Part One: A Flow Reactor with In-Line Analytics: Design and Implementation

Part Two: In-Line Derivatization of Protic Compounds for GC/MS Reaction Monitoring

Kristina Somerville

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Abstract

The creation of an automated synthetic reaction system with in-line analytics is presented in two parts. First, the design and implementation of a flow reactor system complete with in-line gas chromatography mass spectrometry (GC/MS) analytical instrument is discussed. The testing and validation of the systems components was completed in order to create a fully automated chemical reaction system capable of running, analyzing and optimizing a synthetic transformation without operator intervention with the system. The results of the synthetic transformation of allyl phenyl ether to 2-allyl phenol are presented to demonstrate the system's capabilities.

Secondly, an approach was developed to combine a sample preparation step known as derivatization with the reactor-analysis system. Many compounds are not directly amenable to GC/MS analysis, and derivatization is used as a sample preparation step to chemically functionalize compounds so that they can be analyzed with GC/MS. The use of derivatization in combination with a reactor-GC/MS system was introduced to help increase the scope of reactions that can be run and analyzed using this approach. The design, validation and use of a flow derivatization setup was completed and combined to the flow reactor and analysis system without interrupting the already established sample transfer system. The hydrolysis of benzonitrile with phthalic acid is used as a model reaction to demonstrate the reproducibility of the derivatization setup.

Acknowledgements

First I would like to thank Dr. Organ for giving me the opportunity to complete my Ph.D. in his lab. It was an honour and a pleasure. Michael Tilley and Guanlng Li – the first section of this thesis would not have been able to happen so quickly and smoothly without your expertise. Thank you for your dedication and time to the project.

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To my family, my mother, father, sister and step parents – you have all throughout my entire life given me unconditional love and support. I sincerely love and thank you for everything you have done. I hope I have made you proud and will strive to continue to do so for the rest of my life.

Lastly, Richard Rucker, my husband, my rock and my best friend. Finishing my research would not have gone as quickly and be as painless as it was if you were not in my life. Thank you for everything you have done and everything I know you will do for me for the rest of our lives. I love you more than anything in this world and I consider it an honor to be your wife.

Dedication

Dedicated to the memory of my Uncle Steve,

From this Willoughby Wimp to the original Pinawa Pansy,

I hope you are doing cartwheels in heaven.

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Abbreviations

µL – microliter

2AP – 2-allyl phenol

APE – Allyl phenyl ether

API - Active pharmaceutical ingredient

BF3 - Boron trifluoride

BMA - N-benzylmethylamine

BMAPP - 1-[Benzyl(methyl)amino]-3-phenoxypropan-2-ol

BSA - Bis(trimethylsilyl)acetamide

BSTFA - Bis(trimethylsilyl)trifluoroacetamide

CFU – Continuous flow unit

CI – Chemical ionization

CTC – Autosampler

DME - Dimethylethoxide

DMI - 1,3-Dimethyl-2-imidazolidinone

DMSO - Dimethylsulfoxide

EA - Ethyl Acetate

EI – Electron Impact or electron ionization

EPP – 1,2-epoxyphenoxypropane

ESI – Electrospray ionization

GC - Gas chromatography

GC/MS - Gas chromatography coupled with a mass spectrometry mass analyzer

HA – Heterocyclic amine

HPLC - High pressure liquid chromatography

HPLC/MS – High pressure liquid chromatography with mass spectrometry mass analyzer

HPLC/NMR – High pressure liquid chromatography with nuclear magnetic resonance spectroscopy detector

HPLC/UV/MS - High pressure liquid chromatography with UV detector and mass spectrometry mass analyzer

L – liter

m/z - Mass to charge ratio

MACOS – Microwave assisted continuous organic synthesis

MDMA – 3,4-methylenedioxymethamphetamine

MeCN - Acetonitrile

MeOH- Methanol

mL – Mililiter

MS - Mass spectrometry

MTBSTFA - N-methyl-N—(tert—butyldimethylsilyl)trifluoroacetamide

NMR - Nuclear magnetic resonance spectroscopy

NPD - Nitrogen phosphorous detector

P - Pump

PDMS - Poly(dimethylsiloxane)

