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Analysis of Residual Solvents in Pharmaceuticals by Comprehensive Two-Dimensional Gas Chromatography

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Analysis of Residual Solvents in Pharmaceuticals by Comprehensive Two-Dimensional Gas Chromatography

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We certify that we have read this thesis and that in our opinion it is sufficient in scientific scope and quality as a thesis for the degree of Master of Science in Chemistry.

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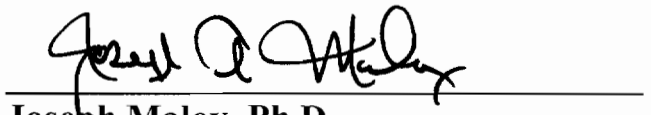

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Abstract

Organic volatile solvents play an incredibly important role in the synthesis, formulation, purification, and manufacturing of consumer pharmaceuticals. The concerns facing the pharmaceutical industry today is the amount of solvent residuals, at the part per million levels that are left behind in the final drug product. There are validated methods used to test at the part per million levels which include gas chromatography, generally with liquid injection, with or without headspace, and gas chromatography by SPME injection. The extreme regulations put forth on the pharmaceutical industry by the International Conference on Harmonization (ICH) require that these residuals and other associated analytes be present only at a safe level and not at a "toxic" level. However, there is no fast one-dimensional gas chromatographic method out there for determining all four ICH residual class solvents that may be present. The current research aims to characterize ICH class one, two, and three residual solvents in a single generic method by using two-dimensional gas chromatography (GCxGC) in under 30 minutes. GCxGC, within the past six years has become the generic method of choice in identifying complex sample matrices containing more then 60 compounds with detection limits ranging from less then 50ppm to 5000ppm. The solvent assay used in this research contained sixty different ICH solvents in a methanol solution and were analyzed on a two-dimensional GCxGC-FID. Standards for the method used were established before all solvents were analyzed together in one solution. Due to the polarity of the analytes optimization of method parameters along with the second dimension column stationary phase polarity used had to be examined. A polar second dimension column produced a final analysis yielding a 2D-GCxGC method (Method 2) that can successfully characterize and identify each of the solvents together in one complex solution, with less than a thirty minute runtime.

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I would like to extend my deepest thanks and gratitude to my mentor Dr. Snow. I came back to Seton Hall in pursuit of a Master in Chemistry even though I had an undergraduate degree in Biology. I was determined to work for Dr. Snow and be part of his research group. He welcomed me into the graduate program, offered me the opportunity to be a Teaching Assistant for the department, and accepted me into his research group. Throughout my graduate tenure Dr. Snow always supported me, he was confident in my ability to teach the undergraduate chemistry students, and believed that I could accomplish whatever goals I set personally and academically. I can not thank him enough for that opportunity along with all the perks, privileges, and support from him and his associates in the scientific community. He taught me how to be a better person, scientist, and wife. The past two years I spent back at Seton Hall were some of the best times in my life. I can not thank him enough and I will always remember the road I spent traveling with him at Seton Hall. Our relationship professionally and personally means the world to me and I appreciate all that he has done for me. Thank you Dr. Snow.

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My family deserves my utmost gratitude especially my husband Craig. The decision to go back to school was a tough one but we worked through it together every single step of the way. All the late nights studying, he supported me through the good times and especially the crazy times. If it was not for the support from my family and them believing that I could accomplish this, my road would definitely have been much harder and lonelier. Thank you so much for your support and unconditional love. Your continuing belief in me helped drive me to complete my research. Without you none of my achievements would have been possible.

This work is dedicated to:

My family

Craig

Mommy, Daddy,
Catherine, Peter and Camo

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Chapter 1

Comprehensive Two-Dimensional Gas Chromatography

1.1 Introduction

Gas chromatography is the premier technique for separation and analysis of volatile compounds and is the most widely used analytical instrument in the world [1]. Gas Chromatography is a separation method in which the components of a sample partition between two phases: one of these phases is a stationary bed with a large surface area, and the other is a gas which percolates through the stationary bed. The sample is then injected, becomes vaporized, and is transported through a column via the carrier gas, usually helium or hydrogen. Analytes partition (equilibrate) into the stationary liquid phase, based on their solubilities at the given temperature. The analytes will then separate from each other based on their relative vapor pressures and affinities for the stationary bed.

Two dimensional gas chromatography or GCxGC uses two columns which are connected together and differ in length, diameter, and polarity. Comprehensive two-dimensional gas chromatography multiplies the resolving ability from a traditional gas chromatograph (1D-GC) to that of another yielding orders of magnitude increase in the separation of chemical compounds in a certain amount of time.

In a GCxGC instrument, there are two ovens. The first oven is a traditional GC oven. The second oven is installed inside the first oven and both can be controlled by the user, with completely different temperature programs. With the secondary oven having its own independent temperature program a much higher resolution can be achieved. The internal

features that connect to two columns including the modulator and the modulation jets will be discussed in more detail later on in this chapter.

1.2 Multidimensional Chromatography

GCxGC is placed in the category of multidimensional chromatography which is a very large and diverse spectrum that includes most chromatographic and extraction methods [2]. A multidimensional gas chromatography separation involves the coupling of two different columns in the separation process with differing stationary phases to obtain an improved selectivity in the separation [3]. The goal is a chromatogram yielding optimum resolution for closely eluting components. Equation 1.1, the master resolution equation, shows that dramatic improvements in separation performance, or resolution, may be obtained by increasing selectivity [2] [4]:

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right) \tag{1.1}$$

Selectivity (α) is the variable from which the dramatic resolution gains needed for increasingly complex samples may be obtained [2]. Multidimensional chromatography invests its whole concept on increasing selectivity through the use of two coupled columns.

Coupled columns (on or off-line), column switching, and sample preparation yield an increase in selectivity [2]. Peak capacity (n) is the next critical variable in GC that relates the number of peaks possibly achieved in a given one-dimensional separation space to the resolution (R), theoretical plates (N), and the retention time frame (t_1 and t_2) [2].

$$n = \frac{\sqrt{N}}{4R} \ln\left(\frac{t_2}{t_1}\right) + 1 \tag{1.2}$$

No matter which calculation is made, a large value for N indicates an efficient column which is highly desirable and a very large retention time frame will only improve one dimensional peak

capacity [1] [2]. Two-dimensional separation substantially increases chromatographic peak capacity because the peak capacities of each dimension are multiplied to gain peak capacity of a coupled system increasing the separation power [2] [5].

1.3 History of GCxGC

In 1991, John B. Phillips and Zaiyou Liu published the first paper on Comprehensive Two-dimensional Gas Chromatography [5]. They stated that "Gas chromatography with serially coupled columns potentially has much greater peak capacity and resolution then conventional single column gas chromatography." Phillips was right and today the technique has proven to be of great value to the petrochemical, forensic, environmental, tobacco, and flavor and fragrance industry because it has significantly improved the resolution of the chemical compositions of their complex mixtures. Back in 1993, Phillips et al published, using GCxGC they were able to successfully separate over 6000 chromatographic peaks in a kerosene sample that traditionally when analyzed on a regular GC only yields less then 100 peaks [6]. In 1994, Liu et al published that they were able to analyze human serum with 15 pesticides present in just under 4 minutes using GCxGC [2].

GCxGC continued to be slowly explored throughout the rest of the 1990s but from 2000 on the interest and research in the field has increased dramatically. Literature searches (sciencedirect.com or scifinder scholar) demonstrate this but GCxGC still is generally present only in the journals committed to the separation sciences. GCxGC has reached the status where it is of great interest to chromatographers, but it has not yet achieved that general use for journal publications in other fields of research [2]. In 2006, M. Adahchour, J. Beens, R.J. Vreuls, and Brinkman reviewed the literature on GCxGC, emphasizing developments in the period 2003 –

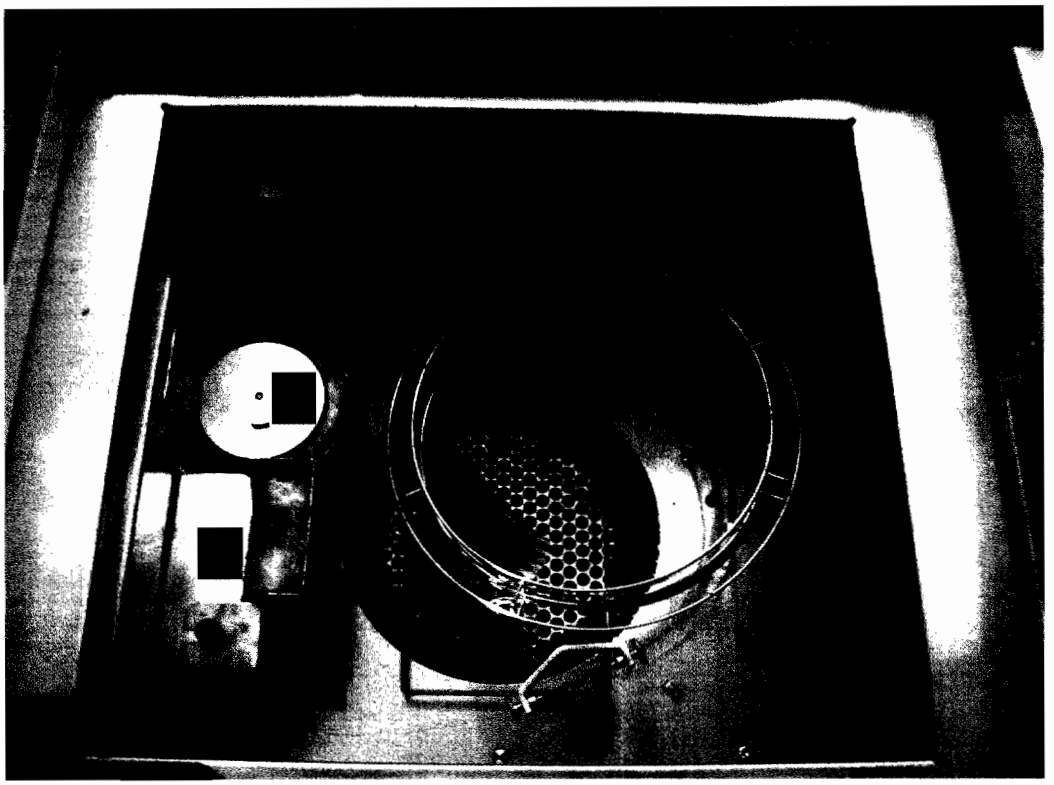
2005 [7]. The review discussed theoretical aspects, trends in instrumentation, column combinations, and detection techniques, with much attention focused on the wide variety of applications and analytical performance.

1.4 GCxGC Instrumentation

GCxGC differs from other types of multidimensional separation techniques, for example heart cutting techniques in which only selected portions of the effluent from the first column are transferred to the second column [5]. The sequential heart-cutting technique can ultimately analyze the entire sample by taking cuts at a succession of different retention times from repeated primary chromatograms, but it also eliminates a majority of the sample before entry into the second column [5]. The difference between GCxGC and heart cutting techniques is that in GCxGC the whole sample (not fractions of it) is subjected to both dimensions of the separation processes in one continuous analysis. It separates substances across a two-dimensional plane [5] keeping the fractions taken narrow to ensure that no information gained during the first separation is lost [7].

A schematic of the inside of a GCxGC oven/system is illustrated in Figure 1.1. This figure is based on the schematic of a single dimension Agilent 6890 GC-FID (Flame Ionization Detector) that is modified by adding a smaller independently temperature controlled secondary oven inside of the primary GC oven. The two columns are connected by a glass press-fit connector. The second (second) column then enters into the secondary oven containing the modulation jets which are controlled by the modulator.

A.



B.

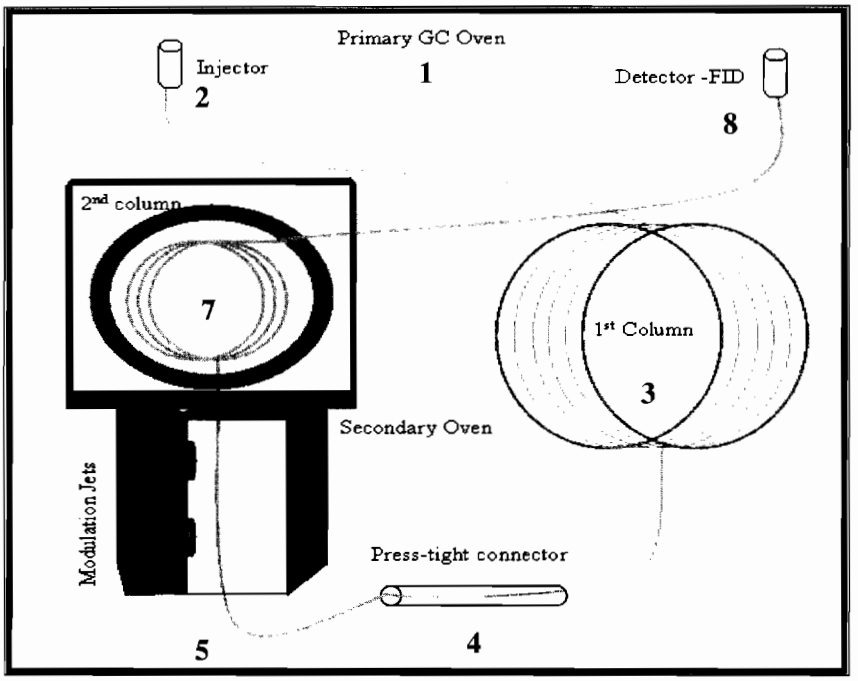


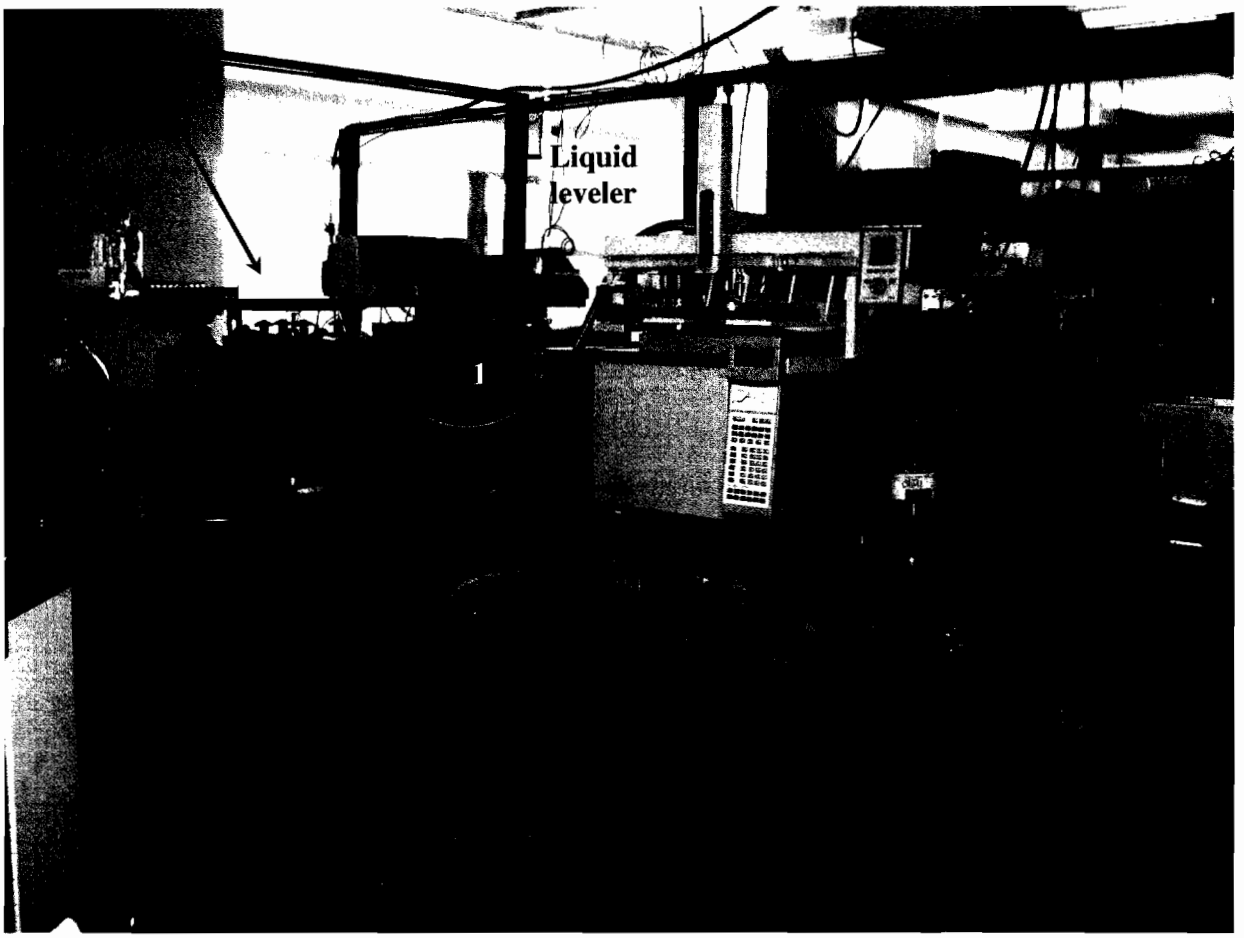
Figure 1.1 – GCxGC Insides

The inside of a GCxGC is the same as a traditional GC except for the addition of the secondary oven. Figure A. is the actual GC oven used in this research and B is a representation drawing of A. **#1** is the oven of an Agilent 6890 GC-FID; **#2** injector inlet – where the sample is introduced into **#3**, the 1st dimension column. **#4** is the press-tight connector that creates the leak free connection between the 1st and 2nd dimension column - **#5**. The 2nd dimension column (**#5**) then enters the secondary oven **#7** where the modulation jets **#6** freeze with liquid nitrogen and heat the column with warm nitrogen air before entering into **#7**. The secondary oven (**#7**) houses the second dimension column. From the secondary oven the 2nd dimension column goes to the detector **#8** – FID (Flame Ionization Detector) and the signal is sent to the computer where the 3-dimensional chromatogram is created using the Leco Chromatof GCxGC software program.

The modulator has been described by many authors to be the "heart" of GCxGC and this can be found at the beginning of the second-dimension column [8]. Effective modulation between the two columns is required for to successful GCxGC. The GCxGC instrument used in this research has a two-stage cryogenic modulator. By referring to Fig. 1.3 the schematics on how the two-stage modulator operates with regards to the sample are seen. The modulator must perform several tasks involved with the transfer of effluent from the first column into the second column [8]. A major role of the modulator is to focus the effluent from the first dimension column and divide it into a series of fractions which are continuously injected as the narrowest possible bands into the second column [8]. Simply put, the modulator collects fractions, focuses them, and injects them onto a second column.

The modulation time is the duration of a complete cycle of modulation and is equal to the time between two consecutive injections into the second column [7]. It must be coordinated with the temperature conditions of both columns to avoid wraparound – compounds eluting from the second dimension after the next modulation cycle begins [8]. These peaks show up on the chromatogram and can interfere with analytes eluting at that specific retention time. Figure 1.4 demonstrates what wraparound peaks look like.

A detailed step by step description of the modulation process is seen in Figure 1.3. As the analyte elutes from the 1st column into the second one it is met by a cold burst of cryogenically cooled nitrogen gas from the cold jet. The cold jet uses liquid nitrogen fed to the modulator from a dewar attached to the side of the GC which is controlled by a liquid leveler and transferred through a hose connected to the liquid N_2 tank (Figure 1.2 GCxGC actual lab setup). The cold jet freezes the analyte from leaking into the second dimension oven and detector. As soon as the cold jet turns off the effluent is met by a hot pulse of Nitrogen air.



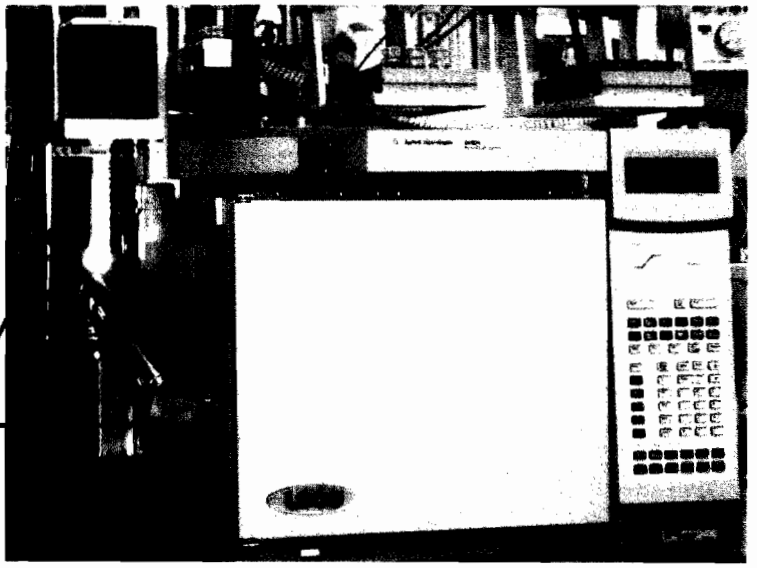


Figure 1.2 – Research Laboratory GCxGC System Setup
The above picture is the complete setup of the GCxGC system used to perform this research. The liquid NO_2 tank (hidden behind computer monitors) has a transfer hose-line which delivers the liquid nitrogen to the dewar attached to the back side of the GC. The transfer hose-line is represented on the above picture by the #1. The liquid nitrogen fills the side dewar is delivered to the GCxGC system from the dewar arm where it is piped directly to the modulation jets.

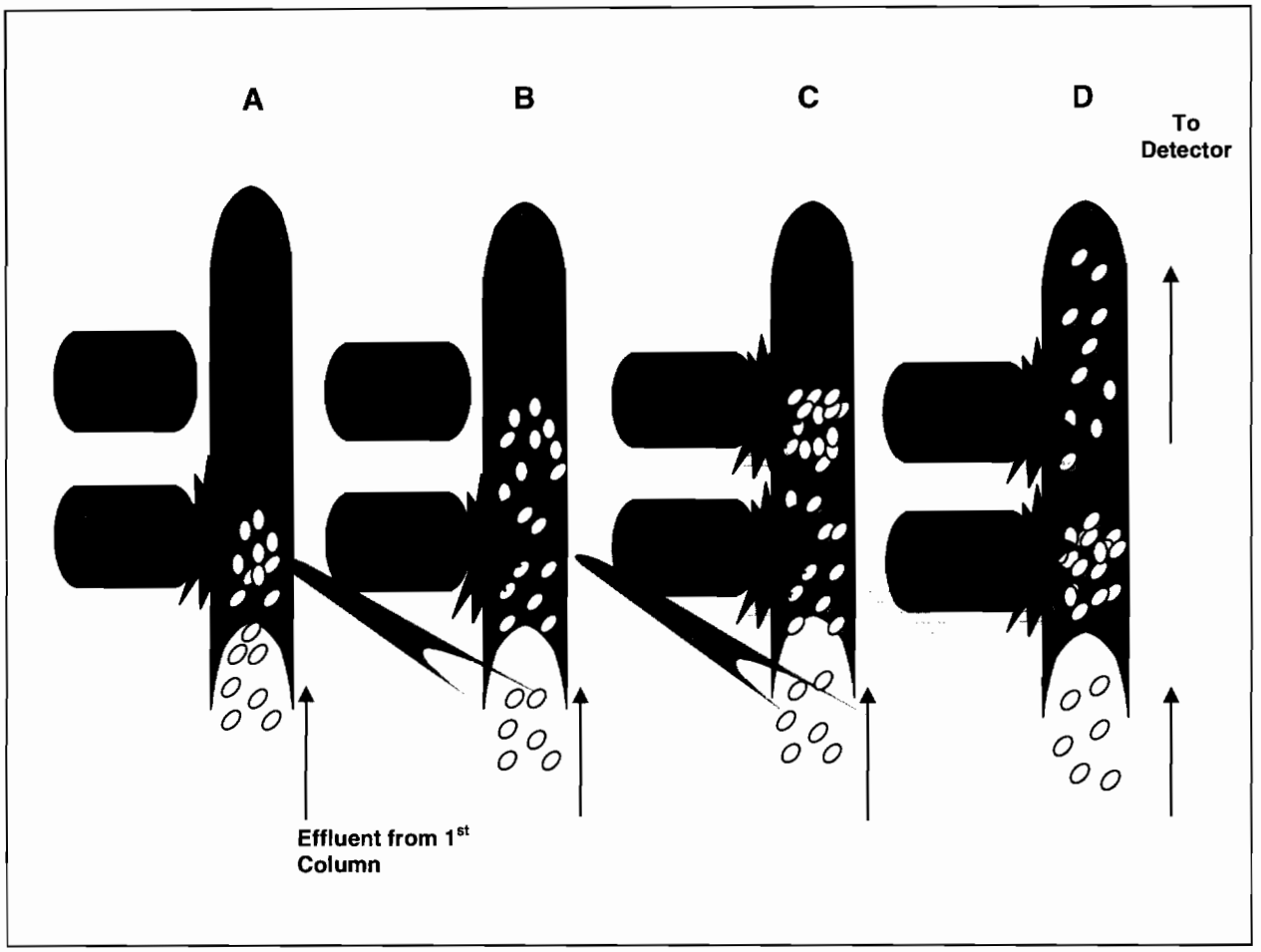


Figure 1.3: Schematic 2-Stage Cryogenic Modulation

A. Effluent from the 1st column rapidly (pulsed) enters the second column and analytes are halted by the 1st Cold Jet (liquid Nitrogen)

B. The Cold jet turns off and the hot jet (Nitrogen air) simultaneously-/-immediately comes on allowing the analytes to move up the column

C. As the hot jet from B turns on the second cold jet turns on at the same time and freezes the analytes released by hot jet.

