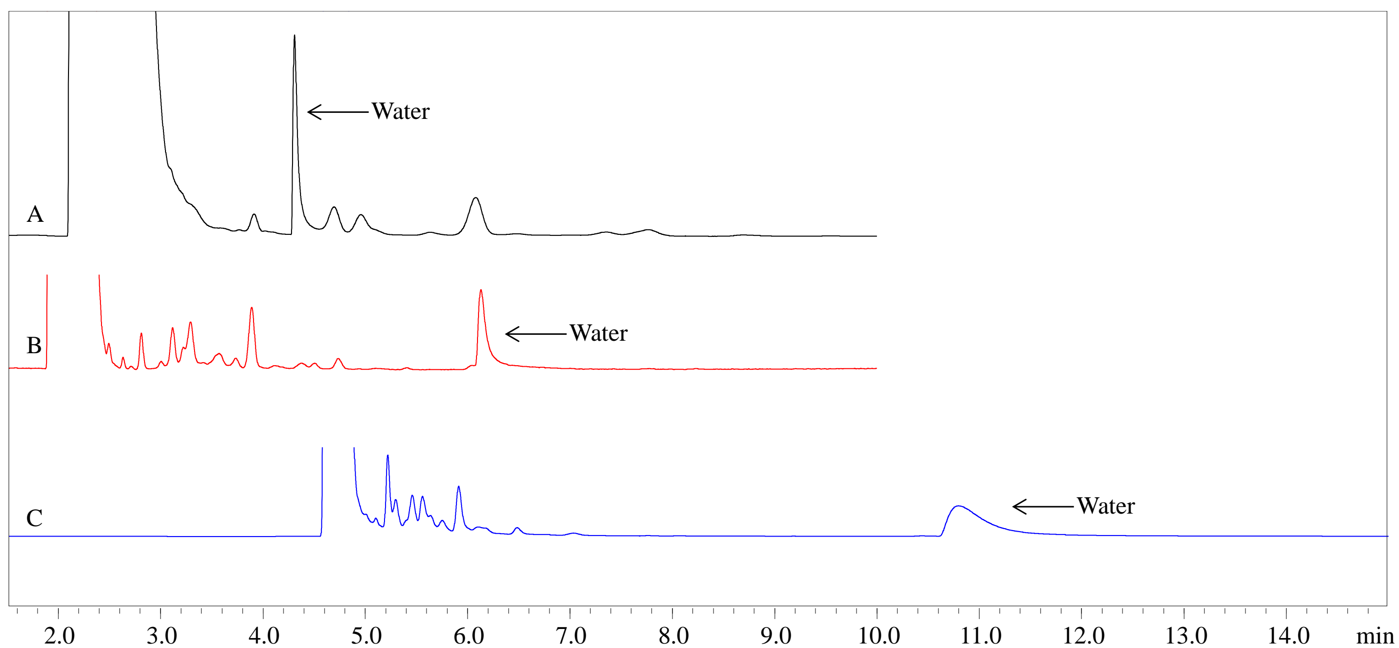

Figure A-8: The analysis of synthetic gun oil (see Experimental) on the Watercol 1460 at 40 ºC, Watercol 1900 at 40 ºC and the Watercol 1910 at 50 ºC are shown in 8A, 8B and 8C respectively.