PFPA - Pentafluoropropionic anhydride

PPM – Parts per million

R - Reservoir

R^2 – Coefficient of determination

RBF – Round bottom flask

SIM – Selected ion monitoring

TBB – Tetrabutylbenzene

*t*BDMCS - *ter*t-butyldimethylchlorosilane

*t*BDMS - *te*rt-butyldimethylsilyl

THF – Tetrahydrofuran

TLC – Thin layer chromatography

TMCS - Trimethylchlorosilane

*t*MDMS - *tert*-butyldimethylchlorosilane

TMS - Trimethylsilyl

TMSA – Trimethylsilylacetamide

UV - Ultra violet detector

V – Valve

VOC - Volatile organic compound

Statement of Author Contribution

This project was highly collaborative with the work being presented, **only in part one,** the result of the cooperation of the whole team. All members have contributed in various degrees to the analytical methods used, to the research concepts, and to the experiment design. These members include myself, Michael Tilley, Guanlong Li, Debasis Mallik, and Michael Organ. The following results were first presented in "A flow reactor with in-line analytics: design and implementation"[1] and some paragraphs below are adapted from this article.

1 Chapter One

1.1 Introduction

Chemical synthesis is employed across different industries to prepare everything from fine chemicals, such as pharmaceutical ingredients and precursors (e.g., over-the-counter medicine), to large scale synthesis of polypeptides for use in biological applications (vaccines, for instance). In an ideal scenario, all chemical transformations would give 100% yield and conversion of final products. However, it is well known 100% conversion in every reaction does not occur, and countless hours are often spent optimizing a procedure until the most efficient pathway to produce a desired compound is determined. Due to the increase in demand for synthetic chemicals and commodities in today's society, there is a significant need to develop more efficient methods for the preparation and purification of reaction products. As a result of this need for efficient but precisely-controlled manufacturing protocols, automated, high throughput applications capable of fast and reproducible reactions are increasingly being adopted in laboratories.

These automated processes can greatly reduce the inherent human errors that occur when multiple chemists are trying to produce a single compound (batch variability) and can increase efficiency of all aspects of chemical synthesis (e.g. reaction, sampling, sample preparation, analysis). Other paradigm shifts in the field of synthetic chemistry also help to increase reaction output. These include: a technique known as continuous flow chemistry, where syringe pumps flow solutions through tubing to run reactions, as well as using microwave irradiation as a reaction heat source instead of other traditional methods. In situations requiring utmost efficiency, typical steps for reaction work-up and analysis are also automated. To further enhance the efficiency of chemical synthetic pathways, the addition of analytical instrumentation

in-line with flow chemistry reactor systems has been developed to increase throughput of both the reactor and analytical instrument.

1.1.1 Flow Chemistry

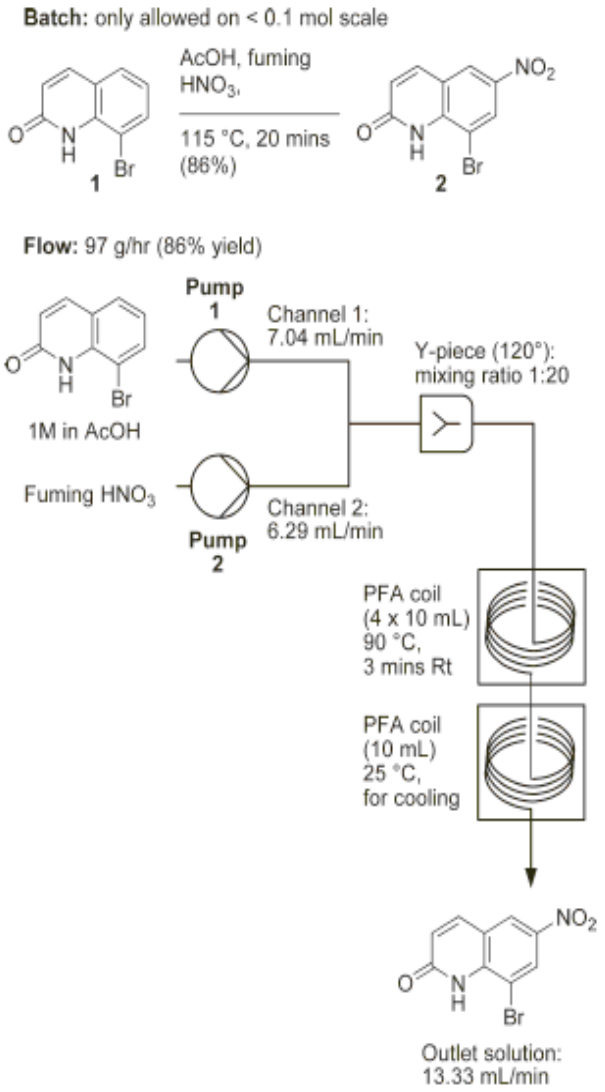
The adoption of flow chemistry into the laboratory was motivated initially due to the need for high yielding, selective, and continuous reactions. Flow systems have since been developed for applications ranging from the creation of nanoparticles,[2] for use in biological testing,[3] and for milligram scale organic synthesis.[4] Flow chemistry reactions are often performed using syringe pumps that are responsible for flowing solutions through a heated reactor zone, and valves, responsible for switching between different process lines to help with delivery and collection of product, are also used.