D. The second hot jet comes on and analytes are released to the detector. At this point the 1st cold jet comes back on, to prevent leakage of 1st dimension column effluent, and the cycle starts all over again. These steps are all part of the continuous modulation cycle constantly occurring for the duration of the run.

The hot pulse desorbs the trapped analytes and they continue up the column again met by one more cold pulse and then another hot pulse before entering the detector.

While the modulation cycle takes place in the second dimension column the separation in the first dimension column proceeds uninterrupted. The first dimension column is a non-polar stationary phase and in order to preserve the integrity of its separation Adahchour et al. states that the narrow fractions subjected to modulation should be no wider than about one-quarter of the peak widths in that dimension, typically 5-30s, and have three to four modulations across each peak [7]. The nonpolar stationary phase separates components largely based on their vapor pressures, volatility, and or boiling points [9]. But it is quite possible that oxygenated and purely hydrocarbon components might have a similar boiling point, which will lead to peak overlap [10]. All sample components that are separated in the first dimension will remain separated in the second dimension. The peaks will become much more resolved as the analytes separate in the second dimension column.

Second dimension times should be in the 2-6s range because the separations occurring in the second dimension are essentially isothermal. The isothermal conditions of the second dimension occur because the second column is housed in a separate oven that can have a temperature program completely different from the main GC oven. The fast separation occurring in the second column yields very narrow peaks and preserves the elution profiles from the first dimension column.

Figure 1.4 – Peak Wraparound
The highlighted red circles shows typical peak wrap around that can occur in GCxGC. This particular peak starts in the last second of the modulation cycle and ends just before the 1st second of the next 4 second modulation cycle. The rest of the analyte peaks on the chromatogram do not display any peak wraparound.

1.5 Modulation Techniques

Optimization of GCxGC techniques has been ongoing on since Phillips developed the first one in 1991. The modulator transfers effluent from the end of the first dimension column to the head of the second dimension column as a repetitive series of pulses [11]. The first modulation interface consisted of a metal-clad, thick-film capillary column that retained the analytes eluting from the first column by phase-ratio focusing and then remobilizing by resistive heating [12]. Phillips' original system was replaced by a thermal sweeper because it was difficult to manufacture and was not rugged enough to allow routine use [12]. The original modulator also had a limited range of analyte volatility [12]. The work following that focused on and replaced the sweeper with cryogenically cooled modulation systems [12-14]. There are several modulation schemes that Adahchour et al. describes in part two of his four part series in Trends in Analytical Chemistry. Those schemes are: thermal desorption modulation, cryogenic modulation, and valve-based modulation [13]. A summary of modulators that are used and or have been used in GCxGC is presented in Table 1.1, with general references for use to better understand these schemes. A pictorial representation of the Modulation schemes diagram can be found in Figure 1.5.

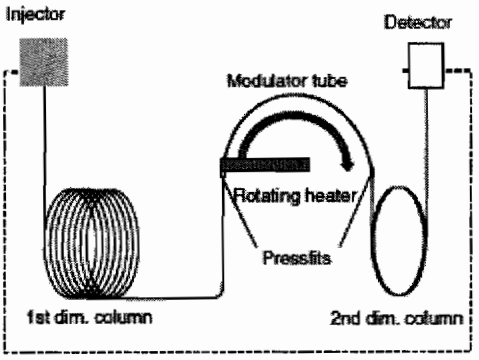
1.5.1 Sweeper – Thermal Desorption Modulator

The main problem with the sweeper was that to achieve rapid heat transfer it was essential that the inside surfaces of the slotted heater moved extremely close to the fragile modulator tube while not cracking or breaking the glass tube [13]. The rotating slot heater had to be maintained 100°C above the primary oven temperature resulting in a maximum first dimension column temperature no more than 230°C [13, 20]. The rest of the sweeper process was time consuming because of the need to optimize parameters including the sweep velocity,

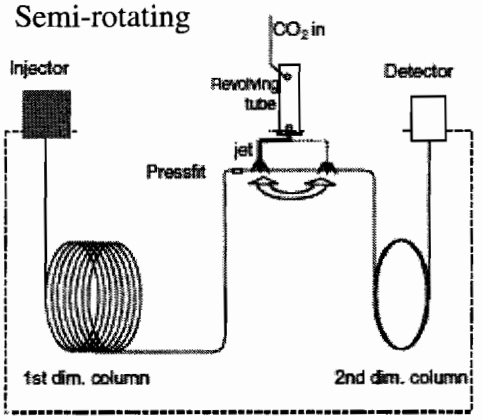
Table 1.1 Summary of Modulation Schemes

Type	Description	Interface	Reference
Valve modulation	Simple multi-port valve between the 1st and second column. Uses a splitting process for fractions of effluent transfer from 1st to second column.	Valve	[13, 15-16, 18]
Differential Flow Modulator	In-line fluidic modulator that generates a succession of pulses by collecting 1st column effluent in a sample loop occasionally switching an auxiliary flow on to flush the effluent from the sample loop into the second column.	Valve	[11, 16, 17]
Thermal Desorption Modulator	Two-stage, metal, coiled column with electrically conductive paint that was heated by a constant-voltage power supply connected to the modulator. Sample is trapped and released two times to achieve narrower peaks.	Electrical current heated and air cooled	[13, 17, 18, 19]
Sweeper	A type of thermal desorption modulator that functions by moving at regular intervals, a small heated element quickly over the modulator capillary. Heating desorbs analytes rapidly into the second column.	Phase ratio	[5, 13, 18, 19]
Cryogenic Modulation	Liquid Nitrogen or liquid carbon dioxide is used to cool, trap, and refocus the analyte either at the end of the first dimension column or the beginning of the second dimension column. Heat is then added to the equation to allow that same column portion to desorb the trapped analytes into the second dimension column.	Liquid N_2, N_2 gas, liquid CO_2	[13, 18]

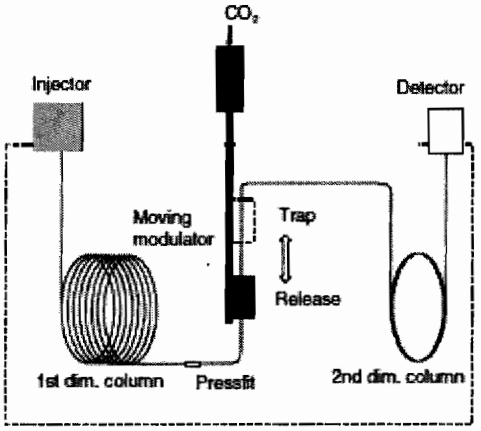
Sweeper Modulator



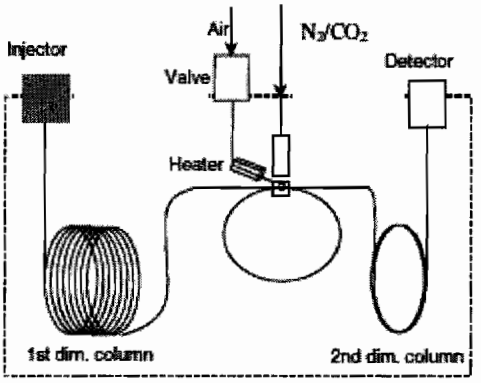
Semi-rotating



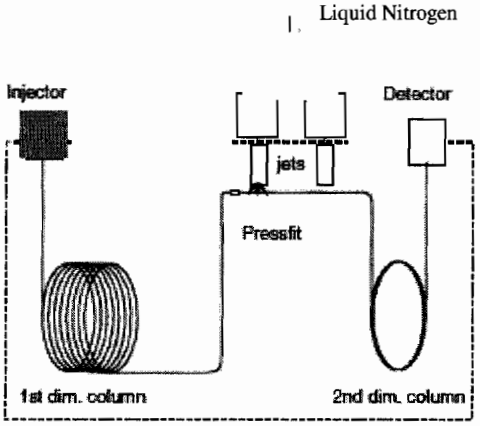
LMCS



2 jet N_2/CO_2



Dual Jet Modulator



4 jet N_2 cryogenic modulator

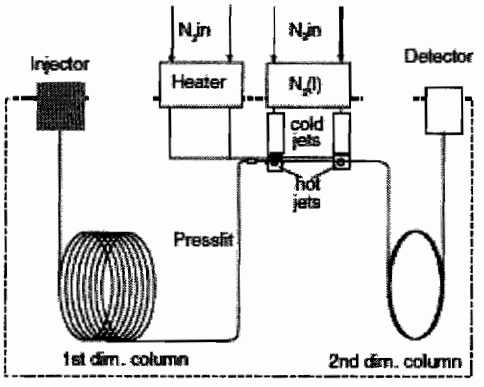


Figure 1.5 Schematics of GCxGC Modulators [13]

the wait time for the heater between cycles, the oven-heater temperature differences, the column wall thickness, and the stationary phase film thickness [13]. Also, the thermal sweeper contained two different column connections. The first connection, using a pressfit connector, was from the first dimension to the modulator tube. The second connection happens at the end of the modulator tube pressfit connector to the beginning of the second dimension column. As the analytes travel out of the first dimension column they are brought forward along the column as a focused band while the sweeper rotates over the modulation tube, and then injects the analytes into the second dimension column as a narrow band where it enters into the detector [21]. A diagram for this type of modulation scheme can be seen by referring to Figure 1.5.

In 2003, Dallüge and Beens, in their first review stated that in all of the 30% of papers published using a sweeper modulation scheme the studies showed "little to be desired with respect to separation performance" [13, 22]. This review even sparked a "set the record straight" debate in the Journal of Chromatography A. The debate started when Gaines and Frysinger [23] commented on the review stating that Dallüge, et al. were wrong about the lack of "robustness" of the thermal modulator and that they have the publications to prove that the temperature ranges given were wrong [23]. In that same issue of the Journal of Chromatography A, a reply was written by Dallüge and Beens [24]. They state that Gaines and Frysinger have "indeed shown that the application range of the Sweeper can be extended, but only by using a distinctly modified and adaptive system" [24]. Today Sweeper modulators aren't readily available to buy commercially; one would have to engineer, build, and retrofit a GCxGC with a homemade one.

1.5.2 Cryogenic modulation

Many different types of cryogenic modulators have been developed over the years and researchers have adopted the use of cooling rather then heating to create the required

retention/release temperature differences [13]. There have been cryogenic modulators made that do require moving parts, i.e. semi-rotating cryogenic modulator [25], longitudinal modulating cryogenic system (LMCS) – a cryogenically cooled trap that oscillates back and forth [26]. Cryogenic modulation employs the use of either liquid nitrogen or liquid carbon dioxide and uses jet based modulators or LMCS types as its interface.

Marriott and coworkers were the first to publish the LMCS type modulator. The LMCS unit uses a cryogenically-cooled trap located around a column segment of 2-3 cm [26]. This cryogenic system uses expanding liquid carbon dioxide (CO_2) for trapping and focusing the analytes in the first 2-3 cm of the second dimension column [25]. The LMCS trap operates at constant temperature, regulated by a digital temperature controller, cooled by the liquid CO_2, and uses an on-off valve to control the flow [27]. The trap is operated by a motor-driven piston that moves the trap into a position that exposes the analyte to the GC oven air [13]. The fraction of effluent that was trapped is then re-volatilized and re-injected as a very thin band using only the heat of the GC oven air [13]. The selective mode of LMCS collects in the cryotrap and rapidly injects the effluent onto the second column. If the second column phase selectivity and efficiency are sufficient the resulting peaks should look very narrow and tall [28].

As time went by several types of jet-based modulators utilizing liquid CO_2 or liquid nitrogen as the cryogen began to dominate the field of GCxGC modulation. Jet based modulators no longer used moving parts like those used in the LMCS modulator. Jet-based cryogenic modulation comes in many configurations – a single-jet, dual-jet, or four-jet modulator. Figure 1.1 shows the traditional setup for a 2-stage cryogenic jet modulation with a step by step explanation shown in Figure 1.3 of what is happening as the jets heat and cool the

analyte entering into the second dimension column. Two-stage cryogenic jet modulation has been previously explained in Section 1.4.

The main advantage of using liquid nitrogen or liquid carbon dioxide is that even small molecules, i.e. gaseous hydrocarbons, can be easily modulated [18]. Very volatile compounds that have boiling points lower than hexane requires liquid nitrogen for cooling in order for them to be properly modulated [13]. Jet modulators using cooled nitrogen gas (-180°C) have the lowest trapping temperature offering the widest application range for the sample containing low boiling points [22]. Liquid nitrogen rather then carbon dioxide for cooling allows the temperature range of 100-500°C, boiling points i.e. C_8-C_{36}, to be increased to about ~160-500°C C_1-C_{36} [13]. This is a true indicator for why GCxGC is very important to the petrochemical industries highly complex samples.

1.5.3 Differential flow modulation

Differential flow modulation is another type of modulator that uses a flow-flush type valve. It generates a series of pulses by collecting primary column effluent in a sample loop (fill position) and periodically redirecting an auxiliary flow to flush the sample loop into the second column [11]. If the flow in the secondary column is greater then flow in the primary column, then the sample loop contents will be flushed in less time than is needed to fill the loop [11, 17]. The slow fill and fast flush sequence over and over again, produces the pulse series [11]. The selective modulation is observed when a small portion of auxiliary flow carrier gas is just large enough to steer the primary column effluent into the sample loop [17]. If too much carrier gas is used, the pulses will become broader and would dilute the primary column effluent [17]. Literature states that under optimal conditions the width of each pulse departing the modulator is given by the modulation period times the primary flow to auxiliary flow ratio [11]. Differential

flow modulation uses the opening and closing of valves ("fill – flush") to release the effluent from column to column. The main problem with differential flow modulation is that the upper temperature limit cannot go above 175°C.

Diaphragm valve modulators require a high flow-rate through the second column and transfer about 80% of effluent from the first into the second column [22, 29]. This type of valve modulation is not cut out for trace analysis because it reaches 'refocusing' by injecting a narrow fraction of the effluent, transferring only 2% from the first into the second column [22]. The rest of the sample is just wasted.

There has been research showing that differential flow modulation can be done without the use of diaphragm valves. This is an improvement from basic differential flow modulation because without the use of diaphragm valves the modulator can have a larger temperature range, a higher flow ratio, and sampling of all the 1st dimension column effluent. Another problem facing this modulation scheme is that if you change your flow rates or modulation periods, the dimensions of the two sample loops must be changed [13].

1.6 Sampling, Inlets, and Injections

All samples injected in gas chromatography may start out as a liquid but after injection in order for them to be analyzed they need to be vaporized. When the sample is injected into the hot inlet (common temperature is 250°C) it is vaporized into a gas which then travels through the column by means of the carrier gas. The most common injection techniques are: split injection, splitless injection, on-column injection, programmed temperature vaporization injection (PTV), and large volume injection. There are also many different sample extraction techniques such as:

solid phase micro extraction (SPME), headspace extraction, membrane-based extraction, pressurized liquid extraction, liquid-liquid extraction, and single-drop extraction. Syringes are typically used for injecting either manually or automatically using an autosampler installed on top of the GC that mimics the manual injection done by a person. Ideally, as stated by McNair and Miller, "the sample is injected instantaneously onto the column, but in practice this is impossible and a more realistic goal is to introduce it as a sharp symmetrical band or plug" [30, 31] by using a small sample size (μl).

1.6.1 Split Injection

Split injection is the easiest injection technique to perform as well as the oldest and simplest [30]. In a split injection, a 50 – 1000 μl gas sample or a liquid injection of about 0.1 – 2.0 μl sample is rapidly injected (delivered) into a heated injection port containing a glass liner about 2-4 mm internal diameter and a couple centimeters long [30, 32]. Since the sample size is so small it is quickly vaporized and about 1-2%, a small fraction of the vapor, enters the column via the inlet. The injected sample enters the inlet through a glass liner where the syringe needle deposits the sample [32]. The other 98% of the vaporized sample flows out through the purge valve along with a large flow of carrier gas. The flow in the purge vent is controlled by a needle valve [32]. The needle valve also regulates the ratio of vent flow to column flow, i.e. the split ratio [32]. The split ratio is the ratio of the volumetric flowrate out of the purge vent to the volumetric flowrate in the column [32]. This provides an estimate of and offers control over the actual volume of sample allowed to enter onto the column. A higher split ratio (200:1) yields a much smaller sample volume injected and usually narrower peaks [32]. The column flow rate is

determined by the pressure drop between the column head at the inlet and outlet at the detector [32].

The many advantages of using a split injection are: small sample size, fast flow rate at the split point (sum of both column and vent flow rates), yields high resolution separation, higher split ratio allows for neat mixtures, and dirty samples can be directly injected when using a glass liner with a wool plug [30, 31]. A disadvantage to using a split injection is that trace analysis can not be done, trace samples are small to begin with so the amount injected onto the column is close to nothing [30, 31]. Also split injections hold prejudice to high molecular weight solutes present in the sample, therefore the sample entering the column is not a true representation of the sample injected [30, 31].

1.6.2 Splitless Injection

The splitless injection uses the same instrumentation as split [33]. The difference is that the purge valve which opens in a split injection is closed during a splitless injection. This is the most common method for trace quantitative analysis since nearly the whole sample is injected onto the capillary GC column [33]. The sample being injected is usually diluted in a volatile solvent (which is a disadvantage) and about 1 – 5 μl are injected into the heated port [30, 31]. Vaporization of the sample occurs and with a flow rate of about 1ml/min it is slowly transported onto the cold column where it is then condensed [30, 31]. The split valve opens about 45 seconds later and the residual vapors are purged out of the system with a flow rate of about 50ml/min [30, 31].

Splitless injections are temperature programmed and your injected sample is subjected to a temperature increase from the start of the run till the end. The splitless inlet is heated to ensure

sample vaporization and mixing with the carrier gas. The temperature program is also a disadvantage in splitless injection because it is time consuming [35]. The injection starts out on a cold column, usually around 30 °C, and then increased according to the temperature program used. Since splitless injections are diluted with a volatile solvent analytes with a boiling point lower then 30 °C may not show up on the chromatogram because they could be hiding behind the solvent peak. The lengthy time required results in sharp peaks [32]. These sharp peaks require optimized instrumental conditions such as the glass sleeve, inlet temperature, injection solvent, column temperature and dimensions, volume, and flowrates [32].

Other types of injection techniques are briefly explained in Table 1.2.

1.6.3 Sampling *Methods and Techniques*

Special sampling methods such as headspace [34], solid phase microextraction (SPME) [37, 38], and pressurized liquid extraction (PLE) [39], can be used with GCxGC systems. These techniques are used before sample injection. Headspace samples come from the area in a vial that contains no liquid or solid. It is that space of air between the bottom of the vial top and the top of the sample inside the vial. Headspace is used to inject samples (liquid or solid) that contain nonvolatile materials by heating the sealed vial/sample to a desired temperature before injecting. There are two types of headspace analysis: static and dynamic [71]. Static analysis samples the analytes under conditions of equilibrium where as in dynamic analysis the analytes are thoroughly extracted from the sample [71]. In static head space a partition between the volatile gas and sample occur and equilibrium is reached.

Table 1.2 Other types of injection techniques

Injection Technique		Reference(s):
On-Column injection	The only inlet for capillary gas chromatography where the sample is not injected into an injection port and then onto the column. A low temperature injection alleviates potential problems in two areas; syringe needle and inlet discrimination in addition to possible reactivity of sample within the inlet. With on-column cool injection the major advantage is also the disadvantage – entire sample entering the column. High-boiling, thermally labile, and reactive compounds excel with this technique. Highly volatile components are no longer lost in the hot inlet. Cold on-column injection exhibits both high resolution and good quantitation	[32, 33, 35]
Programmed temperature vaporization injection (PTV)	A split or splitless inlet that is cold at injection where the inlet is programmed to rapidly increase in temperature to transport the injected sample onto the column. Both split and splitless injections can be done with the inlet cooled, large volume injections all at once or over time can be performed. The most resourceful of all GC inlets the PTV inlet can operate as hot split and splitless; cold split and splitless; and cold split and splitless vent (large volume injection).	[31, 32, 35]
Large Volume injection	Sample volume of 100µl can be injected. Injection of large volumes using cold splitless solvent vent technique. This type of injection can be performed as one large volume injection or in smaller increments prior to entering the column.	[32, 33, 35]

The syringe removes a portion of the volatiles from the headspace and injects. The vial tops are sealed tightly to ensure that the volatile sample in the headspace will not leak out of the vial. Certain samples require specific vials, for example arson samples are collected in a new unlined 1 gallon paint cans [34], and beverage bottles for flavors and fragrances, etc. [35]. In dynamic headspace the sample never reaches equilibrium because the gases in the headspace are constantly being removed from the vial [71].

Solid phase microextraction (SPME) is an adsorption technique where polar analytes stick to the outside of a nonpolar fiber (dimethylpolysiloxane) rather then absorbing into the fiber. The SPME fiber is a small fused silica fiber coated with a thin film of stationary phase [35]. The fiber is exposed to the headspace or submerged directly into the liquid sample (extraction) and then injected into the heated GC inlet where the analytes are thermally desorbed into a capillary GC column [35, 36]. SPME has been used to extract volatiles from strawberries [37], measure sulfur vapors inside an oven cooking roast beef [38], and detect trace contaminants in environmental soil samples.

Pressurized liquid extraction (PLE) uses high-pressure heated liquids and gases such as, methanol/water mixtures and carbon dioxide respectively for static and continuous extraction [33]. It has been used to extract polycyclic aromatic hydrocarbons (PAHs) from soil [39], food analysis, as well as many other very complex sample matrices [33].

1.7 The Column

Column selection in GCxGC is similar to that of traditional gas chromatography. The ability of the column is defined by its capacity to theoretically separate a multitude of components [40]. Two-dimensional gas chromatography columns are to be orthogonal meaning

that it is necessary to use columns that impart separation mechanisms independent of each other. The orthogonality from column one to column two is necessary since analyte elution and separation is based on the intermolecular interactions taking place between the compound and the stationary phase. Intermolecular interactions taking place must be different from column one to column two. The interactions typically arise from polar-polar interactions, dispersion forces, dipole-dipole interactions, and are collectively described to be the chemical potential $\Delta\mu^{\circ}$ [41]. The chemical potential derives from the potential for the compound to be carried from the mobile to the stationary phase [41]. GCxGC columns are usually non-polar for the first dimension and polar for second dimension, therefore, the separation mechanism in the second is not correlated with that in the first [29]. That last statement according to J.B. Phillips in his last manuscript before his untimely death is true even if the two stationary phases are chemically similar and would, in one-dimensional chromatography, produce similar chromatograms [29].

The first dimension column separation mechanism depending on polarity can separate based on analyte volatility and separations by boiling point can occur. The second dimension column separation is usually dependant on the interaction(s) of the column polarity and also by the analyte volatility [7, 22]. The narrow individual fractions eluting from the non-polar first dimension column will contain analytes that share the same chemical potential or similar volatilities. The second dimension separation is very fast and alters the chemical potentials of those analytes co-eluting from the first column and separating them on the second column essentially under isothermal conditions [22].