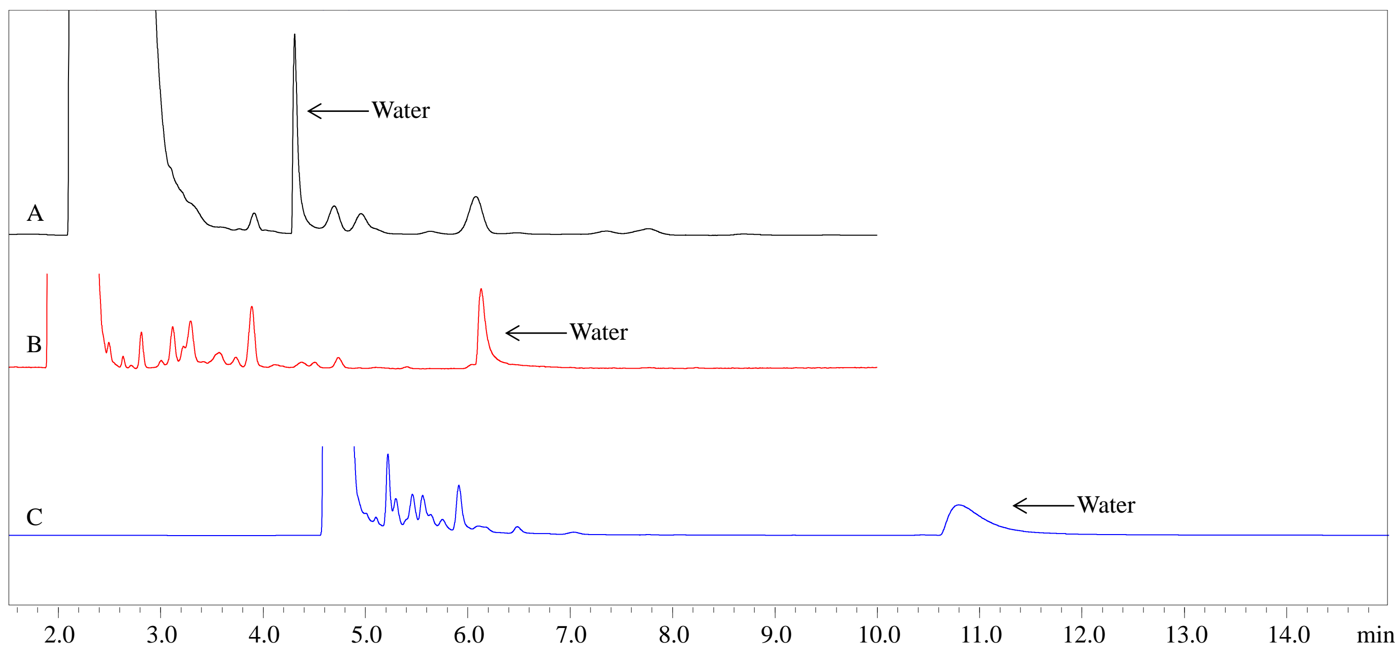

Figure A-9: 9A shows the analysis of Remington oil (see Experimental) on the Watercol™ 1460 at 70 ºC. 9B shows the chromatogram of Remington oil on the Watercol™ 1900 at 70 ºC. The final chromatogram in 9C is the examination of Remington oil at 90 ºC on the Watercol™ 1910.