One advantage that flow chemistry has over traditional batch synthesis can be attributed to the separation of starting materials and movement of the solutions. Flow chemistry helps to avoid issues like decomposition and side or by-product formation due to the fact that starting materials in a flow reaction are usually introduced into the reactor stream from multiple inputs; the use of different reagent streams keeps the vast bulk of the starting materials separated until just before they are ready to undergo reaction. Products in flow reactions are flowed away from the inlet and incoming starting materials, thus eliminating the intermingling of starting materials and products during the reaction. Alternatively, traditional batch style chemistry has limitations if a reaction takes a long time to complete, if final products are reactive, or if a reaction is prone to side product formation. Since all reaction components remain in the round bottom flask (RBF) throughout the entire batch reaction, newly formed product can commingle and react with intermediates or starting materials that are in vast excess at the beginning of a transformation, leading to the possible formation of unwanted side products. Additionally, if a reaction requires

heat, there is a higher chance for decomposition or side product formation to occur in batch. In light of these advantages, flow chemistry has become a platform for improving synthetic efficiency.[5]

Flow reactor volumes are usually much smaller than batch reactor volumes (µL in flow vs mL or multi-litre for batch reactions) and are limited to producing a small amount of product at any given instant in time. This small reactor volume is especially beneficial if the reaction is exothermic. As, at worst, a small amount of heat is released continually from a reaction performed in flow, instead of a large, possibly dangerous amount of heat all at once, such as when large quantities of chemicals undergo an exothermic reaction in a RBF.[6] As an example, Figure 1 depicts a flow reactor capable of delivering 97g/hr of product while the same experiment run in batch reaction has a best case scenario limited to ~58 g/hr due to its exothermic nature.

Figure 1 - Comparison of the reaction time, concentration and temperature for batch and flow reactions of the exoterhmic nitration of 8-bromo-1H-quinolin-2-one reaction.[6]



Even though flow reactions are done on a smaller scale, they can still meet the large scale output required for synthetic applicability. Continuous flow conditions or parallel synthesis can be used to run reactions indefinitely, or for as long as is needed to produce a required amount of compound. This type of synthesis, known as scaling-out a reaction,[7] can be used to produce both large and small scale amounts of product under the same conditions; an advantage over batch

synthesis as often batch synthetic transformations require additional method development when changing from small to large scale production.

The advantageous qualities of continuous flow chemistry are further enhanced by the use of microwave irradiation as a heat source for promoting chemical reactions. Microwave heating has been demonstrated to heat a reaction more homogenously through dielectric heating (using a molecule's dipole moment to "excite" and heat it) compared to conductive heating (physical transfer of heat) that is used with oil and sand baths. Microwave irradiation as a heat source has been demonstrated in both flow[8] and batch format[9] to have positive effects on reaction outcomes.