The polarity of the stationary phase in column one and column two depends on the application and analytes injected for separation. The stationary phase of a nonpolar first dimension column used for example can be a 5% diphenyl and 95% dimethylpolysiloxane or

100% dimethylpolysiloxane column. There is not a typical second dimension column other then knowing what polarity you want. Some of the types of second dimension columns used are 35-50% phenylene – 65-50% dimethylpolysiloxane, polyethyleneglycol, phenyl-methylpolysiloxane (carborane), and cyanopropylphenyl-dimethylpolysiloxane [22]. The first column is chosen the same as you would for a typical one-dimensional GC separation, based upon the analytes being separated (if known). The choice for the "best fit" second dimension column paired with the chosen first dimension may take some time to find the combination that yields the best results. Results may be obtained using a non-polar ~ polar column separation but may not necessarily achieve the same with a polar ~ non-polar or a polar ~ polar column combination [22]. This shows that yes the separation is based primarily on volatility and polarity but also demonstrates that volatility plays a major role in the second dimension [22]. Different column sets, depending upon the compounds in your sample mixture, can offer optimum results with regard to; between-group and within-group separations, resolution of chlorinated and brominated analogues, and separation of planar from non-planar groups of analytes [7]. Examples of this from literature are shown in Table 1.3.

If columns for the first and second dimension are of similar polarity there really is no second dimension separation taking place and the analytes become distributed along a diagonal in the 2D plane. By referring to Figure 4.2 (Chapter 4) an example of this type of separation is demonstrated. In GCxGC the optimal chromatograms show analytes spread out across the two-dimensional plane with complete separations taking place in the second dimension. As this allows for clarity and assurance gained in terms of between-group and within-group separation for the most compatible column combination. In some applications,

Table 1.3 Column Selection (A brief look)

Commercial Name 1st-D	Commercial Name 2nd-D	Stationary Phase of 2nd Dimension Column	Group Separations	Ref.
DB-1	STX™-500	Phenyl polycarborane-siloxane	Separation based on the number of halogens. The high temperature phase of the 2nd dimension column is appropriate for the analysis of analytes with high boiling temperatures, brominated flame retardants and polychlorinated Biphenyl (PCB) congeners.	[7] [72]
DB-1	RTX®-35MS	65% phenyl-methylpolysiloxane	Polychlorinated *n*-alkanes (PCAs) and polybrominated diphenyl ethers (PBDEs) were successfully separated from all classes tested	[7] [73]
DB-1	RTX™-2330	90% biscyanopropyl/10% phenylcyanopropyl polysiloxane	Results within-class separations, especially non-aromatic compounds as PCAs, toxaphene, and organochlorine pesticides. Highly polar column	[7] [74]

DB-1 stationary phase is 100% methylpolysiloxane (no polarity)

*DB-1 is manufactured by Agilent J&W columns a division of Agilent Technologies www.agilent.com

the separation of the target analytes may, in most cases, interfere with the stationary phase of the column.

GCxGC chromatographers can agree that a system becomes orthogonal if it presents independent measures of molecular properties [42]. Cordero, et al. [42] conducted a study that looks closer into the influence of selectivity tuning of the stationary phase of the second dimension on orthogonality in a comprehensive GCxGC system. Ryan, et al [43] addressed the issue of maximizing use of the available separation space in two-dimensions. He states that the degree of orthogonality will depend upon how well or poorly correlated the stationary phases are of the two columns [43]. He does this by pairing the first dimension column with a polar and a non-polar column in the second dimension. The choice of different column sets brings up the point that not all GCxGC separations are "truly" orthogonal but should be looked at as the degree of orthogonality or "partial" orthogonality [43]. The separation space available is defined by assessing how much of the actual space is used with respect to the components in the sample [43].

Adahchour et al [44], published work that uses a highly polar column in the first dimension and a less polar one in the second dimension. They state that with such reverse column combinations it might be expected to yield much better chromatographic behavior on a whole and separation of the polar analytes can be more easily achieved. They did find this to be true with certain samples such as diesel oil, olive oil, and vanilla extract. Their results showed improved sensitivity and peak shapes.

1.8 Detectors

The detectors most familiarly coupled with regular GCs have been used with GCxGC. Flame ionization, electron capture, and mass spectrometers are commonly used with GCxGC systems. It has been stated previously that the separation in the second dimension must be fast and the elution of all analytes modulated as a narrow pulse should be over and done with before the first compounds contained in the next pulse reach the detector [13]. The type of modulator used will determine the peak widths as well as the columns used because of the temperature program, gas flows, and second dimension retention time [13].

1.8.1 Flame Ionization Detector

Flame Ionization Detector (FID) is the most widely used gas chromatographic detector. The column effluent is ignited in a small hydrogen-oxygen flame which produces some ions in the process [45]. The signal is born from the ions produced which in turn form a small current. The flame ionization detector is a mass-sensitive detector [75]. It responds to the number of carbon atoms entering the detector per unit time where the response is proportional to the number of carbon atoms, instead of the compound weight or moles [75]. A schematic of an FID is shown in Figure 1.6. The small jet is surrounded by a high flow of air to support combustion of the analyte [45]. Changes in the flow of air to the detector can affect the area of response, too much flow will blow out the flame and too little flow will not allow the detector to ignite. For the flame processes to be effective a balance between all the air flows and diffusions must be achieved [75]. Disturbances to the flame must be avoided to ensure proper operation of the detector and baseline stability [75]. The temperature of the FID needs to be 150°C or higher because water is produced during the combustion process and so the high temperature is needed

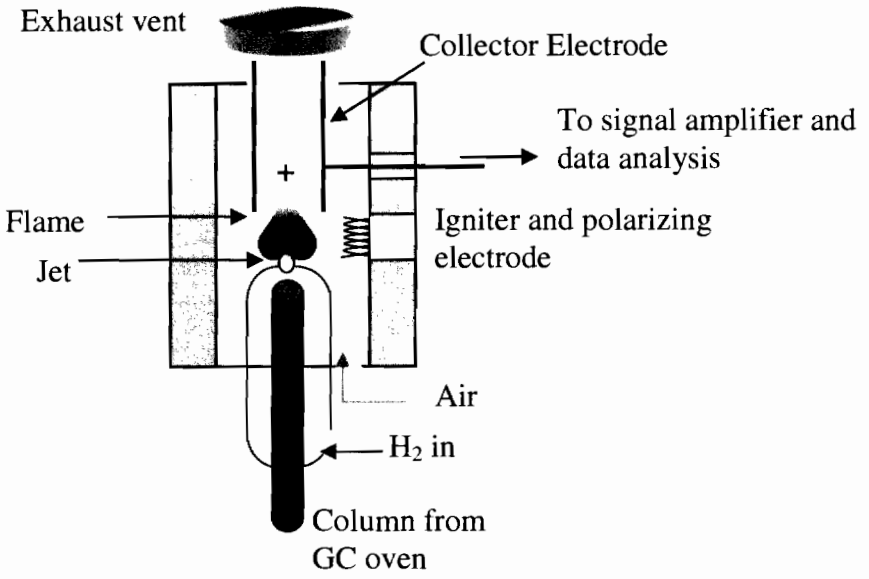


Figure 1.6 FID schematic
The FID (flame ionization detector) consists of a small flame burning at the end of a jet. As the analytes reach the flame, electrically charged species are formed and collected at an electrode. The electrode is set a few volts above the flame producing an increase in current proportional to the amount of carbon in the flame. The resulting signal is amplified by an electrometer and data collected is sent from the detector to the data analysis software which then produces a typical chromatogram [75].

to prevent condensation. For an FID to run efficiently the gases, helium, hydrogen (30-40 ml/min) and air (300 ml/min), must be pure and free of any organic content. Impurities in the carrier gases will interfere with the analysis of the sample. It is a very rugged detector with a loss of detection of 10^{-11} (50 ppb). An FID shows excellent linearity at about 10^6 and data acquisition is performed at 50 Hz and 200-300 Hz [13, 44].

GCxGC-FID has been used in the forensic analysis of ignitable liquids in fire debris [47] and is now being used in the analysis of fast pyrolysis oils to obtain maximal chromatographic output for the analytes [46]. It has also been used in the analysis of essential oils, diesel fuel, strawberry volatiles [37], environmental forensics [48], polycyclic aromatic hydrocarbons [39], and petrochemicals [22].

1.8.2 Time-of-flight Mass Spectrometry (TOF-MS)

There are many publications that couple GCxGC with time-of-flight mass spectrometry and it has become the detector of choice in GCxGC. GCxGC TOF-MS provides structural information, has a much higher level of sensitivity, and can identify unknown compounds in samples tested. GCxGC TOF-MS offers an unrivalled peak resolution and a much greater sensitivity then conventional GC/MS [38]. GCxGC TOF-MS can be used in environmental, food [38], flavor [44], fragrance, and petrochemical analysis [76].

1.9 Data Acquisition and Results

In GCxGC chromatograms are shown in either a three dimensional (3D) surface plot or a contour plot. There is an increase in the peak capacity of GCxGC because the retention times for each dimension are independent, peak capacity of the retention plane is equal to the product of the peak capacities of the two dimensions [29]. The peak capacity for the first column looks

similar to one-dimensional GC chromatograms. Peak integration for GCxGC uses the same traditional integration techniques to generate peak areas in the second dimension peaks and then summing the peak areas for each peak [49]. The second dimension peaks will increase and then decrease according to how the primary peak distribution varies on the first column [41]. A visual explanation on how a GCxGC chromatogram is created can be found by referring to Figure 1.7.

The colorful plots of GCxGC chromatograms draw the viewer in but for some it is hard to distinguish a traditional GC peak as oval-shaped. Figure 1.7 A shows three overlapping first dimension peaks. As the three overlapping peaks are modulated the chromatogram in B is formed. Four slices of each peak are formed (modulation) and then plotted (part C) across a two dimensional plane with the first dimension on the x-axis and the second dimension on the y-axis. Each contour peak (oval-shaped) overlaps in the first column but are completely separated in the two dimensional space. In order to form the three dimensional chromatogram the software program will calculate peak retention times and integrate. In Figure 1.7D the software has drawn the contour plot where the peak markers (black squares) represent the top of each peak. The color and shadings of contour and surface (three-dimensional peaks) represent the intensity in the peak height. The wire frame contour plot (E) depiction shows those four slices seen in B as four ovals inside each other. To understand how the three dimensional peaks forms from the contour plot, imagine picking up the center oval and each other one following, like stretching out an accordion. This forms the three dimensional peaks in F. The fundamental understanding of GC separation whether it is two dimensional or not demands your understanding of the GC method. The concepts of column efficiency and separation become challenging and are now compared with the performance of the two columns working simultaneously and continuously – the post-

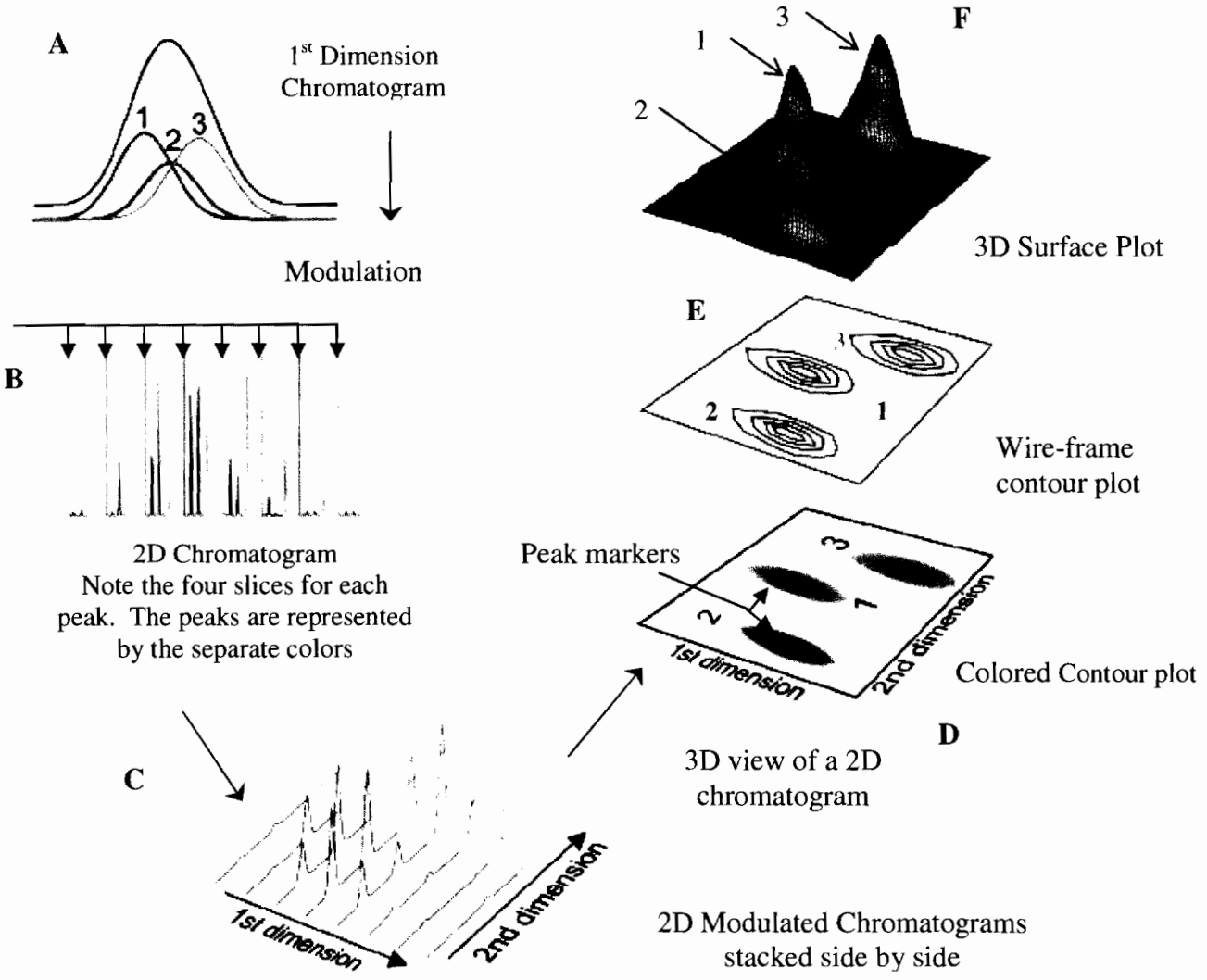


Figure 1.7: Generation of a GCxGC chromatogram – A visual explanation
***Adapted from reference [7]**

run reports are no longer as simple as they used to be since there are multiple peaks reported in the analytical results for each sample [41]. The retention times are more complex since the analyst has two numbers for each peak that relates to the modulation period in addition to the first-column retention. The software and programs allow this to be analyzed in a faster more easily understandable fashion. The retention table generated by the software includes the retention time of the first and second dimensions together, retention of each dimension alone, peak area, peak height, signal to noise ratio (S/N), and many other options if the method running is told to report. Sometimes their may be a peak on the chromatogram but no retention time in the retention table. This is due to the fact that the method's S/N is set higher then the S/N for that actual peak. The peak can be added to the table by a series of easy steps using the chromatographic plot. Libraries similar to those found on a GCxGC-MS can be made using the GCxGC-FID. If the analyst runs standards those can be added as reference standards and the program when told to will check the current results against the reference standards and if the reported result matches or is +/- 2 points of the reference it will give the name in the retention table. If there is no reference standards the software will not check against them when reporting the results. However in GCxGC-MS there are extensive mass spectral libraries just as there are in traditional GC-MS.

The two-dimensional space represents the baseline of the chromatogram. When viewing a GCxGC chromatogram the spaces where there are no peaks represents a true detector baseline or electronic noise level [41]. In conventional petroleum samples which are very complex and unresolved the response causes the detector's baseline to rise and fall throughout the run because the true electronic baseline is never obtained [41]. Instead a chemical baseline comprising small

response to many overlapping components directly indicates that there is more confidence in peak area measurements in the GCxGC run results [41].

GCxGC has grown since Philips developed it back in 1991. The major application areas which will benefit from the use of GCxGC are biological, clinical and drug testing, pharmaceutical, food or natural products, environmental, and the forensic fields. GCxGC in the pharmaceutical industry is addressed in this thesis for residual solvents. The next chapter will examine residual solvents, what they are, why they play such an important role in the development and manufacturing of the pharmaceutical drugs, and the worldwide testing method used to test for these. GCxGC is not currently being used routinely in the pharmaceutical industry and this work will demonstrate that GCxGC can be an effective tool for pharmaceutical analysis.

Chapter 2

Analysis of Residual Solvents in Pharmaceuticals

2.1 What are Residual Solvents?

Residual Solvents, also known as Organic Volatile Impurities (OVIs) are one of the three main types of impurities in pharmaceutical products. The three different categories for impurities in pharmaceutical products according to the International Conference of Harmonization (ICH) are [50]:

1. Organic impurities (process-and drug-related)
2. Inorganic impurities (manufacturing process)
3. Residual solvents

Solvents are routinely used in the synthesis and process chemistry of drug substances; they are the vehicles for the preparation of solutions or suspensions in the new drug substance [50, 51]. They cannot be completely removed from the drug substance material and their presence is not wanted. There is no therapeutic benefit from the presence of residual solvents and some solvents are known to cause unacceptable toxicity as well as hazards to the environment so there should not be any trace of them present in the final product [51]. However, these solvents may be required in manufacturing and needed to enhance stability, bioavailability, and delivery of active drugs [52]. Determining residual solvents is essential for the release testing of all active pharmaceutical ingredients and may be a critical parameter in the synthetic process [51]. Appropriate selection of the solvent for the creation of drug substance may boost the yield, or determine attributes such as crystal form, purity, and solubility [53].

2.2 Regulatory and ICH Pharmacopeias

Regulatory compliance is essential to the manufacturing of pharmaceuticals. The United States Pharmacopeia (USP) sets the standards in the US and the FDA enforces those standards. There are two main aspects of regulatory compliance the first being residual solvent levels and the second aspect is public standards set forth by the Pharmacopeias of ICH.

The International Conference on Harmonization (ICH) was created to harmonize the requirements for pharmaceutical industries on a global scale. Those requirements include method validation, stability of drug product, and impurities. The impurities in product and the methods to test for them is an issue that is the basis for this research. The acceptable maximum levels of residual solvents that can be left back according to worldwide regulatory standards were initially derived for patient safety considerations [55]. The creation of ICH brings together the regulatory authorities from the United States (USP), Europe (EP), and Japan (JP) also including scientific experts from the pharmaceutical industry. The ICH states on their website their mission statement and purpose [56]:

> The purpose is to make recommendations on ways to achieve greater harmonization in the interpretation and application of technical guidelines and requirements for product registration in order to reduce or obviate the need to duplicate the testing carried out during the research and development of new medicines. The objective of such harmonization is a more economical use of human, animal and material resources, and the elimination of unnecessary delay in the global development and availability of new medicines whilst maintaining safeguards on quality, safety and efficacy, and regulatory obligations to protect public health. Facilitate the dissemination and communication of information on

harmonized guidelines and use such as to encourage the implementation and integration of common standards.

According to ICH guidelines they have categorized residual solvents into three different classes which are based on the solvent's level of toxicity. Being that there is no therapeutic benefit from residual solvents the drug products should contain nothing higher then can be supported by safety data. The three classes of residual solvents are [53]:

1. Class 1
   - Severely toxic and should be avoided at all cost
2. Class 2
   - Less severe toxicity still with potential adverse effects on patients
3. Class 3
   - Less toxic solvents and should be used instead when practical

The United States Pharmacopeia (USP) like ICH is the official public standards-setting authority for all prescription and over-the-counter medicines, dietary supplements, and other healthcare products manufactured and sold in the United States [57]. It is an independent science-based public health organization. USP has set standards for production of quality products and their standards are used and recognized in over 130 countries. The mission statement of the USP is found on their website [57] and states:

> USP supports health of the public by developing and publishing quality standards and information for healthcare as well as medicine related products and practices. Their standards and information aid doctors and patients to maintain the improvement of issues related to health and healthcare.

The USP is recognized by the United States Food and Drug Administration (FDA). It has become the official compendium for drugs marketed in the US. All drug products marketed in the U.S. must conform to USP standards. The standards, tests, and assays are the applications that make up the numbered chapters in the USP guidelines. USP <467> is the general chapter which includes the method that is used to test for residual solvents. It has been revised constantly resulting in many supplemental and monograph changes. The original residual solvent 467 method only focused on seven solvents and applied only to drug substances and excipients [58]. Gas chromatography direct injection or headspace is the testing method used.

The first proposal to amend USP <467> was in 2003 and the revision was to be implemented July 1, 2007 but was extended to July 1, 2008. Some of the revisions have been implemented under the Interim Revision Announcement (IRA) and can be used at the present even though the new method is not. A chromatographic revision stating that the split ratio can be modified in order to optimize sensitivity fell under the IRA [58]. Some of the main points in the revision of USP 467 are the safety of patients (the driving force), performing testing only for solvents likely to be present (known solvents used during production and manufacturing), and the limits for acceptable concentrations listed in the Chapter are for drug products, not for its components [58]. In years to come other solvents may be added to the lists and solvents in the classes may be moved to another and the limits and personal daily exposure (PDE) may change based on new safety data. Table 2.1 is the list of other residual solvents that may have a growing interest. Those solvents could someday be called Class 4 Residual Solvents. Any new solvent used must be approved before it can be used in the drug manufacturing process.

There are certain assumptions about residual solvents used in the synthesis and formulation of pharmaceutical products that need to be taken into account when determining exposure limits [53]. ICH lists five reasons [53]:

1) Patients take pharmaceutical drug products everyday to treat their diseases and ailments or as preventative measures to halt infection and disease.
2) Life-time patient exposure risk assessment is not necessary for most pharmaceutical products but may be more of a working hypothesis to reduce risk to human health.
3) Residual solvents, bottom line, are unavoidable components in pharmaceutical production and will 99.9% of the time be part of drug products.
4) Exceptional circumstances which must be approved can only be the reason for residual solvents that exceed the recommended levels.
5) Data from toxicological studies that are used to determine acceptable levels for residual solvents should be created using the appropriate protocols described in, for example the EPA and the FDA red book.

Table 2.1 Other Residual Solvents*

1,1-Diethoxypropane	Methyl isopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Solvent hexane
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

- No toxicological data found to base a PDE
- These solvents may become Class 4 Residual Solvents upon growing interest

**United States Pharmacopeia, Method 467: Residual Solvents, 2007*

2.3 Class 1 Residual Solvents

Organic solvents play an essential role in the drug-substance and excipients manufacture (e.g. reaction, separation, purification) and in drug-product formulation (i.e. granulation and coating) [59]. There are only five chemicals that make up Class 1 and they are: Benzene, Carbon Tetrachloride, 1,2-Dichloroethane, 1,1-Dichloroethene, and 1,1,1-Trichloroethane. According to ICH Guideline for residual solvent impurities solvents in Class 1 should not be used to manufacture drug substances, excipients, and drug products because of their harmful environmental effect [53]. However, sometimes their use is necessary in order to produce a specific drug for a specific therapy and the levels allowed are regulated. When the solvents are used the validated procedures set forth by the USP are to be employed. The concentrations of Class 1 solvents allowed to be present are listed in Table 2.2 [53, 58].

There has been some recent research on Class 1 residual solvents in pharmaceuticals using headspace-programmed temperature vaporization-fast gas chromatography-mass spectrometry [55]. They employed a sensitivity method in which different injection techniques were compared. In the pharmaceutical industry the quality control/quality assurance (QA/QC) testing method for residual solvents is static headspace-gas chromatography and a simple sampling technique of direct injection [55, 60]. The problem with direct injection is that less volatile sample compounds can remain on the column stationary phase decreasing the column lifespan and cause interferences with further analyses performed [60]. Exploring new ways to improve sensitivity keeping headspace instrumentation the researchers used a programmed temperature vaporizer inlet to inject the samples of Class 1 residual solvents into the column [55].

Table 2.2 Class 1 solvents – allowed concentration levels

Solvent	Concentration allowed (ppm)*~	Avoidance
Benzene	2 ppm	Carcinogen
Carbon tetrachloride	4 ppm	Toxic and environmental hazard
1,2-Dichloroethane	5 ppm	Toxic
1,1-Dichloroethene	8 ppm	Toxic
1,1,1-Trichloroethane	1500 ppm	Environmental hazard

**International Conference on Harmonization (ICH) of Technical Requirements for the registration of Pharmaceuticals for Human Use, Q3C: Impurities: Guideline for Residual Solvents, Step 4, July 1997*
~United States Pharmacopeia, Method 467: Residual Solvents, 2007

2.4 Class 2 Residual Solvents

Class 2 contains 26 residual solvents, Table 2.3, and should be limited and specifically tested in pharmaceutical products because of their inherent toxicity [53, 58]. Table 2.3 lists the PDE and concentration limits for all 26 residual solvents which range from 50 ppm to 3880 ppm. Whenever class 2 residual solvents are used or produced in the manufacturing process they are to be identified and quantified [58]. Analytical precision should be determined as part of the validation of the method.