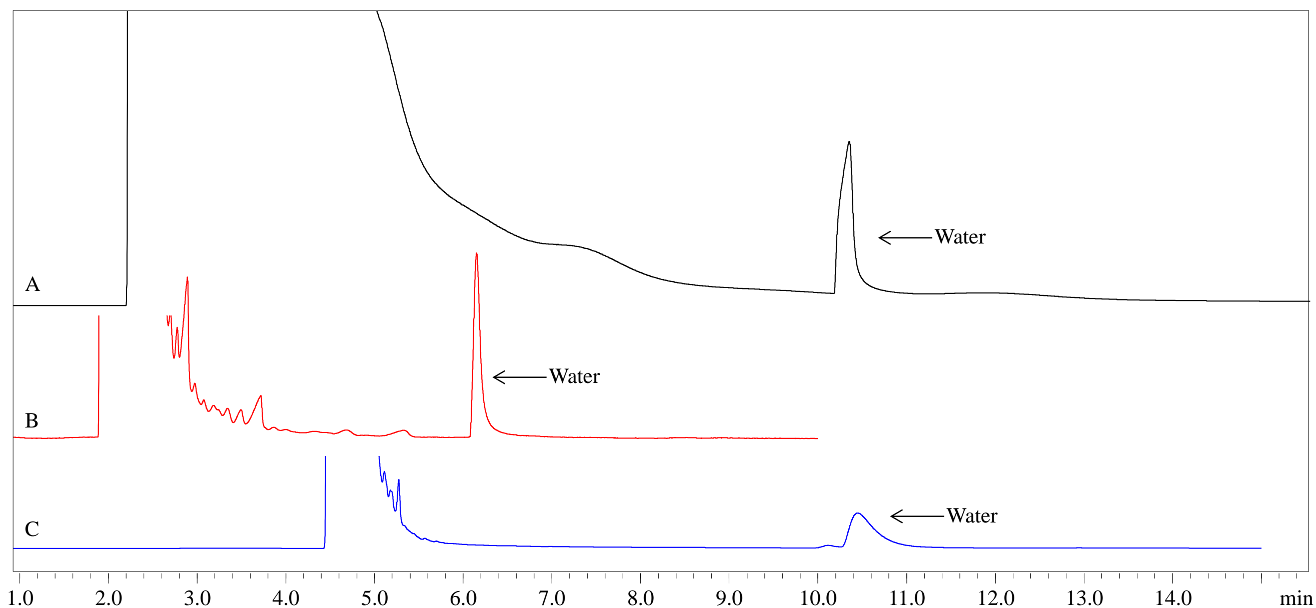

Figure A-10: The chromatograms for the analysis of WD-40 (see Experimental), 10A is on the WatercolTM 1460 at 40 ºC, 10B is on the WatercolTM 1900 at70 ºC and 10C is on the WatercolTM 1910 at 90 ºC.

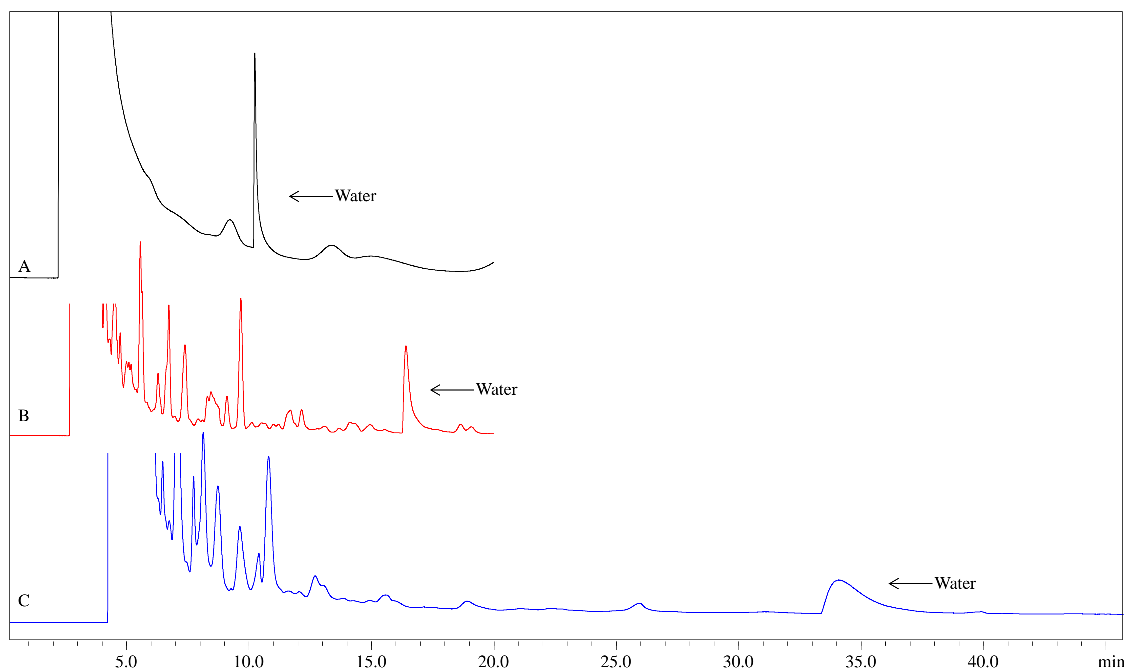

Figure A-11: 11A shows the analysis of light sour crude oil (SRM 2721) (see Experimental) on the Watercol™ 1460 at 50 ºC. 11B shows the chromatogram of light sour crude oil (SRM 2721) on the Watercol™ 1900 at 50 ºC. The final chromatogram in 11C is the examination light sour crude oil (SRM 2721) at 50 ºC on the Watercol™ 1910.

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