1.1.2 Automated Technologies

Flow chemistry also has another advantage over batch synthesis; the process is easily automated. Automated technologies are present in many everyday factories and manufacturing processes. From everything including car manufacturing to sorting small objects into containers, automated technologies allow for quick, reproducible motions that can be relied on to yield the same outcome every time a task is performed. When considering the possible intersections of chemical synthesis and automated technologies, it is apparent that automation can offer more benefits than just reproducibility. As mentioned earlier, flow chemistry is beneficial for reactions that are exothermic. Automated technologies add an extra layer of safety into flow chemistry reactions; even if something were to go wrong in an automated flow reaction, using automation means no human is required to be near the reaction when it is occurring. If something were to happen during a flow reaction, there would be a less likely chance that a person would be hurt when compared to reactions that are run under the supervision of a chemist. The increase in chance of getting injured is simply because a chemist is normally present during the times in which something is most likely to happen in batch (i.e. adding solutions or chemicals to the

reaction) but with automated technologies responsible for programming the pumps and valves to add reagents to the reaction, injury can be avoided.

Automated technologies can be used when several sample preparation steps are required to maintain reproducibility and accuracy, but also when the sample preparation includes toxic reagents to eliminate human contact with dangerous materials. Automation can also be used to eliminate skilled workers from menial tasks that can be done with automated software. Using automated technologies can also help stream line the combination of two different techniques, for example a flow chemistry reactor system and analytical instrument. With software that is capable of controlling more than one system at a time, combination systems capable of automated reaction running and analysis can be created to improve throughput and decrease the amount of down time that is required between reacting and analyzing a sample.

1.1.3 In-Line Reaction Monitoring

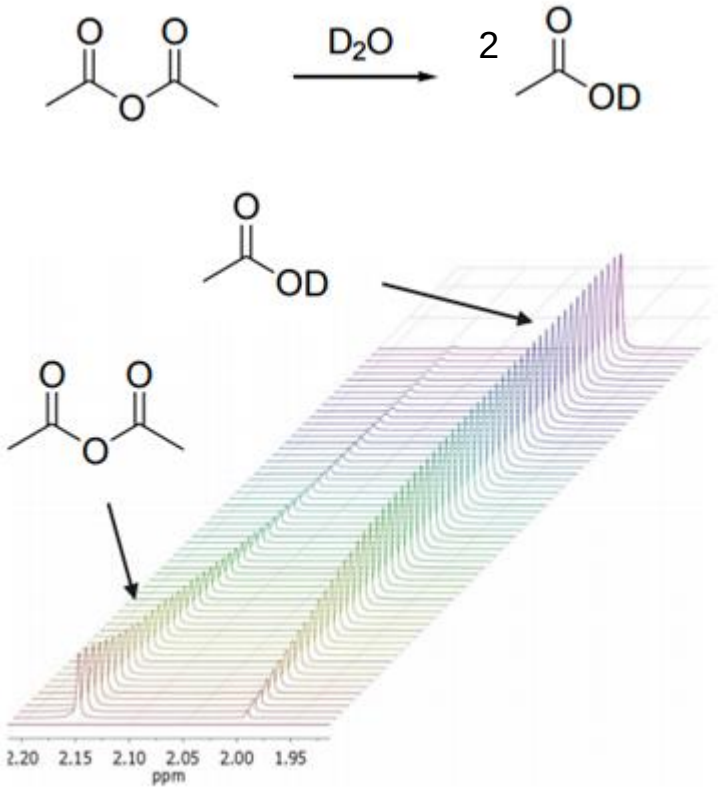
Reaction monitoring is an essential part of both batch and flow chemical synthesis. Without finely tuned and calibrated analytical instruments, being able to quantify the progression of a reaction would be much more difficult. Automated technologies have made it easier to produce sample preparation sequences that are typically required before analysis. Automation allows these sequences to be completed quickly and accurately to help speed up the analysis process. However, no matter how well automation works for the reproducibility of sample preparation steps, if the compounds in a sample cannot be analyzed using the chosen analytical instrument, no amount of automation will enhance the analysis. The choice of analytical instrument to be used to monitor a reaction is crucial in getting the best, most accurate information from the sample. Decisions on analytical instrument to be used should be done so

considering (among other things) the type(s) of samples being analyzed, purpose of analysis (qualitative vs. quantitative) and cost and efficiency of the procedure.