The ICH guideline has two different approaches when setting limits for class 2 solvents; the first approach is to be used when the PDE cannot be estimated in a drug because the dose is not known or very inconsistent [59]. The defined concentration limits are calculated based on the daily intake or daily dosage of a "theoretical" drug product mass of 10g [59]. The second option should be employed if the drug product exceeds the 10g mass. Application of option 2 is to be used when the dose is known and then the PDE and dose value can be used the estimate the acceptable concentration in that specific pharmaceutical product [59]. These same two options are similarly stated in USP 467. The two options in USP 467 for determining levels of class 2 residual solvents in drug products are: option 1 components of drug product (drug substances and excipients) meets the concentration limits listed in Table 2.3 has a daily dose that does not exceed 10g; option 2 if one of the components of the drug product exceeds the concentration limits in Table 2.3 or the daily does exceeds 10g then the daily exposure to a solvent (calculated as the sum of the components contributions) is to be less then the PDE for each specific solvent [58].

The USP gives pass/fail examples of option 1 and 2 with Acetonitrile – a major player in pharmaceutical drug production.

Table 2.3 Class 2 Residual Solvents[*⁂] – daily exposure and concentration

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichlorothene	18.7	1870
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
Methylene chloride	6.0	600
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran (THF)	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
Trichloroethylene	0.8	80
(o-, m-, p-) Xylenes[♦]	21.7	2170

[♦⁂] xylenes – 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethyl benzene

**United States Pharmacopeia, Method 467: Residual Solvents, 2007*
⁂International Conference on Harmonization (ICH) of Technical Requirements for the registration of Pharmaceuticals for Human Use, Q3C: Impurities: Guideline for Residual Solvents, Step 4, July 1997

Equation used to calculate concentration required for Option 1

$$\text{Concentration (ppm)} = \frac{1000 \times \text{PDE}}{\text{dose}} \quad (1)$$

Example 1&2 – USP 467 [58]

The PDE for acetonitrile is 4.1 mg/day, Table 2.3, with 410 ppm – which meets the limit for option 1. The sample tested is 5.0 g drug product per day and is composed of two excipients:

Component	Amount in formulation (g)	**OPTION 1** Acetonitrile Content-Limit (ppm)	**OPTION 2** Daily Exposure (mg)
Drug Substance	0.5	409 (pass)	0.20
Excipient 1	0.6	350 (pass)	0.21
Excipient 2	2.5	600 (exceeds 410 ppm)	1.5
Drug Product	5.0	617 (exceeds 410 ppm)	**3.08** **PASS**
United States Pharmacopeia, Method 467: Residual Solvents, 2007			

- Drug substance and excipient 1 acetonitrile content-limit is 409 ppm and 350 ppm respectively, which is less then the 410 ppm/day - it meets the limit for option 1
- Excipient 2 and drug product acetonitrile content-limit is much greater then 410 ppm/day and so they do not meet the limit for option 1
- The drug product is below the 4.1 mg/day daily exposure limit for acetonitrile – meeting the limit of option 2
- Drug Product – PASSES

Component	Amount in formulation (g)	**OPTION 1** Acetonitrile Content-Limit (ppm)	**OPTION 2** Daily Exposure (mg)
Drug Substance	0.5	506 (exceeds 410 ppm)	0.25
Excipient 1	0.6	400 (pass)	0.24
Excipient 2	2.5	970 (exceeds 410 ppm)	2.42
Drug Product	5.0	1106 (exceeds 410 ppm)	**5.53** **FAILS**
United States Pharmacopeia, Method 467: Residual Solvents, 2007			

- Drug product does not meet option 1 or option 2
- Excipient 1 meets the 410 ppm limit for Option 1
- Drug substance and excipient 2, do not meet option 1 and are much higher then 410 ppm
- Drug product failed and so the manufacturer can test to see if manufacturing reduced the level of acetonitrile in drug product below 410 ppm – drug product can then pass

United States Pharmacopeia, Method 467: Residual Solvents, 2007

2.5 ICH Class 3 Residual Solvents

There are 28 solvents in class 3 and they are less toxic and do not pose a large risk to human health. For all class 3 solvents, Table 2.4, the PDE is no more then 50 mg/day at a concentration limit of 5000 ppm for daily doses not exceeding 10 g of product [58]. The only time class 3 solvents are to be identified and quantified is if the manufacturer exceeds more then 50 mg/day. If only class 3 solvents are present, they may be quantified by loss on drying, with quantitation by gas chromatography if the level exceeds 5000 ppm.

USP and ICH state that Class 3 residual solvents cause no hazards to human health. That statement is only in regards to the levels normally accepted in pharmaceuticals [53]. However, long-term exposure, toxicity, and carcinogenicity for some of the class 3 solvents have not been studied and are not known. The data available for the class 3 solvents suggests that they are less toxic in short-term studies and negative in genotoxicity studies [53]. The accepted levels for daily exposure are no more then 50 mg/day at 5000 ppm or 0.5% under the same conditions of option 1 for the class 2 solvents [53]. Acceptable higher amounts can be used as long as they are realistic in relation to manufacturing capability and good manufacturing practice (GMP) [53].

Modern drug-manufacturing processes use Class 3 solvents as much as possible for re-crystallization and conditioning. The amount present in the final product is generally 0.5% and causes analytical challenges. Class 3 together with lower quantities of class 1 and 2 solvents requires analytical columns with large sample capacity [59]. To avoid unwanted effects such as back-flush and injection discrimination low split ratios are necessary in certain cases and careful optimization is required [59].

Table 2.4 Class 3 Residual Solvents*⁂

Solvent	PDE (mg/day)	Concentration Limit (ppm)
Acetic Acid		
Acetone		
Anisole		
1-Butanol		
2-Butanol		
Butyl acetate	All Class 3 Residual Solvents	
tert-butylmethyl ether	50 mg or less is acceptable,	
Cumene	no more than that	
Dimethyl sulfoxide		5000 ppm daily dosages
Ethanol		not greater than 10g of
Ethyl acetate		product
Ethyl ether		
Ethyl formate	Less toxic and lower risk of	
Formic acid	Human health	
Heptane		
Isobutyl acetate		
Isopropyl acetate		
Methyl acetate		
3-Methyl-1-butanol		
Methylethylketone		
Methylisobutylketone		
2-Methyl-1-propanol		
Pentane		
1-Pentanol		
1-Propanol		
2-Propanol		
Propyl acetate		

**United States Pharmacopeia, Method 467: Residual Solvents, 2007*
⁂International Conference on Harmonization (ICH) of Technical Requirements for the registration of Pharmaceuticals for Human Use, Q3C: Impurities: Guideline for Residual Solvents, Step 4, July 1997

2.6 Residual Solvent Pharmaceutical Testing Methods

There are many different analytical techniques that have been used to test for residual solvents. The main technique used and required by the USP is GC because it is easy to use, highly selective and sensitive, sample prep is very simple, and can be automated very easily [59]. Headspace sampling, direct injection and solid-phase microextraction (SPME) are the three main types of GC procedures used to test for residual solvents. FID is by far the most preferred detector to use because it allows for low detection limits, wide linear dynamic range, ease of operation and it is very reliable and robust [59]. A mass spectrometer (MS) can bring spectrometric identification to the table when coupled with the GC. In order to achieve optimal results the column used can affect results whether using headspace gas chromatography or gas chromatography mass spectrometry.

Regulatory agencies and pharmacopeias require head space gas chromatography as the most fitting technique for residual solvent testing for active substances and formulations soluble in water [59]. In USP 467 Class 1 and 2 solvents have three different main testing procedures: A, B, and C as well as other analytical techniques. The organic-free water specified in those procedures produces no significantly interfering peaks on the GC chromatogram [58]. For water-soluble Class 1 and 2 samples the USP requires standard stock, standard, and test solutions to be prepared and ran on headspace GC-FID. The rest of the chromatographic method parameters for each procedure are represented in Table 2.5 and the headspace operating parameters can be found in Table 2.6 [58]. For water-insoluble Class 1 and 2 samples the USP requires standard stock, standard, and system suitability solutions to be ran on a headspace GC-FID. The same chromatographic method, Table 2.6, is used except the procedures themselves change. Since the samples tested are water-insoluble for Procedure A Class 2 standard stock and

Table 2.5 USP 467 Chromatographic method – Class 1 and 2 Solvents

	Procedure A	Procedure B	Procedure C
Column	0.32 mm × 30 m fused silica 1.8 µm layer of phase G-43 (6% cyanopropylphenyl, 94% dimethylpolysiloxane) or 0.53 mm × 30 m wide-bore column coated with a 3.0 µm layer of phase G43	0.32 mm × 30 m fused silica 0.25 µm layer of phase G16 (WAX – polyethylene glycol) or 0.53 mm × 30 m wide-bore column coated with a 0.25 µm layer of phase G16	0.32 mm × 30 m fused silica 1.8 µm layer of phase G-43 (6% cyanopropylphenyl, 94% dimethylpolysiloxane) or 0.53 mm × 30 m wide-bore column coated with a 3.0 µm layer of phase G43
Carrier Gas	nitrogen or helium linear velocity 35 cm/s	nitrogen or helium linear velocity 35 cm/s	nitrogen or helium linear velocity 35 cm/s
Injector Temperature	140° and 250°	140° and 250°	140° and 250°
Split – Ratio	1 : 5	1 : 5	1 : 5
Temperature Program	40° for 20min, 10° per min to 240°, hold at 240° for 20 minutes	50° for 20min, 6° per min to 165°, hold at 165° for 20 minutes	40° for 20min, 10° per min to 240°, hold at 240° for 20 minutes
Procedure	Separate equal volume injections according to headspace parameters in Table 2.6 of Class 1 and 2 standard solutions and test solution. Measure responses for the major peaks and compare to the peaks in Test Solution. If any class 1 and 2 peaks are ≤ the peaks in the Test Solution then go to Procedure B to verify peak identity.	Separate equal volume injections according to headspace parameters in Table 2.6 of Class 1 and 2 standard solutions and test solution. Measure responses for the major peaks and compare to the peaks in Test Solution. If the peak(s) response in test solution of Procedure A is/are ≤ to a corresponding peak in class 1 and 2 go to Procedure C to quantify otherwise the sample meets the requirements	Separately inject equal volumes following procedure in Table 2.6 of the standard, test, and spiked test solutions and measure responses of major peaks. Calculate the ppm of each residual solvent found in the sample using this formula: $5(C/W)[r_u/(r_{st}-r_u)]$ C = conc. (ppm) of USP ref. std. in std. solution, W=mass (g), r_u =test solution & r_{st} = rtn. time in spiked test solution.

**United States Pharmacopeia, Method 467: Residual Solvents, 2007*

Table 2.6 USP 467 Headspace Operating Parameters

	Headspace Operating Parameter Sets		
	1	2	3
Equilibration Temperature	80°C	105°C	80°C
Equilibration time	60 min.	45 min	45 min
Transfer-line temperature	85°C	110°C	105°C
Carrier gas: nitrogen or helium at an appropriate pressure			
Pressurization time	30s	30s	30s
Injection volume	1 ml	1 ml	1 ml

**United States Pharmacopeia, Method 467: Residual Solvents, 2007*

stock solutions are diluted with 1, 3-dimethyl-2-imidazolinone (DMI), the test stock solution is diluted with dimethylformamide (DMF), and the test solution 2 is diluted with 1,3-dimethyl-2-imidazolinone. Test solution 2 is used to identify DMF and/or *N, N*-dimethylacetamide (*N, N*-DMA) in the sample being tested. The procedures used are similar to those for the water-soluble samples, i.e. if the retention time for the peaks are greater then or equal to a corresponding peak in standard, and test solutions then proceed to procedure B to be identified, otherwise the sample meets the requirements of the test [58]. For Procedure B of water-insoluble samples the standard, suitability, and standard stock solutions are to be prepared as directed in Procedure A for water-soluble samples. But the class 2 standard stock solutions C is prepared as is directed for Procedure A and class 2 system suitability solution and the chromatographic method is to be done according to procedure B – water-soluble samples [58]. If the peaks are greater then or equal to the corresponding peaks in the standards then proceed to procedure C to be quantified otherwise the sample meets the test requirements. In procedure C, standard solution 1 has to have a final concentration of 1/20 of the value stated in Table 2.2 and 2.3. The test solution and test stock solution follows the same prep as did in Procedure A but a separate spiked test solution for each peak identified and verified by Procedures A and B is to be prepared and tested on the GC. Separate injections of equal volumes for the standard, test, and spike test solutions are to be ran and recorded. The amount of residual solvent present in ppm is to be calculated using equation 2.1: C = the concentration (ppm) of the correct USP Reference Standard in the Standard Solution, W = weight (g) of the sample, r_u = peak responses of each residual solvent from test solution 1 or 2, and r_{st} = peak responses from spiked test solution 1 or 2 [58].

$$5(C/W)\left[\frac{r_u}{(r_{st}-r_u)}\right] \qquad 2.1$$

If only Class 3 residual solvents are present the level is to be determined by USP ⟨731⟩ Loss on Drying (LOD). If the LOD value is greater then 0.5% a water determination should be performed on the sample using USP ⟨921⟩ Water determination [58]. If it is greater then 50 mg/day then the residual solvent should be identified and quantified as is done in Option 1 [58].

There are many challenges with the static headspace GC residual solvent analysis [59]. In the chromatographic separation of all ICH solvents there are some solvents that do not cooperate. These solvents either consistently co-elute or react with the stationary phase of the column resulting in their non-presence on the chromatogram. Some ways to remedy this may lie in the column choice because the stationary phases, film thickness (megabore), and column lengths affect the selectivity of analyte separation. Head space – gas chromatography can at times take much longer then a typical gas chromatographic injection and so the sample turn-around and separation speed in the method needs to be re-evaluated by changing the flow rates (higher), using a more efficient column, and changing the temperature program as well as the pressure program [59]. Certain solvents that have a low solubility in water are inefficient and exhibit low sensitivity when analyzed using head space – gas chromatography. To improve the efficiency of the sample injected reducing the sample volume and maximizing the headspace volume as well as reducing the split ratio should yield more desirable results. Optimizing the headspace extraction parameters, i.e. conditioning time, loop-injection parameters, together with injector temperatures and liner volume is another way to improve sensitivity [59]. The sample matrix, depending on which one used (DMSO, methanol, water), can cause many interferences on the chromatogram because of the impurities that it may contain or chemical reactions it may have with sample components. The last major challenge facing head space – gas chromatography is the reproducibility. In the pharmaceutical world any method used must be

reproducible not only in the research laboratory but also in quality control and manufacturing. Some solvents react with the septa in the vial and the inlet septa generating high RSD values, resulting in the use of another type of septa and or vial [59].

2.7 Other testing methods for Residual Solvents

Headspace solid phase microextraction (HS-SPME) has become more popular and currently the method of analysis in many different research studies as well as a current one in the analytical chromatography group at Seton Hall University [59, 61, 62]. In HS-SPME the liquid sample being tested is adsorbed onto a fiber until the fiber adsorption capacity is reached and equilibrium between the HS and fiber is achieved [59]. The fiber is then placed into the injector where the analytes are desorbed from the fiber and injected onto the column. HS-SPME applications in residual solvent analysis for the pharmaceutical industry are not used or not used as much as seen in other fields, because HS-SPME is not yet recognized by the USP.

The next chapter will discuss how GCxGC was used to successfully detect residual solvents using one single method.

Chapter 3

Determination of Residual Solvents

3.1 Research Introduction

The challenge in developing a single comprehensive gas chromatographic method for pharmaceutical solvents presents itself as there are about 60 compounds in classes 1-3 and the allowable concentration limits may range over 3 orders of magnitude (2-5000 ppm for a 10 g dose of the drug) within the same sample [64]. While there has been considerable work and there are numerous residual solvents methods reported in the literature, a single comprehensive method encompassing both challenges in one run does not currently exist. Most methods were designed for individual problems and involve the separation of 3-10 solvents with run times of 10-30 minutes or more. The large number of compounds coupled with varying chemistry demands the use of separations run on multiple columns of differing selectivity if a single method to separate all of them in a reasonable amount of time, as described in USP <467>, is desired.

Throughout this research we demonstrate the use of GCxGC for comprehensive separation of the class 1, 2 and 3 ICH pharmaceutical residual solvents. We discuss the significant additional selectivity and separation advantages from using the second column and demonstrate that comprehensive two-dimensional gas chromatography can perform the identification, confirmation and quantitation steps required by ICH and described in USP <467> in a single experiment.

3.2 Experimental

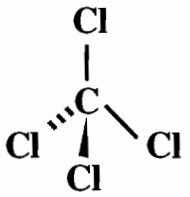
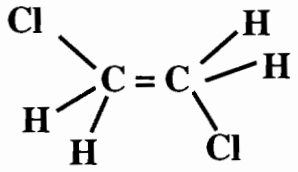
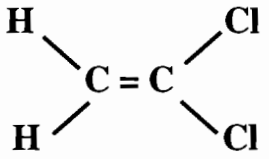
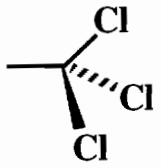
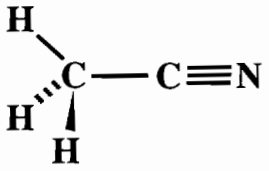
3.2.1 Chemicals and Reagents

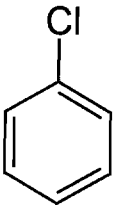
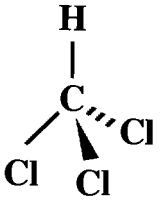
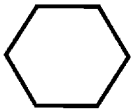
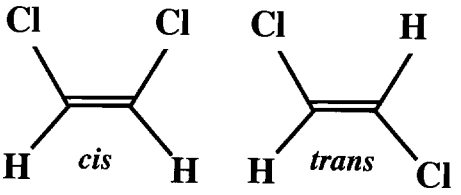
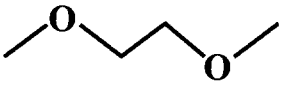
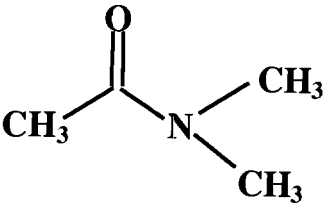
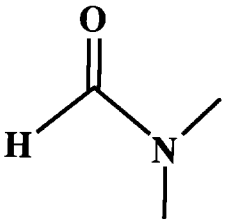
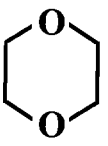
All the class 1, 2, and 3 solvents as well as the diluents were purchased from Sigma-Aldrich (St. Louis, MO) and were used without further purification. Table 3.1 shows all the class 1, 2, and 3 solvents as well as their chemical and structural formula that were used to perform this research. The purity of those solvents was either of GC grade or HPLC grade. The highest level of purification was purchased in order to minimize the trace amounts of contaminants that are sometimes found in the solvents which then show up on the GC chromatogram. The solvents used as the diluents were mainly Methanol as well as DMSO (dimethyl sulfoxide). Stock solutions of each individual residual solvent were measured using calibrated manual or electronic pipettes.

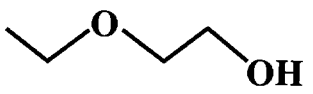
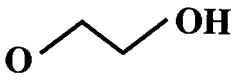
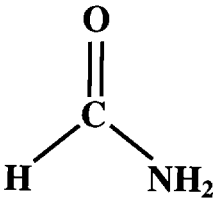
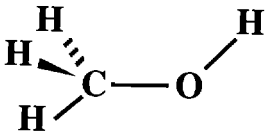
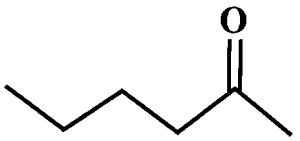
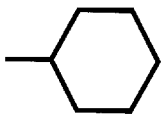
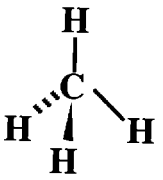
3.2.2 GCxGC Instrumentation

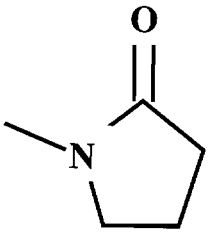
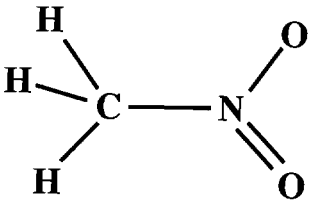
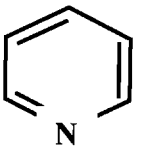
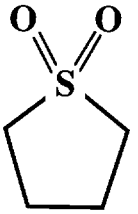
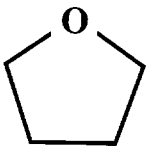
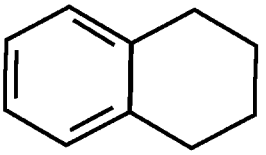
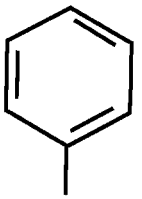
All the work was performed on Leco GCxGC-FID system (Leco Corporation, St. Joseph, MI) using Chroma-TOF FID software for both instrument control and data analysis (Leco Corporation, St. Joseph, MI). The modulator, secondary oven, and associated GCxGC electronic and pneumatic components were installed into an Agilent 6890 Gas Chromatograph Flame Ionization Detector (Agilent Technologies, Wilmington, DE). All injections were done using a CTC Analytics Combi-PAL autosampler (Leap Technologies, Carrboro, NC) which was software controlled using the Chroma-TOF FID software. The modulator utilized a liquid nitrogen dewar to deliver the cold air from the jets. The liquid nitrogen was controlled by the Model 186 Liquid level controller (AMI- American Magnetics, Inc., Oak Ridge, TN) which could pump liquid nitrogen from the LS240 tank into the dewar attached to the GC manually or software controlled. Both manual and software controlled delivery of liquid N_2 was used in this

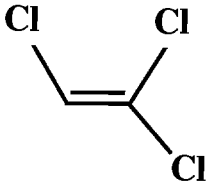
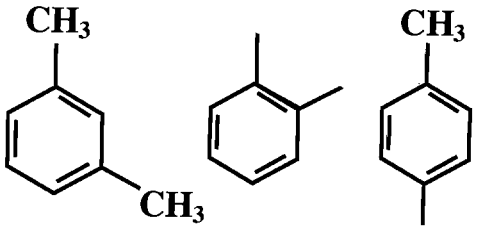
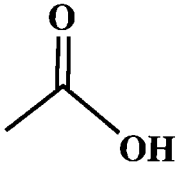
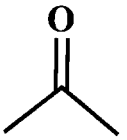
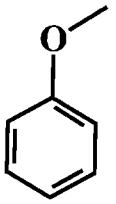
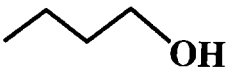
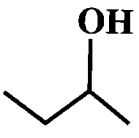
Table 3.1 List of Solvents Used

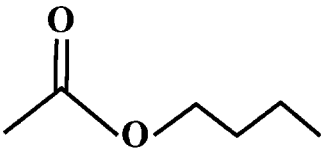
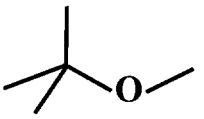
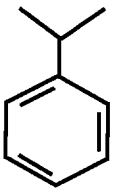
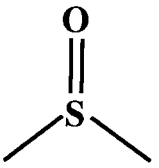
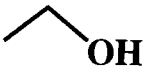
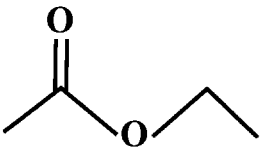
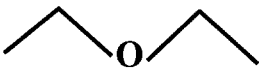
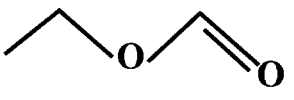
Solvent	Chemical Formula	Structure	Class
Benzene	C_6H_6		Class 1
Carbon Tetrachloride	CCl_4		Class 1
1,2-dichloroethane	$C_2H_4Cl_2$		Class 1
1,1-dichloroethene	$C_2H_2Cl_2$		Class 1
1,1,1-trichloroethane	CH_3CCl_3		Class 1
Acetonitrile	CH_3CN		Class 2

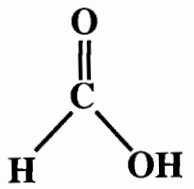
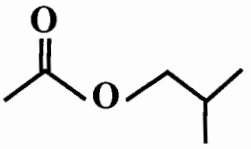
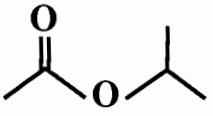
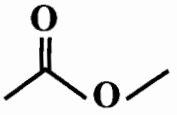
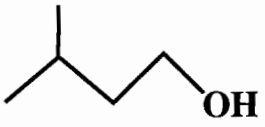
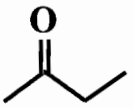
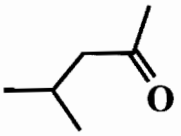
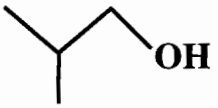
Chlorobenzene	C_6H_5Cl		Class 2
Chloroform	$CHCl_3$		Class 2
Cyclohexane	C_6H_{12}		Class 2
1,2-Dichlorothene	$C_2H_2Cl_2$		Class 2
1,2-Dimethoxyethane	$C_4H_{10}O_2$		Class 2
N,N-Dimethylacetamide (DMA)	C_4H_9NO		Class 2
N,N-Dimethylformamide (DMF)	C_3H_7NO		Class 2
1,4-Dioxane	$C_4H_8O_2$		Class 2

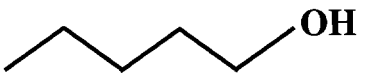
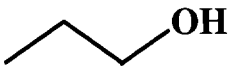
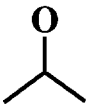
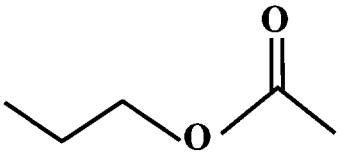
2-Ethoxyethanol	$C_4H_{10}O_2$		Class 2
Ethylene glycol	$C_2H_4(OH)_2$		Class 2
Formamide	CH_3NO		Class 2
Hexane	C_6H_{14}		Class 2
Methanol	CH_3OH		Class 2
2-Methoxyethanol	$C_3H_8O_2$		Class 2
Methylbutylketone	$C_6H_{12}O$		Class 2
Methylcyclohexane	C_7H_{14}		Class 2
Methylene chloride	CH_2Cl_2		Class 2

N-Methylpyrrolidone	C_5H_9NO		Class 2
Nitromethane	CH_3NO_2		Class 2
Pyridine	C_5H_5N		Class 2
Sulfolane	$C_4H_8O_2S$		Class 2
Tetrahydrofuran (THF)	C_4H_8O		Class 2
Tetralin	$C_{10}H_{12}$		Class 2
Toluene	C_7H_8		Class 2

Trichloroethylene	C_2HCl_3		Class 2
(o-, m-, p-) Xylenes	C_8H_{10}	 *m*-Xylene *o*-Xylene *p*-Xylene	Class 2
Acetic Acid	CH_3COOH		Class 3
Acetone	CH_3COCH_3		Class 3
Anisole	$C_6H_5OCH_3$		Class 3
1-Butanol	$C_4H_{10}O$		Class 3
2-Butanol	$C_4H_{10}O$		Class 3

Butyl acetate	$C_6H_{12}O_2$		Class 3
tert-butylmethyl ether (MTBE)	$C_5H_{12}O$		Class 3
Cumene	C_9H_{12}		Class 3
Dimethyl sulfoxide (DMSO)	C_2H_6OS		Class 3
Ethanol	C_2H_5OH		Class 3
Ethyl acetate	$C_4H_8O_2$		Class 3
Ethyl ether	$C_4H_{10}O$		Class 3
Ethyl formate	$C_3H_6O_2$		Class 3

Formic acid	CH_2O_2		Class 3
Heptane	C_7H_{16}		Class 3
Isobutyl acetate	$C_6H_{12}O_2$		Class 3
Isopropyl acetate	$C_5H_{10}O_2$		Class 3
Methyl acetate	$C_3H_6O_2$		Class 3
3-Methyl-1-butanol	$C_5H_{12}O$		Class 3
Methylethylketone	C_4H_8O		Class 3
Methylisobutylketone	$C_6H_{12}O$		Class 3
2-Methyl-1-propanol	$C_4H_{10}O$		Class 3
Pentane	C_5H_{12}		Class 3

1-Pentanol	$C_5H_{12}O$		Class 3
1-Propanol	C_3H_8O		Class 3
2-Propanol	C_3H_8O		Class 3
Propyl acetate	$C_5H_{10}O_2$		Class 3

research. Figure 1.1 shows the actual instrumental setup used in this research. The inlet was a split/splitless and the detector used was an FID. The carrier gas was helium in constant pressure mode (method 1) with column pressure at 25 psi. Constant flow mode was used for method 2 and had a column flow of 2.8 ml/min. The FID temperature was 250°C with air makeup at 450 ml/min and hydrogen at 40 ml/min. The pressure of nitrogen gas used to deliver the hot pulse was around 15 psi. The data acquisition from the FID detector had a rate of 100/sec because the data coming from the second dimension was very rapid and it was needed in order to construct the chromatograms correctly.

3.2.3 Columns and Temperature Programs

The 1st dimension column used for all analysis was an RTX-5 MS (Restek, Bellefonte, PA) fused silica 5% diphenyl and 95% dimethyl polysiloxane. The RTX-5 column was 30 m in length with an internal diameter (ID) of 0.25 mm and a 1 μm film thickness. The second dimension column used during the first half of the research was a moderately polar DB-17 (Agilent Technologies, Wilmington, DE) 50% dimethyl polysiloxane and 50% diphenyl polysiloxane. The second dimension column is cut and installed manually into the second dimension oven and its length is much smaller then that of the first dimension. The second dimension column length is about 1 m (+/- a few centimeters) and has an internal diameter of 0.10 mm with a 0.1μm film thickness. Helium was the carrier gas used for all analysis. For the second half of the research a more polar second dimension column was used. The column used was an RTX-Wax (Restek, Bellefonte, PA) polyethylene glycol 1.1 m length, 0.25 mm internal diameter, and had a 0.5 μm film thickness. There were two different temperature programs used in this research. The temperature programs corresponded with the column's max temperature, according to the manufacturer's column specifications. For GCxGC method 1 the RTX-5

column had a max temperature of 350°C and the DB-17 max temp was 300°C. The primary oven temperature ramp started at 35°C held there for 4.00 minutes and then ramped up 10°C per minute to 240°C. The secondary oven's initial temperature was 75°C for one minute then ramped up 10°C per minute to 280°C. In GCxGC method 2 the primary oven initial temperature was 35°C hold for 10 minutes then increase 10°C per minute to 220°C. The secondary oven initial temperature was 75°C hold for 10 minutes then ramp up 10°C per minute to 250°C and hold for 1 minute. The temperature programs and column specifications are also illustrated in Table 3.2. The inlet temperature for both methods 1 and 2 was set at 250°C.

3.2.4 Modulation

The modulation scheme installed in the GCxGC used in this research was a dual jet modulator. Liquid Nitrogen was used for the cold jet and the hot jet was a pulse of warm Nitrogen air. Figure 1.3 in Chapter 1 demonstrates the operation of the modulation jets best. Each jet can be a hot or cold pulse, depending on the modulation scheme used.

The modulation scheme used in Method 1 was programmed to maintain a temperature 40°C higher then the main (primary) GC oven, not the secondary one. The modulator operated the jets continuously throughout the entire run, from injection to finish. The scond dimension separation time was 2 seconds with the total of 4 pulses, 2 for the hot and 2 pulses for the cold jet. The modulator gave a 0.40 sec hot pulse and a 0.60 sec cooling time between stages. The modulation scheme used in Method 2 was programmed to maintain 40°C higher then the main oven. The second dimension separation time was 4 seconds with two 0.40 sec hot pulses and a cooling time between stages of 1.60 seconds. The modulation scheme is also found in Table 3.2.

Table 3.2 GCxGC Method Parameters

	Method 1			Method 2		
1st Dimension Column	RTX-5MS 5% diphenyl 95% dimethyl polysiloxane			RTX-5MS 5% diphenyl 95% dimethyl polysiloxane		
2nd Dimension Column	DB-17 50% dimethyl polysiloxane 50% diphenyl polysiloxane			RTX-WAX polyethylene glycol		
Carrier Gas	Helium			Helium		
Inlet Type	Split/Splitless			Split/Splitless		
Inlet Mode	Split			Split		
Column Mode	Constant Pressure			Constant Flow		
Column Pressure / Flow	25 psi (column pressure)			2.8 mL/min (column flow)		
Split Ratio	100			100		
Split Flow (mL/min)	117.4 mL/min			284.4 mL/min		
Total Flow (mL/min)	118.6 mL/min			287.3 mL/min		
Inlet Temperature (°C)	250°C			250°C		
Oven Equilibration Time	1 minute			1 minute		
Temperature Ramp – Main Oven	Rate °C/min	Temp	Duration	Rate °C/min	Temp	Duration
	Initial	35°C	4.0 min	Initial	35°C	10 min
	10°C/min	240°C	0	10°C/min	220°C	0
Temperature Ramp – 2nd Oven	Rate °C/min	Temp	Duration	Rate °C/min	Temp	Duration
	Initial	75°C	1 min	Initial	75°C	10 min
	10°C/min	280°C	0	10°C/min	250°C	1 min
Modulator Temperature Offset	40°C higher then main oven			40°C higher then main oven		
2nd Dimension Separation Time	2 sec			4 sec		
Hot Pulse	0.40 sec			0.40 sec		
Cool Time between stages	0.60 sec			1.60 sec		
Detector	FID			FID		

3.3 Sample Preparation

3.3.1 Method 1

ICH class 1, 2, and 3 solvents were prepared in methanol and in dimethyl sulfoxide (DMSO). All the solvents were prepared in methanol before injection on the GCxGC. Manual and digital pipettes were used to pipette 50µl of each solvent into 50mL of methanol. The methanol was measured using analytical grade graduated cylinders and placed into 100mL washed and methanol rinsed glass bottles. Class 2 solvents were placed in 4 groups and class 3 solvents were placed in 5 groups. The solvents in each group were arranged according to their increasing boiling points. There was only one group for class 1 because there are only 5 solvents in that class. Each class was then placed in one group and injected onto the GCxGC. After the mix for each class was analyzed the three classes were combined into one large mixture and injected.

Dimethyl sulfoxide was the other diluent used for this method and was only used with the class 2 solvents. Three groups of class 2 solvents were prepared with varying concentrations. The concentrations of each solvent were determined according to the USP OVI Solvent (United States Pharmacopeia Organic Volatile Impurities) standards sold by Restek. Restek solvent concentrations for mix A, B, and C were given in µg/mL and placed in dimethyl sulfoxide, 1mL/ampul. The concentrations given were converted into µL of analyte / mL of solvent (methanol). The solutions were made 20 times more concentrated then the Restek mixes. The concentrations used compared to the Restek ones are in Table 3.3

Table 3.3 Residual Solvent Concentration vs. Restek

Residual Solvent	Restek Concentration* 1mL/ampul DMSO	Calculated volumes used µl/100mL of DMSO	Actual volumes used µl/100mL of DMSO
Class 2 Mix A			
Acetonitrile	2.05 µg/mL	5.21 µl/100mL	5.20 µl/100mL
Chlorobenzene	1.80	3.24	3.25
Cyclohexane	19.4	49.8	50.0
cis-1,2-dichloroethylene	4.70	7.32	7.30
trans-1,2-dichloroethylene	4.70	7.47	7.50
1,4-dioxane	1.90	3.67	3.70
Ethylbenzene	1.84	4.24	4.25
Methanol	15.0	37.8	40.0
Methylcyclohexane	5.90	15.3	15.0
Methylene chloride	3.00	4.25	4.25
Tetrahydrofuran	3.45	7.75	7.80
Toluene	4.45	10.2	10.2
m-xylene	6.51	15.0	15.0
o-xylene	0.98	2.18	2.20
p-xylene	1.52	3.53	3.50
Class 2 Mix B			
Chloroform	300 µg/mL	400 µl/100mL	400 µl/100mL
1,2-dimethoxyethane	500	1100	1100
n-hexane (C6)	1450	4400	4400
2-hexanone	250	600	600
Nitromethane	250	400	400
Pyridine	1000	2000	2000
Tetralin	500	1000	1000
Trichloroethylene	400	500	500
Class 2 Mix C			
2-ethoxyethanol	800 µg/mL	1700 µl/100mL	1700 µl/100mL
Ethylene glycol	3100	5500	5500
Formamide	1100	1900	1900
N,N-dimethylacetamide	5450	11600	11600
N,N-dimethylformamide	4400	9300	9300
2-methoxyethanol	250	510	510
N-methylpyrrolidone	2650	5100	5100
Sulfolane	800	1200	1200

**Literature Catalogue #59085A 2006 Restek Corporation*

3.3.2 Method 2

Methanol was the only solvent used as the diluent for method 2. The components in each mixture were kept the same as the DMSO groups. Each of those mixtures was divided into smaller groups containing only 3-4 components in each. The concentrations of each solution were 1000ppm, 20μL of solvent in 20mL of methanol. Mix A was broken into 5 different groups (Mix1A, 2A, 3A, 4A, and 5A), mix B was comprised of 2 groups (1B, 2B), and mix C had 3 groups (1C, 2C, 3C). Each group was injected into the GCxGC and standard retention times were recorded. The same was done for class 1 and class 3 solvents. The mixes for class 3 were labeled class 3 mix A (1A, 2A, 3A), class 3 mix B (1B, 2B), and class 3 mix C (1C, 2C, 3C). Since there is only 5 solvents in Class 1 only two mixtures were made, 1A and 2A. The components of each mixture can be found by referring to Table 3.4.

The mixtures in each class were run separately to determine the retention time of each solvent, characterize and assign peaks. If any solvent did not show up on the chromatogram the specific analyte was ran alone to determine its retention time, and then ran again in the group to verify retention time and assign the peaks. For class 2 each mixture was combined and ran on the GCxGC, 5 mL of Mix 1A, 2A, 3A, 4A, and 5A made up Mix A and the same was done for Mix B and C. Mix A, B, and C, 2 mL of each mixture (all class 2 components) were combined and ran together to determine the retention times. The same was done for classes 1 and 3. Class 2 and 3 were combined and ran to characterize and assign peaks and finally class 1, 2, and 3 were combined in one large mixture and analyzed on the GCxGC. All the results are discussed in the following chapter.

Table 3.4 Mixture Components

1000 ppm Solutions - 20μL solvent in 20mL of methanol

Class 1

Mix 1A	Mix 2A
1,1-dichloroethene	benzene
1,1-trichloroethene	carbon tetrachloride
1,2-dichloroethane	

Class 2

Mix 1A	Mix 1B	Mix 1C
acetonitrile	chloroform	2-methoxyethanol
cyclohexane	1,2-dimethoxyethane	1-methyl-2-pyrrolidone
p-xylene	pyridine	sulfolane
Mix 2A	**Mix 2B**	**Mix 2C**
Methylene chloride	trichloroethylene	N,N-dimethylacetamide
1,4-dioxane	nitromethane	N,N-dimethylformamide
Chlorobenzene	2-hexanone	formamide
	tetralin	
Mix 3A		**Mix 3C**
cis-1,2-dichloroethylene		2-ethoxyethanol
Toluene		ethylene glycol
o-xylene		
Mix 4A		
trans-1,2-dichloroethylene		
methylcyclohexane		
m-xylene		
Mix 5A		
tetrahydrofuran (THF)		
ethylbenzene		

Class 3

Mix 1A	Mix 1B	Mix 1C
ethanol	pentane	ethyl formate
2-methyl-1-propanol	heptane	ethyl acetate
2-methyl-1-butanol	THF	propyl acetate
cumene	dimethyl sulfoxide	methylisobutylketone
Mix 2A	**Mix 2B**	**Mix 2C**
2-propanol	t-butylmethyl ether	methyl acetate
2-butanol	acetone	methylethylketone
1-pentanol	acetic acid	butyl acetate
	anisole	
Mix 3A		**Mix 3C**
formic acid		ethyl ether
1-butanol		isopropyl acetate
1-propanol		isobutyl acetate

Chapter 4

Results and Discussion

GCxGC technology allows the use of two different separation mechanisms to increase the separation power of the chromatographic system [65]. GCxGC separation increases peak capacity and improves the chromatographic resolution of difficult mixtures [66]. This increase in separation power is achieved through the use of two different columns where the whole sample from injection to detection is pushed through both columns. If the second dimension column demonstrates considerably different chemistry than the first dimension column, an additional separation channel is provided. The additional separation channel and the higher separation power was the main reason for using GCxGC to perform this research.

4.1 Results – Method 1

Method 1 demonstrates the use of a non-polar (RTX-5) first dimension column and a moderately polar second dimension column (DB-17). Figure 4.1 shows the separation of 57 class 1, 2, and 3 solvents in methanol at the 1000ppm level. The large long peak at the beginning of the chromatogram is the methanol solvent peak. The first peak beyond the solvent peak is completely separated and not hidden by solvent peak whereas in one dimensional GC it can be. There are many critical pairs that require the two-dimensional capability. The chromatogram in Figure 4.1 is a contour plot where the view is looking down onto the 3-D separation surface. The first dimension retention time (in seconds) is indicated along the x-axis (bottom) while the shorter second dimension time is along the y-axis (side). The oval shaped images on the

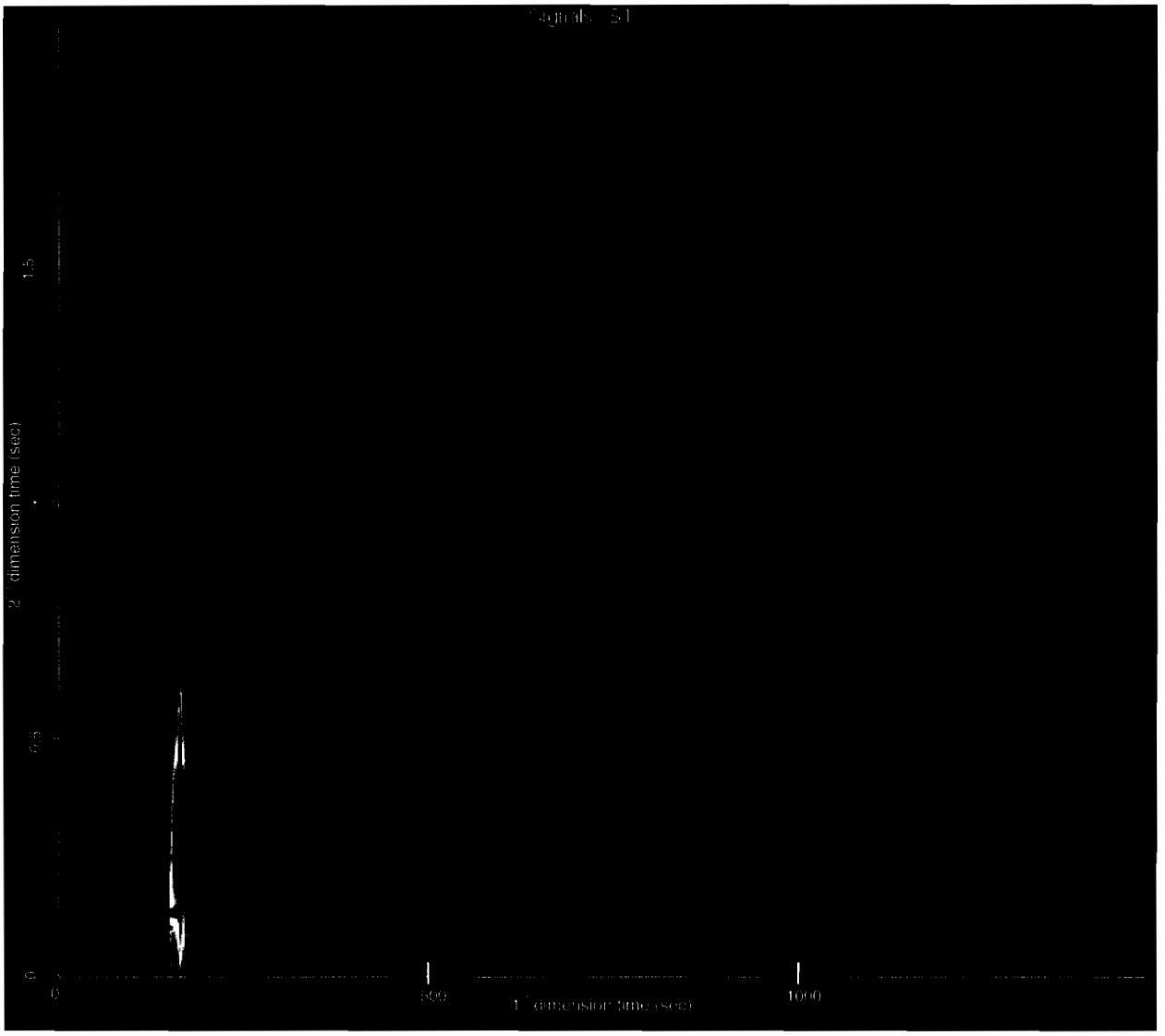


Figure 4.1 – Class 1, 2, 3 (Method 1)
Typical 2-Dimensional chromatogram using the non-polar 1st dimensional column and the mildly polar second dimension one. The black dots indicate the peak as well as the peak height. The difference in placement of peaks on the chromatogram is determined by the second dimension time. The 1st dimensional peak separation is good but the 2nd dimension separation needs improvement.

chromatogram represents the peaks. As explained previously in Chapter 1 those peaks are formed by the slicing which occurs during the modulation process. The black squares in the center of each peak indicate the peak's retention time as well as the peak height. Peak tailing can occur in two-dimensional chromatography and is represented by lines extending from the top of each oval shaped peak, this is also called peak wraparound. Peak fronting can be seen in two-dimensional chromatography and will make the peak (oval) look thicker then the others on the chromatogram. Figure 4.1 shows some minor fronting at the carbon tetrachloride peak. That specific peak looks wider then the rest and has a blurry spot protruding from the right side representing the fronting. Higher concentrated analytes, such as the methanol solvent peak in Figure 4.1, have much larger peaks extending across the entire second dimension (y-axis). In traditional gas chromatography more concentrated analytes have larger peak heights then those at lower concentrations. The same concept applies to two-dimensional gas chromatography it is just represented by a large broad peak that wraps around into the next modulation cycle.

The retention times for class 1 solvents are found in Table 4.1 along with each solvent's normal boiling point. The retention tables for class 2 and 3 are Tables 4.2 and 4.3 respectfully. Figure 4.1 shows some of the solvents from retention tables 4.1, 4.2, and 4.3. The elution order of solvents did not match their order in increasing boiling points. This may be from intermolecular interactions happening between the analyte and column stationary phase (column coating). The total run time for method 1 is about twenty-five minutes.