1.1.3.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy (NMR) is a technique that uses strong magnets to manipulate the spin of active nuclei. It is a commonly-used technique that many, if not all, organic chemists have used at one point to monitor the progression of a reaction. One example of an in-line NMR reaction monitoring system was developed through a joint effort between the University of Kansas, Pfizer and Bruker Biospin.[10] Their goal was to "develop a system that can provide real-time reaction monitoring capability with value added information for better process understanding", which was highlighted in a Royal Society of Chemistry publication.[10] In one of several examples in this publication, a simple yet clear illustration of the use of flow NMR techniques to monitor a reaction is shown in Figure 2. Here the conversion of acetic anhydride to acetic acid in D_2O solvent was studied. Using a flow probe inserted into the bottom of a normal NMR instrument, a temperature controlled flow cell inside the probe was used as the reactor. By

Figure 2 - Flow NMR analysis used for monitoring the conversion of acetic anhydride to deuterated acetic acid.[10]



using syringe pumps, a solution was pushed through the flow cell and the reaction proceeded. Proton NMR was able to monitor the decreasing peak of the upfield-shifted methyl hydrogens on the anhydride which, over time, decrease as the downfield-shifted methyl peaks of the acid arise.

Unfortunately, NMR techniques are inherently lower in sensitivity compared to other analytical techniques, and often a larger sample size is required to receive good signal. More advanced multidimensional techniques have been developed to harness the power of NMR to delve deeper into the structural connections, atomic locations and coupling partners inside molecules.[11] However, one of the problems with NMR is that normally all components of a mixture are being analyzed at once. If starting materials and final products have similar structures, peak overlap can make it hard to quantify the reaction progression. To aid in the pursuit of monitoring a reaction using in-line analytics, some type of sample separation is necessary before analysis in order to get quantitative information from the sample.

1.1.3.2 Chromatography

Chromatography, meaning "to write with colors" - literally translated from its Greek roots *chroma* and *graphein* - is a method for separating mixtures into their individual components. The invention and earliest use of chromatography was done by biologist M. S. Tswett in 1905.[12] Tswett separated different coloured pigments (hence the name) in plant matter using calcium carbonate and various solvents.

Chromatography is done using a mobile phase and a stationary phase. Not surprisingly, the mobile phase in chromatography is a gas or liquid that *moves* through a stationary phase, often referred to as a column, permanently bound to a solid support scaffold. The interactions between compounds with the two phases of chromatography (among other things) dictate how well

compounds will be separated in a mixture. Currently, many different types of chromatography are used as analytical separation techniques.

1.1.3.2.1 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a bench top, small scale, qualitative method of reaction monitoring. Knowing where starting materials and final products show up on a TLC plate, one looks for an increase in product spot and a decrease or disappearance of starting material spot to monitor a reaction. TLC is a tool that most chemists use on a daily basis as it is quick, inexpensive and easy to do. However, TLC is not for high throughput automated use by any means.

1.1.3.2.2 High pressure (performance) liquid chromatography (HPLC)

High pressure (performance) liquid chromatography (HPLC) is a technique that uses high pressure syringes to deliver samples through separation to analysis using liquid as a mobile phase. With more advanced systems, HPLC can switch between columns with different stationary phase chemistries, can use multiple solvents with varying polarity, and systems can change between flow rates and gradients to help thoroughly separate the components of a sample. Often coupled with an HPLC is an ultra-violet spectroscopy (UV) detector and a mass spectrometer (MS) mass analyzer. The combination of HPLC/UV/MS gives information on mass and fragmentation of the compounds seen eluting from the column. In recent years, literature has surfaced with HPLC/MS being used as a reaction monitoring instrument.[13] And unsurprisingly, there have also been recent cases of NMR and HPLC being combined together for reaction monitoring purposes.[14] HPLC/MS and HPLC/NMR both have advantages, but for the creation of a new reactor and analysis system, both can be time consuming and costly to first initiate. A related technique – gas chromatography mass spectrometry (GC/MS) - can be used just as easily

for monitoring reactions and its cost, performance, maintenance and upkeep is much better suited for in-line reaction monitoring.