Method 1 demonstrates the basic characteristics of a comprehensive two-dimensional gas chromatogram. It is evident in Fig. 4.2 that most of the analytes are separated in the first dimension, requiring additional selectivity in some cases, one of which is illustrated on the chromatogram and shown in the inset. The moderately polar column showed

Table 4.1 Retention Times Class 1 Method 1

Solvent	BP °C	Retention Time (sec)
1,1-dichloroethene	31.7	272
1,1-trichloroethane	74.1	480
1,2-dichloroethane	83.5	486
Benzene	80.1	510
Carbon Tetrachloride	76.1	512

Table 4.2 Retention Times Class 2 Method 1

Solvent	BP °C	Retention Time (sec)
methanol	64.6	188
acetonitrile	81.6	272
dichloromethane	39.8	278
nitromethane	101.2	384
chloroform	61.7	428
1,2-dimethoxyethane	85	476
2-methoxyethanol	124.6	492
cyclohexane	80.7	496
N-methyl-pyrrolidone	202	1140
ethylene glycol	195	574
formamide	210	576
1,2,2-trichloroethene	86.7	538
1,4-dioxane	101.1	558
2-ethoxyethanol	135.6	594
methylcyclohexane	101	636
pyridine	115.2	624
toluene	110.6	666
N,N-dimethylformamide	153	722
2-hexanone	127	720
Chlorobenzene	130	836
σ-xylene	140	854
N,N-dimethylacetamide	166.1	864
tetralin	207	1238
sulfolane	285	1280

Table 4.3 Retention Times Class 3 Method 1

Solvent	BP °C	Retention Time (sec)
ethyl ether	34.6	252
pentane	36.1	
t-butyl methyl ether	55.2	344
acetone	56.2	236
methyl acetate	56.9	286
tetrahydrofuran	66	450
ethyl acetate	77.1	422
ethanol	78.3	204
Methylethyl ketone	79.6	390
2-propanol	82.4	244
isopropyl acetate	90	504
1-propanol	97.2	326
heptane	98.4	570
2-butanol	99.5	396
formic acid	100.7	186
propyl acetate	102	596
2-methyl-1-propanol	107.9	444
methylisobutyl ketone	117.4	642
1-butanol	117.6	506
acetic acid	117.9	378
isobutyl acetate	118	692
butyl acetate	126.1	756
3-methyl-1-butanol	130	630
1-pentanol	137.9	684
ethyl formate	148	270
cumene	151	930
anisole	154	916
dimethyl sulfoxide	189	888

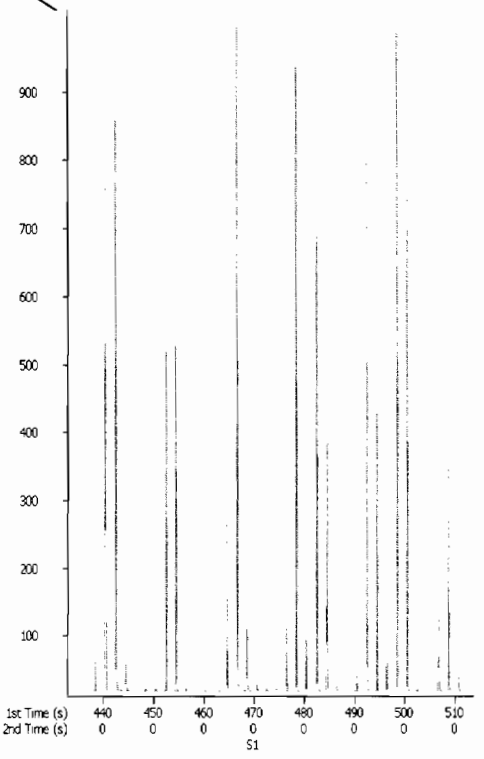


Figure 4.2 – GCxGC chromatogram using a moderately polar second dimension column. Insets show a three-dimensional plot and the actual FID trace for a portion of the chromatogram.

some of the needed separation but not all of it. The overall second dimension separation time is not utilized across the whole plane of the chromatogram yet the peak widths in the second dimension show good resolution. The second dimension separation is, however, highly efficient, with overall peak widths about 100 milliseconds and there is no loss in the first dimension resolution.

The methanol solvent peak, long peak at the beginning of the chromatogram, may have coeluted with early eluting solvents. To determine if this was taking place, dimethyl sulfoxide was used in place of methanol as the base solvent in the mixtures. Dimethyl sulfoxide is a higher boiling point solvent and elutes much later on the chromatogram, usually about 850-890 seconds or 14 minutes, according the method and temperature program used. The advantage of using dimethyl sulfoxide as the diluent allowed us to see if any of these solvents coeluted with the methanol solvent peak. Class 2 solvents were used first and the concentrations used were based on USP OVI Solvent standards in DMSO (Restek, Bellefonte, PA). The actual concentrations that were used were double the amount in the Restek standards. For this research all class 2 mixes were 1000 ppm solutions, 20µl solvent in 20ml of methanol.

Early eluting solvents, such as acetonitrile and methylene chloride, which can sometimes be hidden by the methanol solvent peak were visible, but for the solvents that come out much later, they were obstructed or hidden by the impurities found in DMSO. It was hard to distinguish between the large DMSO solvent peak in the center of the chromatogram and its impurities. Fig. 4.3 is a chromatogram of Class 2 Mix C in 100 mL of DMSO. This chromatogram shows the large DMSO solvent peak and the impurities that interfere with the later eluting solvents. Those solvents that come before the DMSO peak are there but not as distinct as the ones seen in methanol. The use of DMSO, especially on a two dimensional GC,

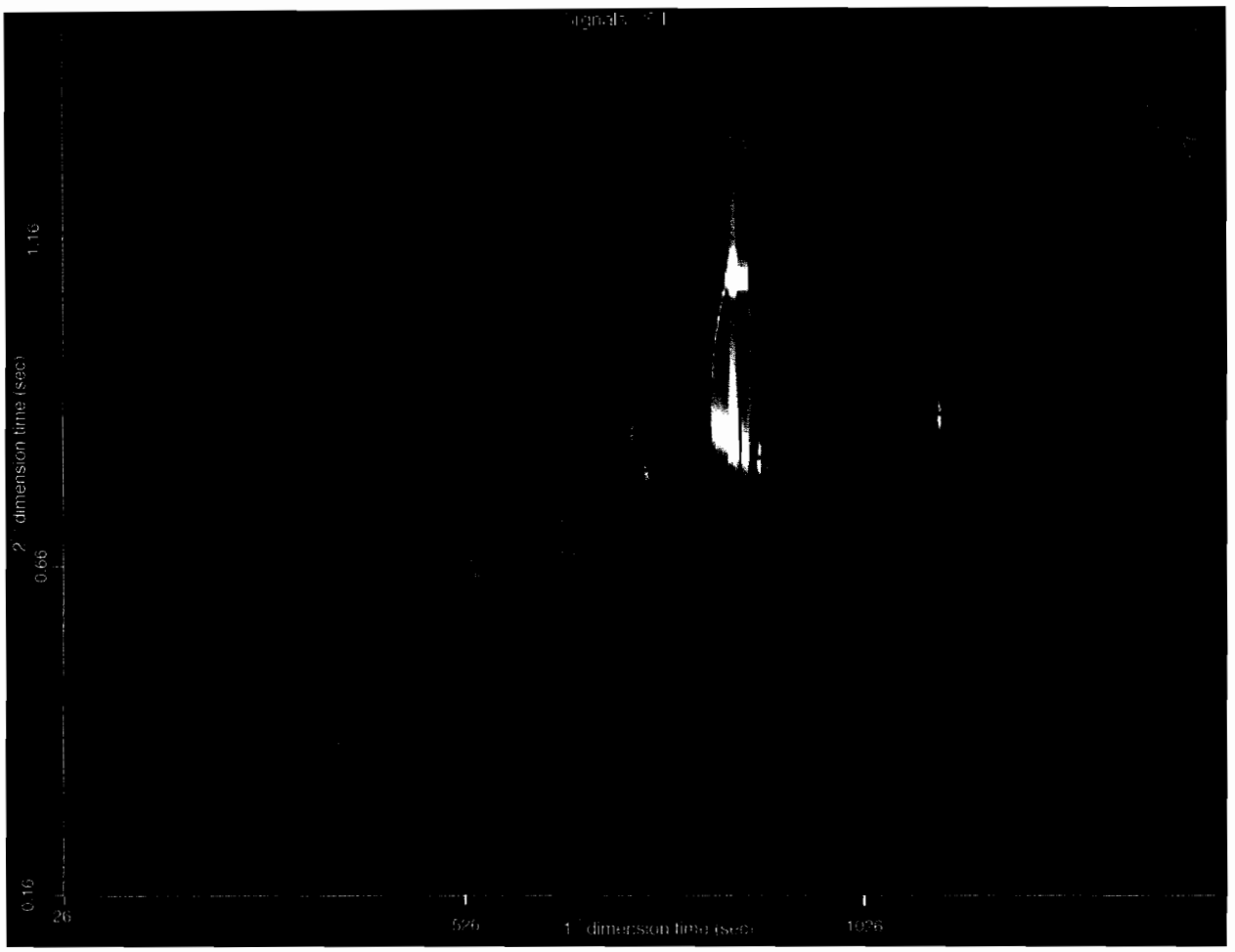


Figure 4.3 – Class 2 Mix C in 100 mL DMSO
The chromatogram shows the large DMSO solvent peak and its impurities. Those solvents which elute after DMSO can not be verified due to the impurities and the retention times obtained are not reliable.

as a diluent is not reliable because of the highly selective and sensitive nature of the GCxGC system, every impurity is picked up. Those peaks that show up on Fig. 4.4 as well as Fig. 4.5 may not show up on a traditional gas chromatogram since the sensitivity of the instrument is not as sensitive as a GCxGC system.

In the pharmaceutical industry for a method to be fully generic, the solvents used need to be flexible in dissolving pharmaceutical compounds [67]. A variety of polar, inert, aprotic and high boiling point solvents have successfully been used for the determination of volatile organic impurities in pharmaceuticals [67]. Dimethyl sulfoxide is a polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a large range of organic solvents as well as water [68]. DMSO has excellent solvating power and is frequently used in reactions involving salts but its high boiling point does not allow for easy evaporations of solutions and so reactions conducted in DMSO are often diluted with water to isolate the organic products [68]. It is very favorable in the pharmaceutical industry because DMSO increases the rate of absorption of compounds through organic tissues allowing for it to be used as a major player in drug delivery systems. Yet DMSO the currently recommended diluent for residual solvent analysis will show the impurities it contains or the decomposition which takes place as the sealed solvent bottle sits on the lab bench top, causing interferences in the traditional methods [64]. Fig. 4.3 is an example of why DMSO should not be the most favorable diluent used in this research. This being the fact that GCxGC sensitivity picks up all impurities which pollute the chromatogram. DMSO also caused difficulty at the GC inlet. The inlet septa would start to decompose at a faster rate then normal and would have to be changed about once a week, to prevent septa pieces inside the inlet liner. With methanol the inlet septa could last for months. The problem of the inlet would then spread down into the inlet glass liner. That too would have to be changed

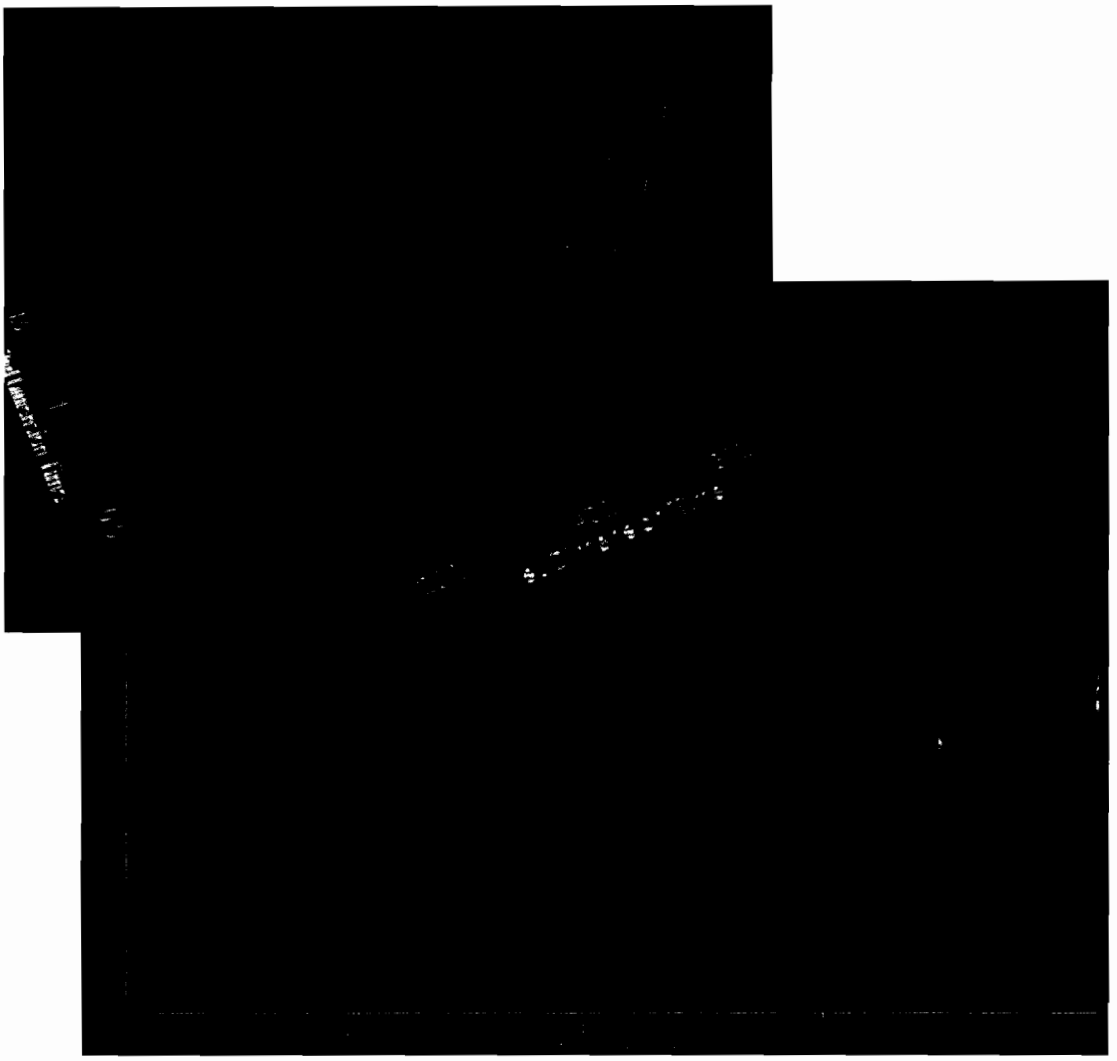


Figure 4.4 – Class 2 Group B
The surface and contour plot chromatograms in this figure are the 1st half (before the solvent peak) of class 2 group B in DMSO. The peaks that are inside of the red circles show how DMSO caused major peak tailing to occur.

frequently because pieces of the septa would fall into the inlet liner and this would also cause some interference on the chromatogram. Injection of DMSO was very slow and so the split ratio was changed from 100:1 to 200:1 but showed no significant difference and so it was changed back to 100:1 split ratio. In Figure 4.4 you can see how DMSO caused peak tailing to occur. Methanol, for this research was the best diluent to use, even though industry is committed to using DMSO in the official method.

4.2 Results and Discussions – Method 2

As it was stated in the previous section DMSO as the solvent diluent did not prove to show successful results for split injection. Instead of trying to get DMSO to work better it was decided to switch back to methanol, evaluate the type of columns used, and change some of the GC method parameters. The first step was to re-evaluate the second dimension column especially its polarity. At the time the DB-17 (50% dimethyl polysiloxane and 50% diphenyl polysiloxane) column was being used, which was not very polar at all. It was brought to our attention that some other researchers doing similar type of tests were using a much more polar second dimension column, a carbo-wax column. To obtain the additional selectivity needed to more fully separate all the analytes, the second dimension column was changed to a very polar, polyethylene glycol-based stationary phase. The new second dimension column used was the RTX-Wax (Restek, Bellefonte PA). With this column type separation should occur across the whole plain with much more distinctive second dimension separation occurring as well as a much higher selectivity in the second dimension.

The first few runs with the RTX-wax column were discouraging and the GCxGC method parameters were examined. The current method was ran at constant pressure and the

temperature program was higher then the new second dimension column maximum. The temperature program on a whole had to be changed. The main oven temperature had to be about 20 to 30 degrees lower then that of the second dimension oven, and so it was changed from 240°C to 220°C. The initial temperature stayed the same (35°C) but the ramp was changed from initially holding for only four minutes to now holding there for ten minutes and ramping up to the above mentioned temperatures. The LECO® GCxGC instrument manual states that the starting temperature of the secondary oven must be at least 5 degrees above the starting temperature of the GC oven because the secondary oven cannot cool as low as the primary oven due to heat from the transfer line and modulator [66]. But since the starting temperature of the main GC oven was not changed the starting temperature of the secondary oven stayed the same and the initial hold time for the secondary oven was changed to from one minute to ten minutes. The ending temperature of the secondary oven had to be lowered from 280°C to 250°C because the max temperature of the RTX-wax column was 250°C. However in the old temperature program once the oven temperature reached the ending temperature of 280°C the run was over, but in the new program the ending temperature was held for one minute. With the entire overall temperature program changes the run time did not get any longer. This was very important to us because the main goal of the research was to create a method that not only tested for all solvents but had a run time under thirty minutes. Second dimension separation time in method 1 was only two seconds. Method two was started using the two second separation time but was later changed to four seconds because there was a large amount of peak wrapping taking place. The four second separation time eliminated peak wrapping and allowed more area for peaks.

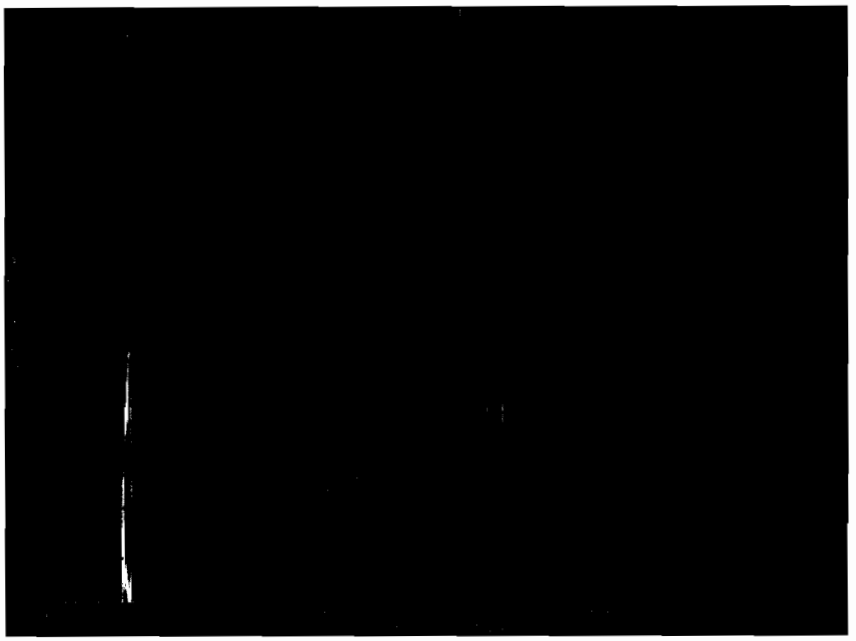
The other major change in the methods was the change from constant pressure mode to constant flow. In constant pressure mode the flow depends on total constraint of combined

columns [66]. An equivalent column is calculated for the initial conditions allowing the GC to calculate precise column and split flows at the initial settings [66]. Constant pressure maintains the column head gauge pressure at the head of the column throughout the run. In the software GC method section it states that if the column resistance changes, the gauge pressure does not change but the mass flow rate does allowing the user to get constant pressure throughout the entire GCxGC run. Since a much more polar second dimension column was being used some of the analytes may become retained in the column stationary phase whereas some may not. This was possibly due to the fact of the varying flow rate throughout the run. Constant flow was explored and found that polar analytes which were becoming stuck in the highly polar column stationary phase were now showing up on the chromatograms. As the temperature increases, in constant flow mode, the actual flow increases relative to the target flow. The results were much better and not as many analytes were held in the column stationary phase.

The change in column stationary phase gave desirable results. The RTX-Wax column provided significantly different chemistry between the two dimensions, giving the appearance of a more orthogonal separation. Chromatogram A in Figure 4.5 shows separation of the Class 2 solvents using the DB-17 column and B shows separation using the RTX-wax column. Figure 4.6 illustrates the important class 2 solvents, dissolved in methanol at 1000ppm. Table 4.4 gives the solvent and retention times for the peak numbers labeled on the chromatogram in Figure 4.6. Take notice to the dramatic difference in the peak locations in the second dimension. This chromatogram gives solid proof that the separation power of GCxGC is greater then traditional gas chromatography.

The distinctive separation between analyte and solvent peak is very important in determining residual solvents that may be present in specific pharmaceutical products. As it was

A



B

Figure 4.5 (A) – Chromatogram of Class 2 solvents using method 1, (B) – Chromatogram of Class 2 solvents using method 2. There is an extremely different separation going on in the second dimension. The more polar column allows the locations of the peaks in the second dimension to be across the whole chromatogram whereas in (A) the peaks go across the chromatogram in a diagonal. Chromatogram B gives evidence that method 2 separation is best.

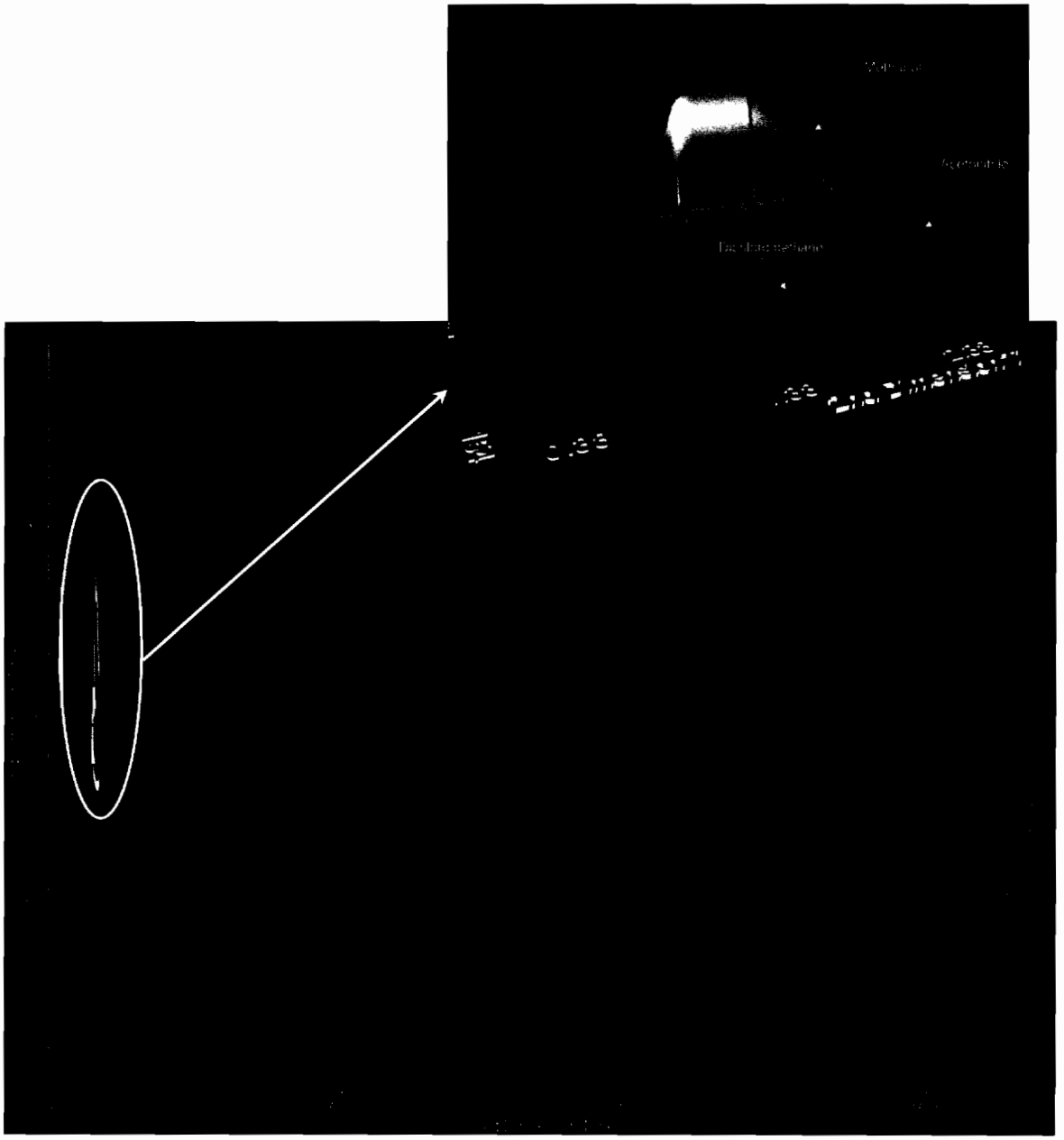


Figure 4.6 – Class 2 Residual Solvents 1000ppm in Methanol
The polyethylene glycol based 2nd dimension stationary phase (RTX-Wax) is much more strongly holding on to the more polar analytes and weakly retaining the less polar and non-polar analytes [64]. The contour plot inset gives a closer look into the complete separation between analyte and solvent peak. The inset shows separation of acetonitrile and dichloromethane for system suitability in USP <467>. In regular GC these peaks may be coeluting with the methanol solvent peak, leaving the researching asking how is this acceptable for the pharmaceutical industry if not all class 2 solvent peaks are visible.