1.1.3.2.3 Gas Chromatography Mass Spectrometry (GC/MS)

Gas chromatography (GC) is a chromatography technique using gas as a mobile phase and (most commonly) a liquid lined capillary column to carry out separations of analytes in the gas phase. A single gas mobile phase (normally helium or hydrogen, heated and dried before entering the GC) is much easier to handle compared to the multiple solvents and additives used in HPLC. Gas phase separation is much easier to do when compared to separation in the liquid phase, and longer column lengths can be used in GC compared to HPLC; the longer column length leads to (for example) the ability to separate structurally similar fatty acids that would be difficult to separate via HPLC.[15] Although this column length increase is advantageous for separation, admittedly GC runs have longer analysis times than HPLC.

A variety of GC capillary columns are available, with different lengths and stationary phase thicknesses and chemistries. These stationary phases range from completely non-polar (100% poly(dimethylsiloxane) (PDMS)) to extremely polar (1,5-Di(2,3-dimethylimidazolium)pentane bis(trifluoromethylsulfonyl)imide on fused silica). Each type of column has benefits and is normally used to separate compounds that have similar polarity to the stationary phase. However, all columns are not capable of being used with all detectors. Highly polar columns normally have higher bleed (stationary phase loss over time) and are unsuitable for the most sensitive detectors (MS); while non-polar columns are ideally suited for low level analysis due to their low bleed levels. Stationary phases, such as intermediate polarity cyanopropyl-based stationary phases, cannot be used with nitrogen phosphorous detectors (NPD) because the bleed profile is much higher due to the presence of nitrogen in the GC column.

Choosing the proper stationary phase is crucial for proper separation and should be done based on the type of compounds being separated, type of detector being used and amount of compound needing to be separated.

Mass spectrometry (MS), when combined with GC, is a technique used to analyze compounds eluting from the GC column and it gives information on the mass and fragmentation patterns of each compound. GC/MS is a very sensitive technique, with sample quantification and detection levels being very small.[16] As analytes elute from the GC column, they are transferred to the mass spectrometer through a transfer line. At the end of the transfer line is a source that, by various methods, fragments the compound into a radical and cation (or anion if negative mode is on). MS analysis gives information on the ions formed only and the radicals are not detected. The information obtained from a MS is in the form of mass-to-charge ratio (m/z). Where m is the mass of the ionic species hitting the detector and z is the charge of that ion (most often +1).

The most widely used type of ionization source for GC/MS analysis is electron impact or electron ionization (EI), where a stream of electrons are introduced to the source via the heating of a filament with electric charge running through it. The compounds eluting from the chromatography column are introduced orthogonally to the electron stream in the source so that these electrons collide with the analytes of interest and molecule fragments are formed. EI is known as "hard" ionization as the compound is almost always fragmented and often the molecular ion ($M^{+\cdot}$) is not visible. The missing M+ hinders the ability to see the molecular mass of the compound but enhances the structural information that can be obtained about the compound. Chemical ionization (CI) introduces a gas (often methane or ammonia) into the source in large excess of the analyte. The fragmentation here undergoes bimolecular reactions of various types (e.g. proton transfer, electrophilic addition, anion abstraction) to form ions. Known