Table 4.4 – Class 2 Solvent Retention Times for Figure 4.6

Peak #	Name	R.T. (s)
1	Solvent Peak	84 , 2.110
2	Acetonitrile	112 , 2.790
3	Methylene Chloride	136 , 1.995
4	trans-1,2-dichloroethylene	160 , 1.485
5	trichloroethylene	172 , 2.995
6	Hexane	196 , 0.915
7	cis-1,2-dichloroethylene	216 , 2.655
8	chloroform	232 , 3.110
9	THF	248 , 1.475
10	1,2-dimethoxyethane, 2-methoxyethanol	300 , 1.925
11	cyclohexane	316 , 1.055
12	Nitromethane	424 , 2.665
13	1.4-dioxane	456 , 3.775
14	methylcyclohexane	508 , 1.165
15	pyridine	624 , 2.560
16	Impurity	680 , 1.470
17	Toluene	700 , 2.105
18	2-hexanone	768 , 1.885
19	chlorobenzene	892 , 1.980
20	ethylbenzene	920 , 1.410
21	m-xylene, p-xylene	932 , 1.405
22	o-xylene	972 , 1.440
23	1-methyl-2-pyrrolidone	1164 , 2.835
24	Tetralin	1296 , 1.400
25	Sulfolane	1352 , 1.705

shown before when DMSO was used (instead of methanol) the impurities take over the second half the chromatogram and peak identification can not be determined. Close examination of Fig. 4.6 shows that the solvent peak, methanol, seen in the upper left corner of the chromatogram, is fully separated from the other analytes. Even though the solvent peak slightly tails in the first dimension it still does not interfere with the other peaks. Potential use of other more common solvents, like those used in HPLC, such as methanol as the diluent for residual solvent analysis can eliminate the use of the recommended higher-boiling solvents like DMSO which contain impurities or decompose causes interferences in the traditional methods.

In the current <USP 467> method the standard for system suitability for the resolution (R_s) of acetonitrile and dichloromethane can be no less then 1.0. Resolution is the degree to which adjacent peaks are separated or the distance between the peak maxima for two solutes [69]. Fig. 4.6 best illustrates this and shows how GCxGC can meet the requirements for resolution better then regular GC. The new USP <467> projected to come out in July 2008, involves up to three steps - identification of solvents on one column, confirmation on a second column followed by a third experiment for quantitation. Our method provides all of those steps in a single injection, and has all of the class 1, 2 and 3 solvents separated. Acetonitrile and dichloromethane are well separated in both dimensions with resolution at about 3 in the first dimension and 12 in the second dimension. The Restek standard USP chromatogram for class 2 shows resolution of 1.35 for acetonitrile. Figure 4.7 is a traditional GC chromatogram of the class 2 USP mix from Restek. The peaks on the GCxGC chromatogram for acetonitrile and dichloromethane are much more retained then those on the traditional chromatogram. The peaks cannot really be seen separated in the GC chromatogram unless zoomed in. Fig. 4.8 is a side-by-

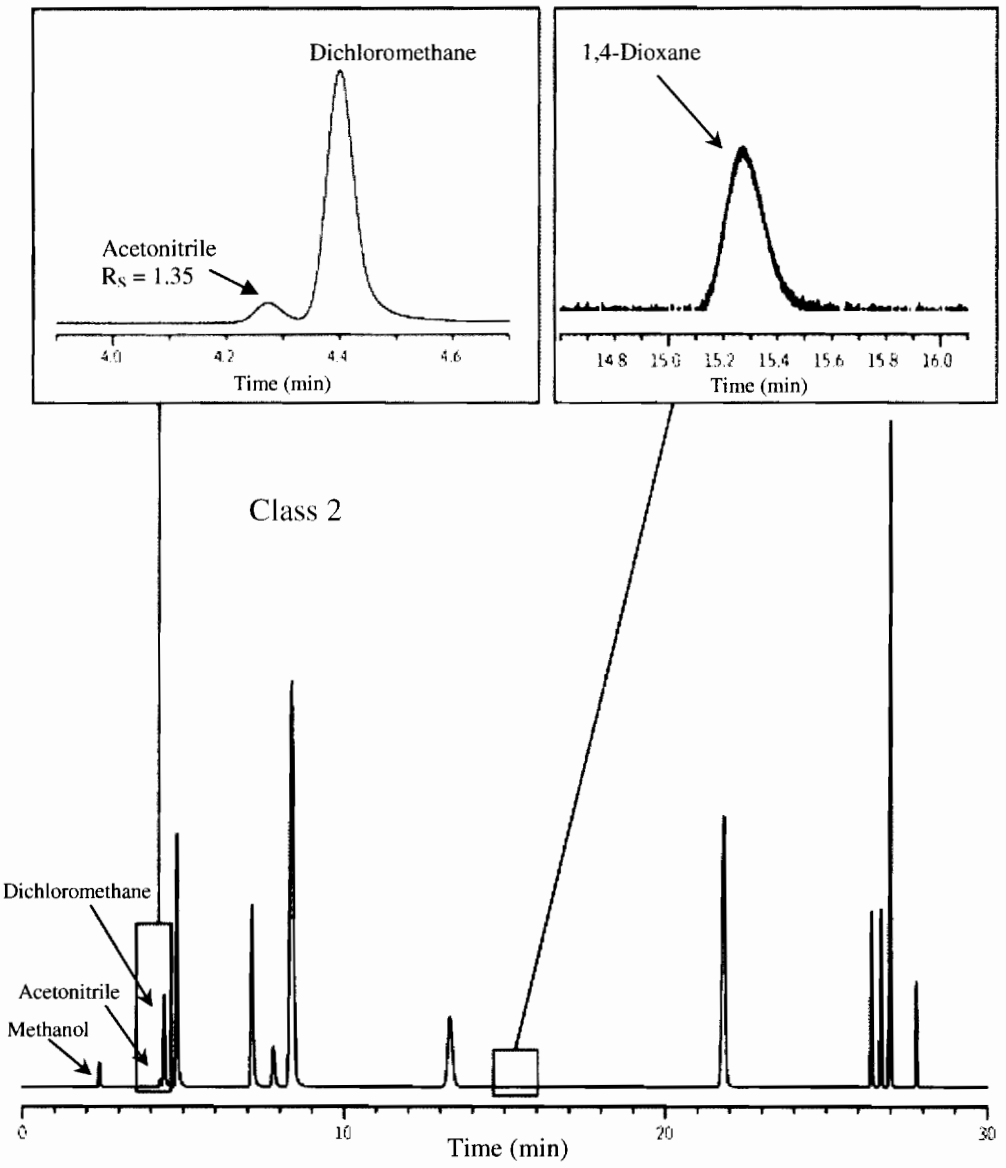


Fig. 4.7 – Restek Chromatogram for USP Residual Solvent Class 2 Mixture A standard solution on an Rtx®-624 column [70].

This column is very similar in polarity to the one used in the 1st dimension for this research. The acetonitrile peak can barely be seen and can be mistaken as fronting of the dichloromethane peak. The R_s for acetonitrile is only 1.35 and you can only see that by zooming in on the chromatogram for that specific peak location. The 1,4-dioxane peak can not even be seen by the naked eye. If one didn't know to zoom in the baseline there you would not see it. In the current research method you can see that all of these peaks are completely separated from each other and are visible on the full chromatogram.

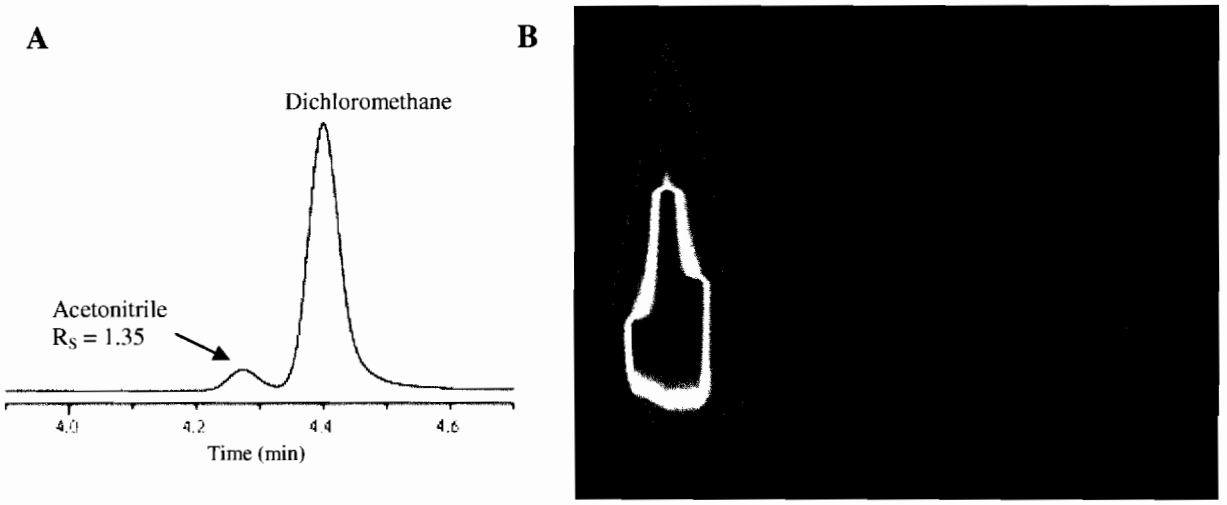


Fig. 4.8 – Traditional GC vs. GCxGC chromatogram of acetonitrile and dichloromethane. Chromatogram A is the Restek [70] and B is the GCxGC one. There is complete separation in the GCxGC chromatogram of acetonitrile and dichloromethane. Also in the GCxGC method (B) separation occurs at 112s or 1.8 minutes and in A it does not separate until 4.2 minutes.

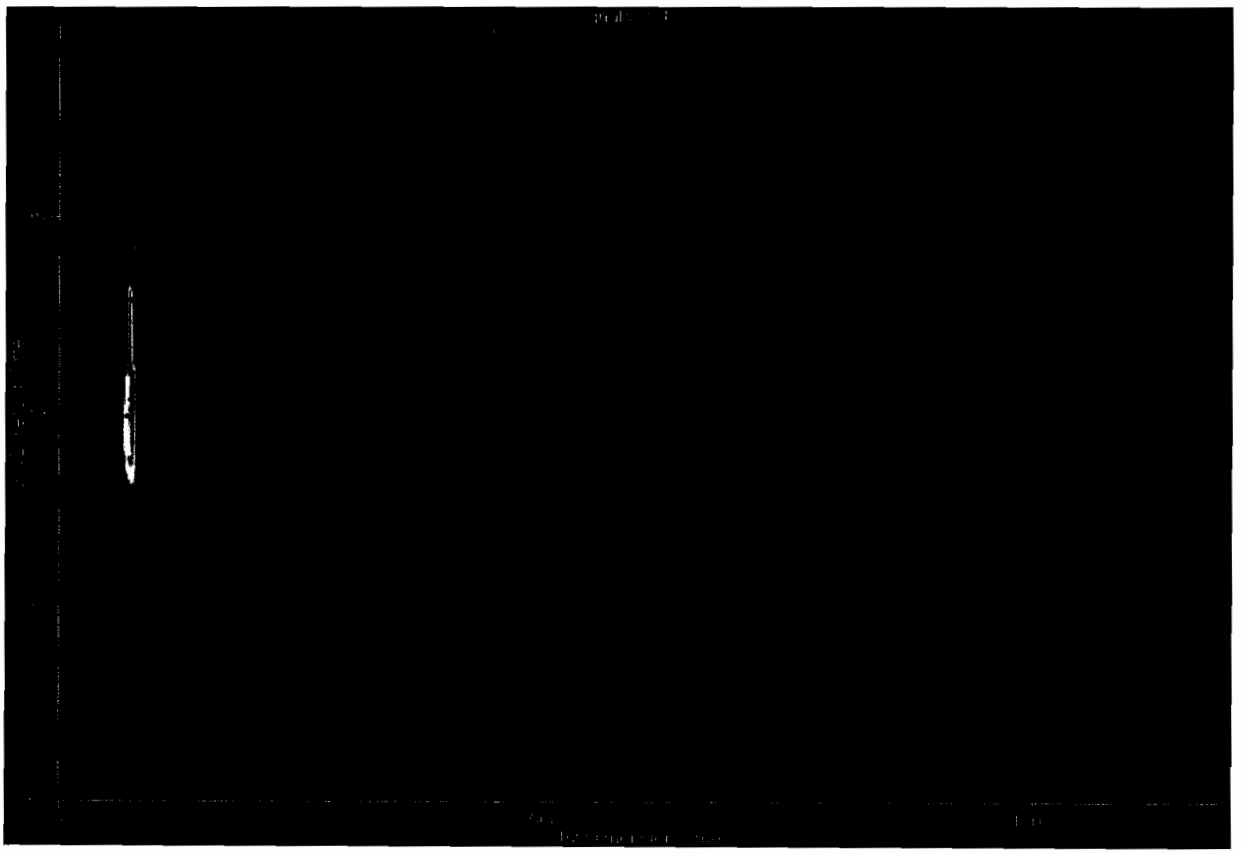


Fig. 4.9 – Class 2 Mix A Two-Dimensional Chromatogram.
Separation of components is much better on this one then on the chromatogram in Fig. 4.7. 1,4-Dioxane is more then just baseline noise here.

side comparison of acetonitrile – dichloromethane in both one and two dimensions. Fig. 4.9 is a comparison of class 2 mix A in two dimensions with the one dimensional class 2 Restek mix A [70]. At the later retention times, the column bleed (seen in Fig. 4.9) commonly associated with most capillary columns is clearly separated in the second dimension from the later eluting analyte peaks. In one-dimensional separation those later eluting analyte peaks would have co-eluted with the column bleed, causing potential problems with quantitation. Column bleed is a common problem when high boiling point solvents are being analyzed because a higher final temperature is needed to see all components.

Ortho-, meta-, and para-xylene all have similar retention times. The three xylenes are higher boiling point solvents and have much later retention times but when all three are together in the same mix only two of them show up. The retention time for each xylene was found by running them in separate mixtures with their class 2 counterparts. This co-elution happens on a regular GC and a GC-mass spectrometer because they share the same molecular weight and boiling point. The only difference in these three isomers of dimethyl benzene is the placement of the two methyl groups on the carbon atoms of the benzene ring (see Table 3.1). When looking at the three xylenes on the chromatogram the peak containing both meta- and para-xylene is much larger and the peak color intensity indicates that it is much more concentrated. The shape of the m-xylene and p-xylene specific peak is the normal oval shaped contour plot peak but if you look closely you can see what looks to be another peak overlapping the first peak. Figure 4.10 highlights the three xylene peaks. Much finer tuning of the method at the specific temperature where the xylenes co-elute may allow complete separation of the two xylene peaks.

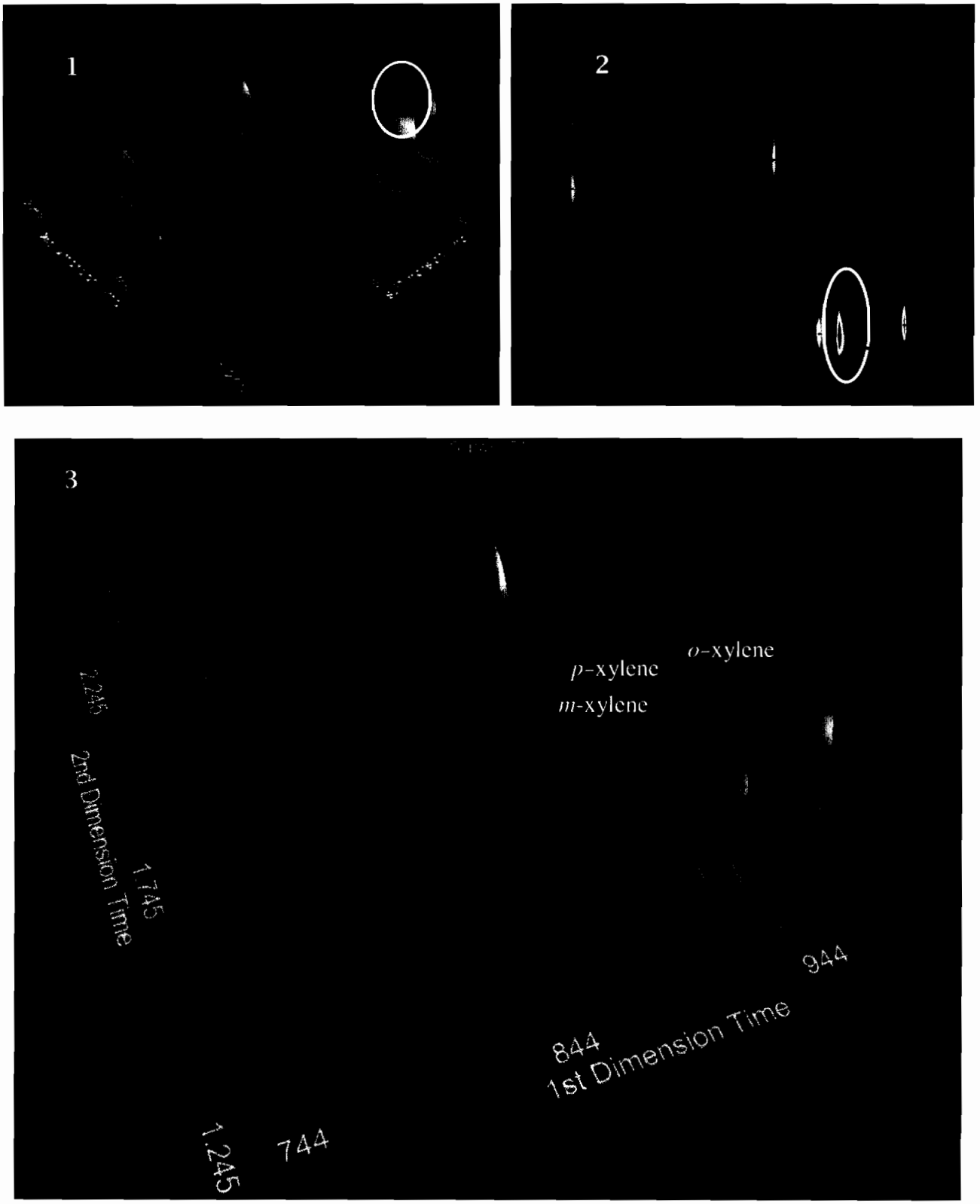


Fig. 4.10 – The Xylenes:
Chromatographic section of Class 2 solvents ortho-, meta-, and para-xylene. The arrow on #2 shows the possible overlapping peak of p-xylene and the color intensity at the center of the peak has more red color. This could be caused by the co-elution of the two xylenes making one large concentrated peak.

Ethylene glycol was another class 2 problem analyte. It would not come out of the RTX-wax second dimension column. The answer to this is very simple being that the second dimension column is 100% polyethylene glycol, which is a long polymer chain of ethylene glycol, ethylene glycol sticks or absorbs into the column stationary phase and does not come out of the column. This goes back to the age old statement of "Like dissolves like." To confirm this, spiked samples of ethylene glycol were analyzed. The smaller concentration mixtures in methanol did not show up on the column, only when ethylene glycol alone (straight out of the bottle) was injected did a wide band show up in the middle of the chromatogram. Ethylene glycol using the current method parameters will not come out of the RTX-Wax column.

Many of the class 3 solvents are polar small molecules that are not strongly retained on the first dimension non-polar column. However, the polar second dimension column greatly facilitates their resolution and demonstrates the great resolving power of GCxGC. Figure 4.11 shows the class 3 solvents. All of the class 3 solvents were not easily separated therefore they are not on the chromatogram in Fig. 4.11. Acetic acid, formic acid and formamide are the problem analytes from class 3. These three analytes are known to often present difficulties in routine gas chromatographic analysis and they were not included in the final mixture. The reason behind leaving them out of the final mixture was because they tail across the entire second dimension and are broad tailing peaks. Before removing acetic acid and formic acid from the mixtures they were ran in their class 3 group mixtures and then each were ran alone in methanol. The concentrations used were the same as those used in the mixtures: 20µl of solvent 20ml of methanol. Nothing came out on the chromatogram for formic acid in methanol and this may be an indication of co-elution with the solvent peak. To prove whether or not this

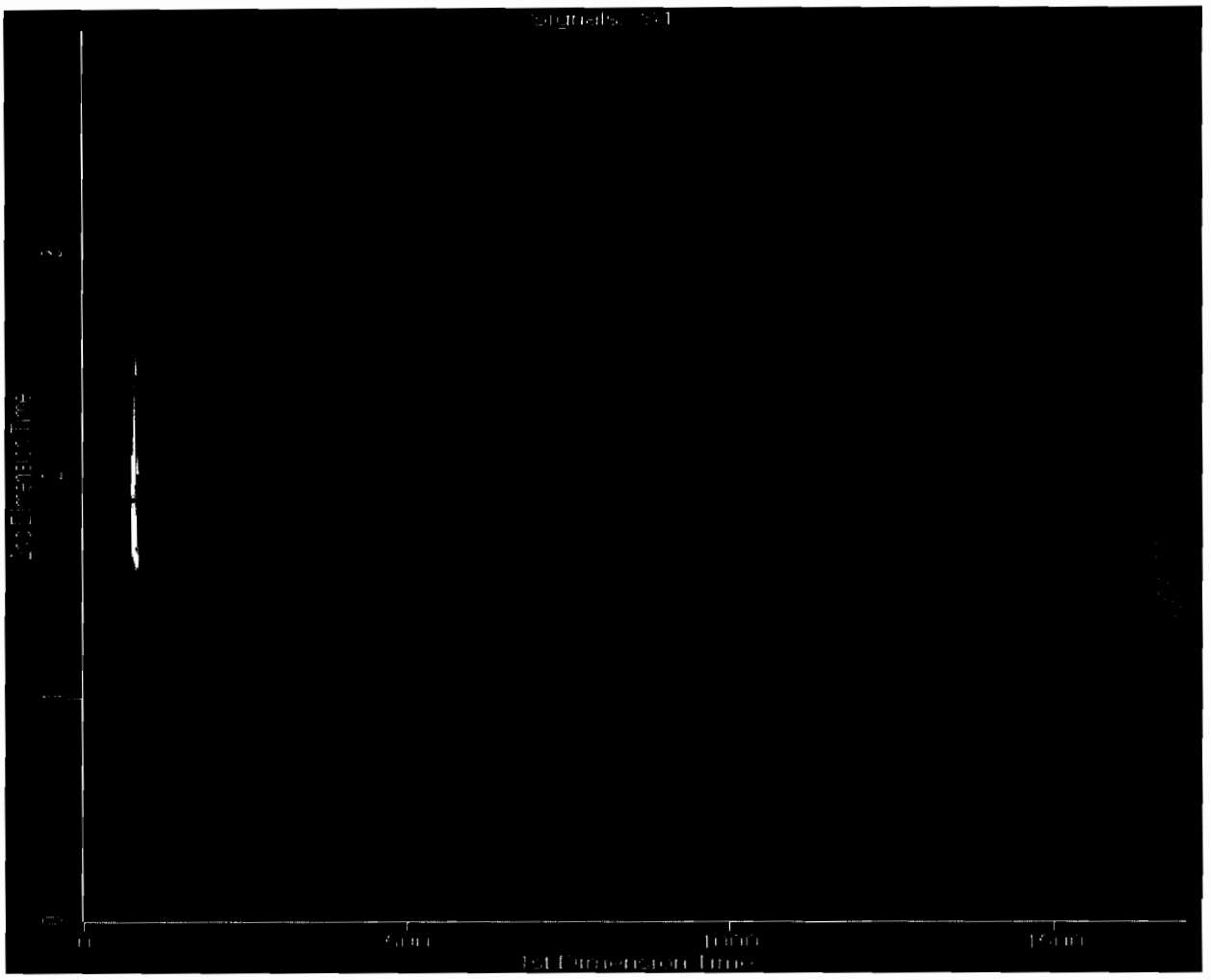


Figure 4.11 – Class 3 Residual Solvents
Chromatogram for the class three residual solvents, notice how the column bleed at the end of the chromatogram does not interfere with the analyte peaks. Good peak retention and resolution is very evident on this chromatogram.

hypothesis is correct formic acid was run again in methanol and in DMSO: 20µl of formic acid in 20ml of DMSO. As of recently, when formic acid was ran using DMSO there were no peaks in prior to the solvent peak. This proved that formic acid whether in methanol or DMSO could not be separated on this column.