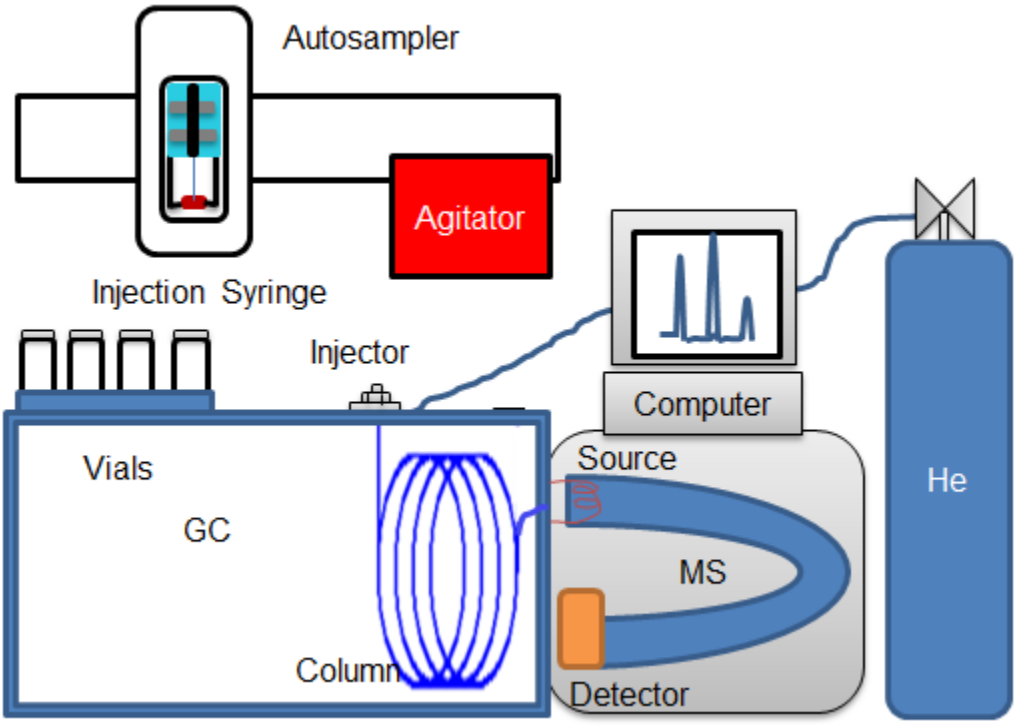
as a soft ionization technique, this method can often give molecular ions to help determine the molecular weight of the compound but less often can give as much structural information about a compound as EI techniques can.

After the source, there are many different configurations of a MS that can occur. Time-of-flight mass spectrometry is a technique that monitors m/z by the time it takes ions to complete the path (with heavier ions taking longer time). Quadrupole and triple quadrupole instruments use electric fields to manipulate the path of ions so that only the analytes of interest are actually detected. Triple quadrupole instruments have the added ability to do a second fragmentation with inert gas (often argon) to gain further knowledge of a compounds structure. The MS's detector records the current produced when an ion passes by or hits a surface. Often an anode is used and peaks in a chromatogram arise from detector current as a function of m/z and electron multipliers can also be used to amplify signal in the detector These are only a few examples of MS configurations and many other types of MS exist. There is also still plenty of research into MS technologies ranging from miniaturization[17] to different ionization methods.[18]

Seen in Figure 3 is a GC/MS instrument. Helium gas is used as a mobile phase and it is flowed through a trap to stop any oxygen or water in the gas from entering the instrument. The inlet to the GC is an injector port that an injection syringe filled with analyte enters. The injection port is heated to aid in vaporization. The analytes travel out of the injection port and into the column under the pressure of the helium mobile phase. Mixtures are separated into their individual components on the column and each component exits the column and enters the MS. Here, ionization occurs in the source, and a detector gives fragmentation information on the eluting compounds. Data is given in a chromatogram where each peak at a certain time

represents a compound, and under each peak is a mass spectrum that displays which m/z values are present.

Figure 3 - Schematic of GC/MS instrument



Discussed below is the design and implementation of an automated continuous flow reactor and analysis system, where reactions are run with syringe pumps, solutions are heated with microwave irradiation and samples are analyzed via an in-line EI triple quadrupole GC/MS instrument.

1.2 Results and Discussion

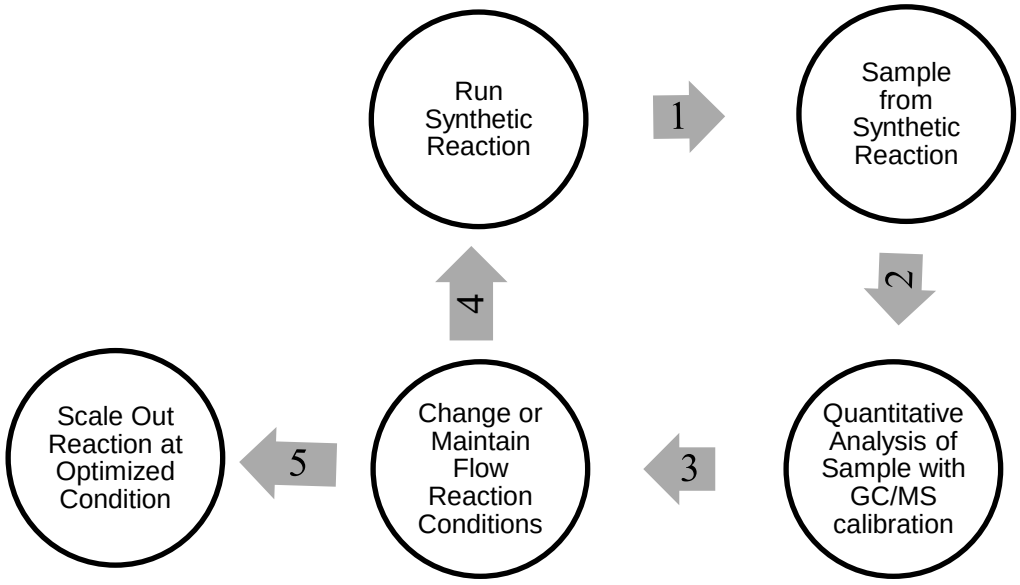
1.2.1 Expected Reactor-Analysis Abilities