Class two and three solvents were focused on the most because they are used in all pharmaceutical compounding and manufacturing. It was very critical to make sure that all the class 2 and class 3 solvents completely separated from each other. The selectivity obtained using GCxGC separation is critical at the early retention times because this is where most of the solvents come out. There are some solvents that co-elute in the first dimension but completely separate from each other in the second dimension.

With the four problem analytes omitted from the mix, all of the solvents from class 2 and 3 were completely separated. Figure 4.12 shows the complete separation of class 2 and class 3 together on the same chromatogram. This was a major achievement because it confirmed our method correct and that the separation power of GCxGC can separate them all in 30 minutes.

The class one solvents had no problems. Benzene and carbon tetrachloride share the same first dimension retention time but their second dimension time completely separates one from the other. There are no coelution issues or bad solvent-column behaviors for class 1 residual solvents. Figure 4.13 is the chromatogram for class 1 solvents.

Some of the class 1, class 2, and class 3 solvents share the same retention time. Class 1 solvents benzene (316sec, 1.550sec) and carbon tetrachloride (316sec, 2.000sec) share the same retention time with the class 2 solvent cyclohexane (316sec, 1.030sec). Figure 4.14 shows a close up of the four solvents with retention time at 112 seconds that co-elute on a regular GC using the same method parameters. Those four solvents with their first dimension retention

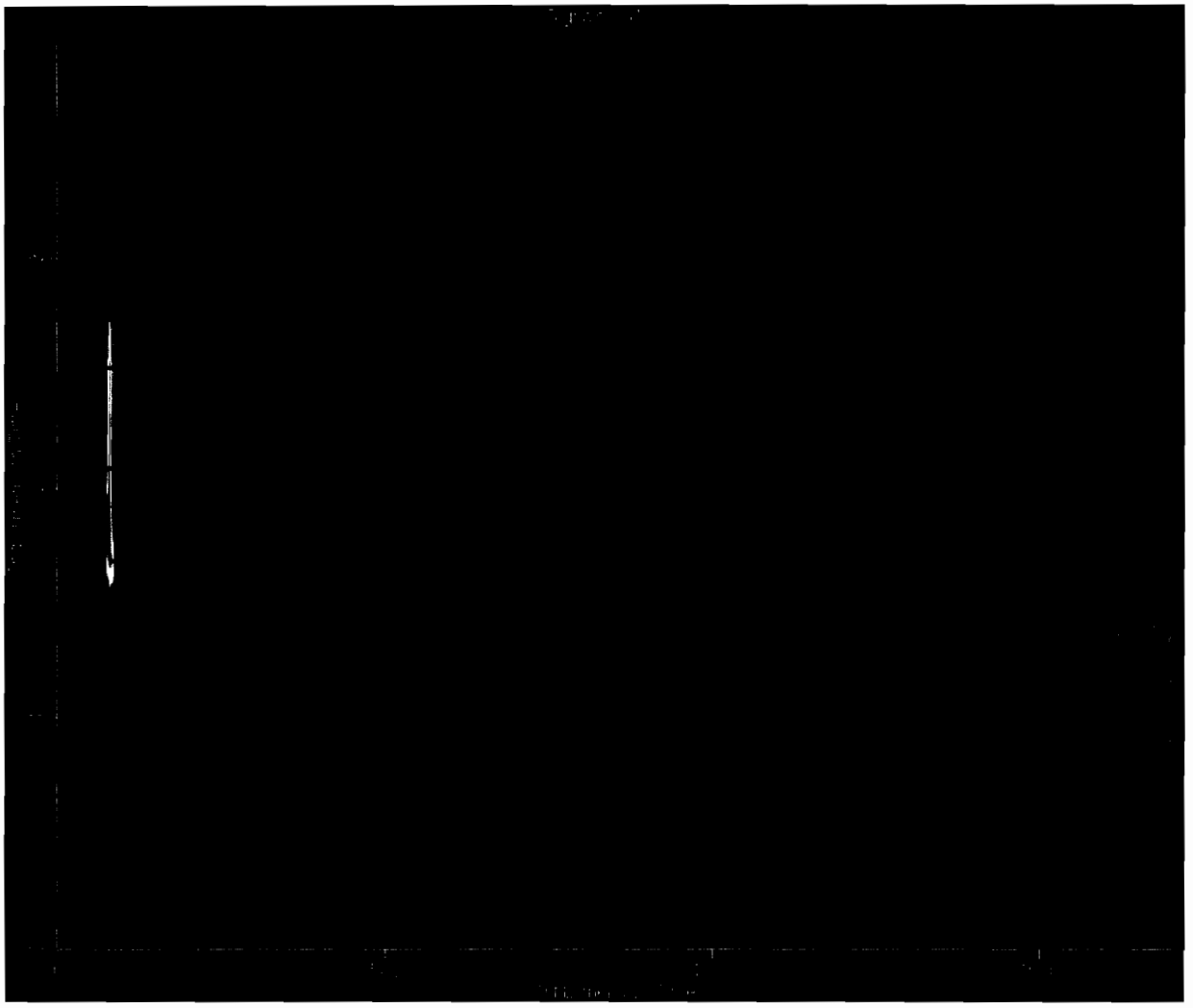


Figure 4.12 – Class 2 and 3 group mix.
The column bleed in this chromatogram shows that it does not interfere with the later eluting solvents.

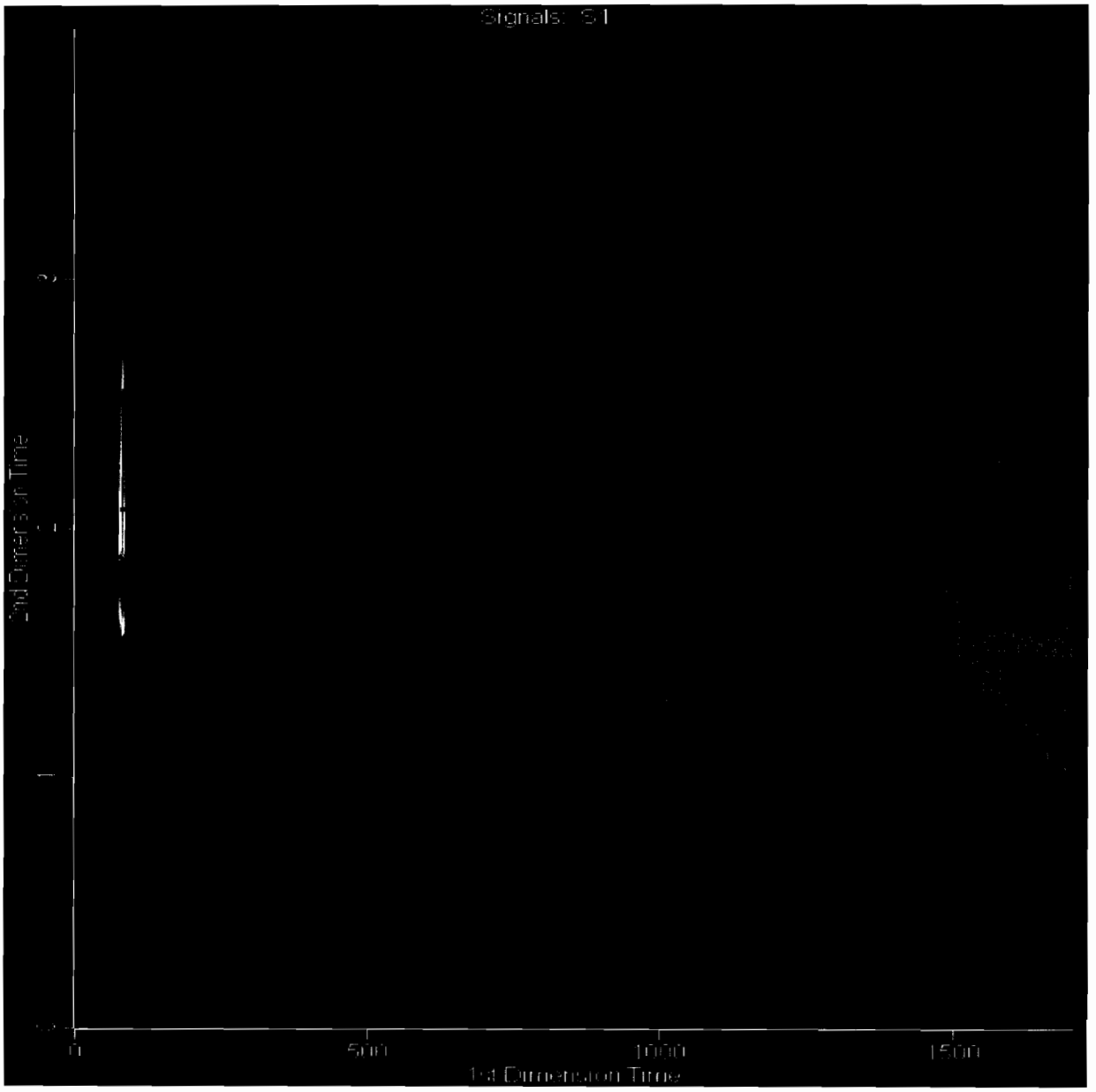


Figure 4.13 – Class 1 Residual Solvents

There is some tailing or wrapping in the second dimension for peak #3. Peaks 4 and 5 overlap but you can distinguish between where 4 starts and 5 ends.

1. 1,1-dichloroethene
2. 1,1-trichloroethane
3. 1,2-dichloroethane
4. benzene
5. carbon tetrachloride

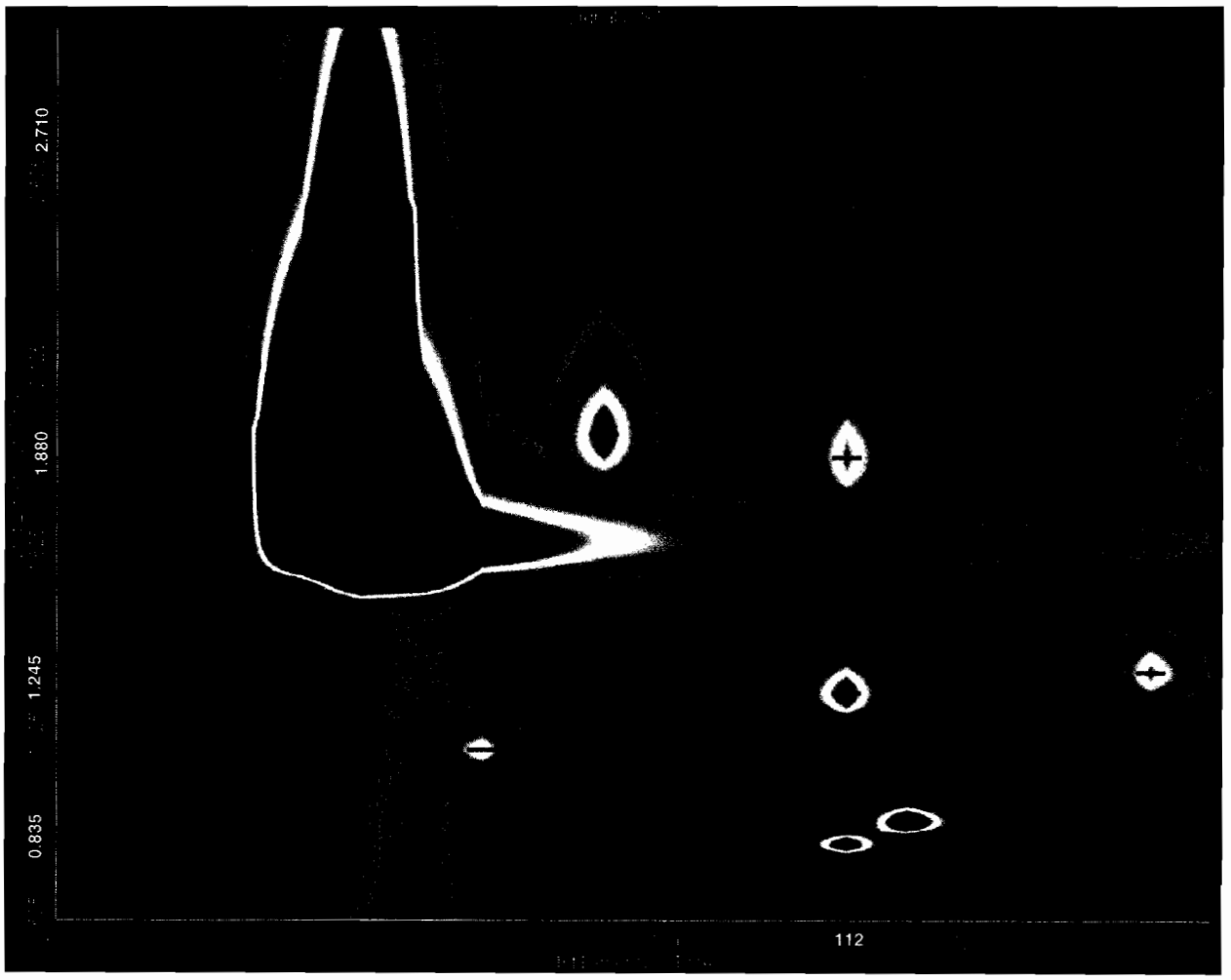


Figure 4.14 – Second Dimension Resolution
Class 2 and 3 solvents that share the same 1st dimension retention time but have different 2nd dimension retention times.

time at 112 seconds are pentane (112sec, 0.835sec), t-butyl methyl ether (112sec, 1.245sec), 2-propanol (112sec, 1.880sec), and acetonitrile (112sec, 2.710sec). Also hexane and methylethyl ketone share the same first dimension time of 196 seconds but their second dimension retention times are 0.885sec and 1.665 sec respectively.

The final investigation of this research put all the class 1, 2, and 3 solvents into one big mixture and attempting to separate them completely on the GCxGC in lesser than thirty minutes. This mixture of the three classes was prepared, leaving out the four problematic solvents and run on the GCxGC. That same mixture was run about 10 times over the course of a few days to check reproducibility in the results. Other then the second dimension times being one tenth or one hundredth of a second different from the previous runs, the first dimension retention times stayed the same.

The polar second dimension column proved to be the best method for separating Class 1, 2, and 3 solvents in one single thirty minute run. Figure 4.15 is the chromatogram for all solvents minus formic acid, acetic acid, formamide, and ethylene glycol. Many of the class 2 and 3 solvents come out early and share very similar retention times. One can see on the chromatogram where the majority of the peaks are located and how the second dimension separation allows for each peak to be distinguishable from the next.

This research has demonstrated that GCxGC has the resolving power to separate the pharmaceutical solvents in a single injection, allowing generic method development in most cases. Although the research performed here was only qualitative, GCxGC might also be used for quantitative studies. Identification, confirmation, and quantitation in a single analysis are the three main requirements in the newest release of USP <467>, and GCxGC is a likely candidate to perform all three steps in a single injection using two columns comprehensively.

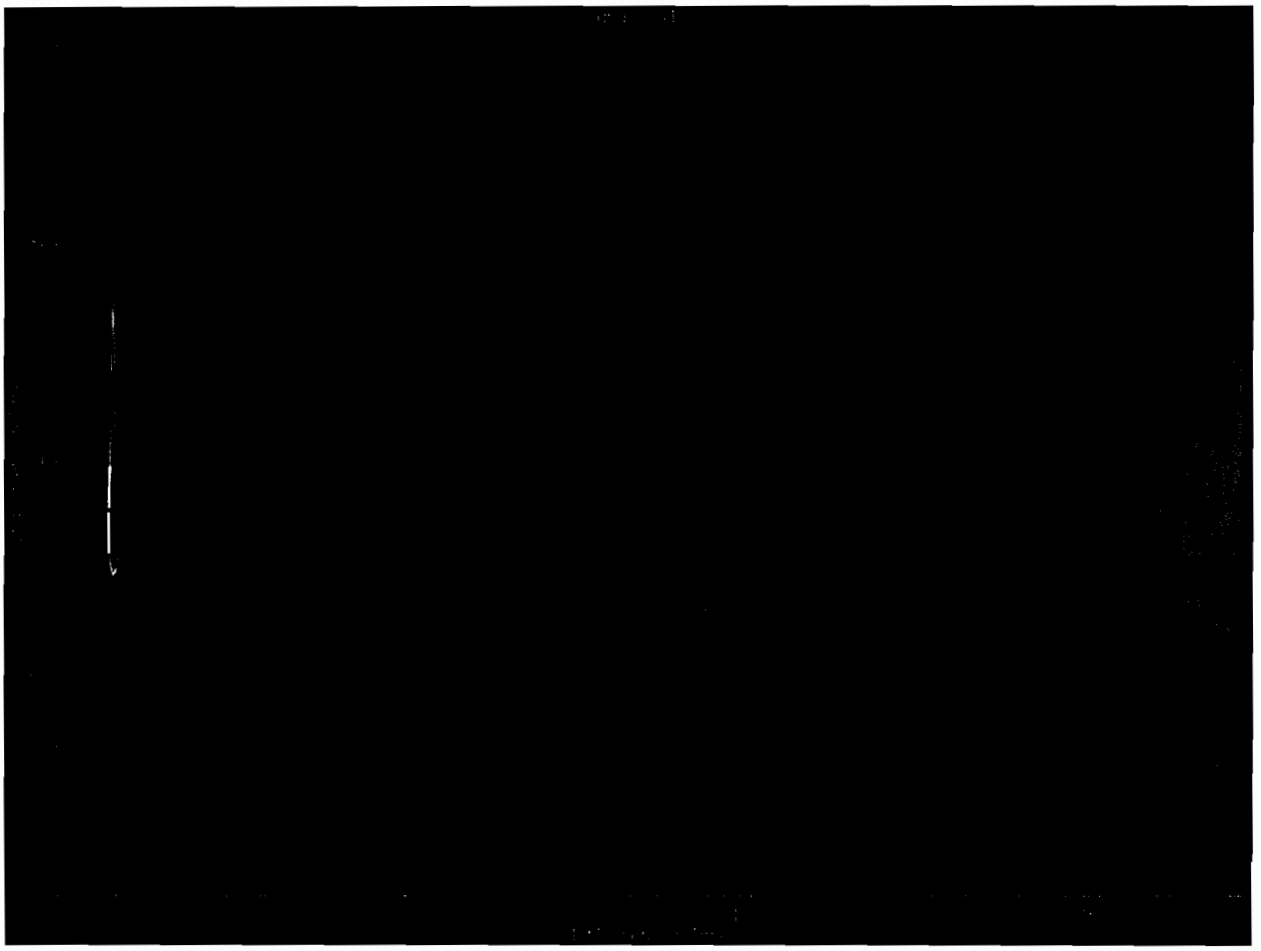


Figure 4.15 – Class 1, 2, and 3 Residual Solvent Chromatogram
The above chromatogram is from the Class 1, 2, and 3 mix that was injected onto the GCxGC using a nonpolar 1st dimension column and a polar 2nd dimension one. Good peak resolution and separation is achieved here. Also the column bleed normally associated with interfering with the later eluting analytes does not co-elute here. The best peak resolution and separation takes place in the 1st half of the chromatogram. Table 4.5 lists the solvents numbered on the above chromatogram.

Table 4.5 – Peak labels from Figure 4.15

Peak #	Name	Retention Time (sec)	Peak #	Name	Retention time (sec)
1	Solvent Peak - Methanol	76 , 1.780	30	isopropyl acetate	320 , 1.610
2	ethanol	96 , 1.875	31	1-butanol	328 , 2.257
3	pentane	112 , 0.810	32	heptane	416 , 0.960
4	MTBE	112 , 1.200	33	nitromethane	424 , 2.490
5	2-propanol	112 , 1.815	34	1,4-dioxane	456 , 3.540
6	acetonitrile	112 , 2.600	35	2-ethoxyethanol	484 , 2.270
7	ethyl ether	116 , 0.870	36	propyl acetate	488 , 2.296
8	ethyl formate	124 , 1.240	37	methylcyclohexane	508 , 1.095
9	1,1-dichloroethene	128 , 0.995	38	3-methyl-1-butanol	588 , 1.795
10	methyl acetate	132 , 1.255	39	methylisobutyl ketone	596 , 2.650
11	methylene chloride	136 , 1.860	40	pyridine	632 , 1.892
12	1-propanol	156 , 3.180	41	Methanol impurity	680 , 1.390
13	*trans*-1,2-dichloroethylene	160 , 1.390	42	Toluene	700 , 1.985
14	acetone	164 , 0.930	43	1-pentanol	720 , 1.095
15	trichloroethylene	172 , 2.530	44	isobutyl acetate	732 , 1.570
16	hexane	196 , 0.860	45	N,N-DMF	764 , 1.501
17	MEK	196 , 1.605	46	2-hexanone	768 , 1.785
18	2-butanol	204 , 2.945	47	butyl acetate	832 , 1.410
19	*cis*-1,2-dichloroethylene	216 , 2.475	48	chlorobenzene	892 , 1.860
20	ethyl acetate	224 , 1.545	49	DMSO	896 , 3.310
21	chloroform	232 , 2.922	50	ethylbenzene	920 , 1.335
22	THF	248 , 1.380	51	m-xylene	932 , 1.330
23	2-methyl-1-butanol	252 , 0.390	52	N,N-DMA	940 , 2.920
24	1,1,1-trichloroethane	280 , 1.495	53	o-xylene	972 , 1.360
25	1,2-dichloroethane	292 , 3.790	54	Anisole	1008 , 1.825
26	1,2-dimethoxyethane	300 , 1.800	55	Cumene	1020 , 1.185
27	cyclohexane	316 , 0.995	56	1-methyl-2-pyrolidone	1168 , 2.615
28	benzene	316 , 1.490	57	Tetralin	1300 , 1.305
29	carbon tetrachloride	316 , 1.915	58	Sulfolane	1352 , 1.305

A major reason why GCxGC is not currently being used to perform residual solvent analysis in the pharmaceutical industry is because the software is not 21 CFR compliant. The software used to analyze the data is not in compliance with the current requirements. The Leco Corporation expects to have the compliance in the future but they do not know of any other reason that the "technique" would not be compliant.

Chapter 5

Conclusions

5.1 - Conclusion

The research conducted provided a qualitative separation and analysis of sixty pharmaceutical solvents. The focus of this research was can a method be developed that was more accurate and efficient in the analysis of residual solvents compared to the current method, USP <467> and keep the run time under 30 minutes. The method was successfully developed and will be challenged to a quantitative analysis of results. A more quantitative analysis of the data and of the method falls under the scope of future research.

Comprehensive two-dimensional gas chromatography was used to resolve 60 solvents generally found in pharmaceuticals. These 60 solvents are listed into three solvent classes by the International Conference on Harmonization. Having a run time of twenty-eight minutes fifty-eight class 1, 2, and 3 solvents in methanol were fully resolved using a 5% diphenyl 95% dimethyl polysiloxane stationary phase in the first dimension column and a polar polyethylene glycol second dimension column.

In order to develop a generic method for residual solvent analysis that was more reliable, reproducible, and faster then the current USP <467> different avenues had to be explored. The first method developed used a "semi" polar second dimension column that did not utilize the full separation power of the GCxGC system. Staying with the first method the diluent was changed from methanol to dimethyl sulfoxide (DMSO) to verify whether or not any analytes were co-eluting or hidden by the methanol solvent peak. DMSO only worked well for solvents before the solvent peak but the later eluting solvents could not be seen because the impurities in DMSO interfered with those in the injected solution. For method one the results with methanol gave

poor peak resolution in the second dimension where as the first dimension peaks did not loose any typical GC resolution.

Development of the second method yielded very favorable and attractive results due to the polarity change in the second dimension column. The polyethylene glycol second dimension column paired with a 5% phenyl polydimethyl siloxane first dimension column using methanol as the diluent allowed for the characterization of class 1, 2, and 3 residual solvents. It gave a much better representation of the solvents and allowed the achievement of a much more improved separation in the second dimension. The polar second dimension column greatly facilitates the resolution of the analyte peaks as well as the highly selective nature of a two dimensional gas chromatograph.

GCxGC will continue to be the instrument used for future research. Currently research is being conducted modeling the exact mixtures stated in USP <467> using headspace SPME (Solid Phase Microextraction) injection. The next step being using the method developed to inject spiked samples of pharmaceutical APIs (active pharmaceutical ingredients) and be able to identify the peaks of solvents used.

This work was recently published in the February 2008 issue of LCGC North America. The current SPME work was presented at Pittcon 2008 in New Orleans, Louisiana by Mandy Danser. It will also be the topic of a plenary lecture and poster presentation at the 32nd International Symposium on Capillary Chromatography and the 5th Annual GCxGC Symposium Riva del Garda, Italy, May 2008.

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