The combination of the reaction system with in-line analytics will further be referred to as a microwave assisted continuous organic chemistry (MACOS) unit. The MACOS system completed was made partially with commercially available products but also with in-house

created software and hardware. Using both types of products was necessary to facilitate the automation of all parts of the system. All commercially available products come with their own set of software and hardware that must be used or mimicked by an outside source in order to function properly. The commercially available products include the GC/MS, microwave, pumps and valves. Accessing and gaining control of these proprietary items was of upmost concern. As one of the main purposes of automating multiple processes is to streamline the connection between the systems, if the commercially available products were not capable of being connected or controlled the same way the in-house created software and hardware was, there would be a large disconnect between parts of this reactor system.

The general process that should be accomplished when an automated reactor and analysis system is used for optimizing synthetic reactions is seen in Figure 4.

Figure 4 – Steps used to optimize and scale out a reaction using automated technology.



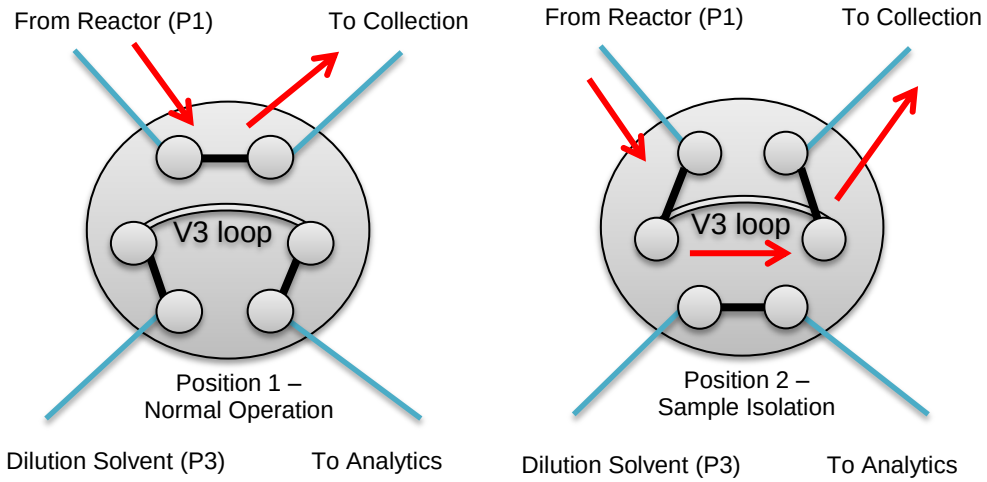
The automated reactor and analysis system should be capable of 1) running a continuous flow reaction and sampling from the reaction stream without interrupting the reaction flow, 2) performing sample preparation and GC injection sequences to quantitatively analyze a reaction sample, 3) changing reactor conditions, 4) restarting the reaction until an optimized amount of product is created and 5) scaling out the product. Logistically, all moving parts of the reactor system need to be under the control of single software. The first steps in creating the system was the creation of software to replace each of the proprietary software, condensing all into a single, central work horse for MACOS operations.

Several tests were undertaken using the automation software and various parts of the reactor system; each test designed to calibrate and/or validate the use of each part of the system under the control of the MACOS software.

1.2.2 Design and Validation

1.2.2.1 Reagent Delivery and Sample Isolation

Figure 5 - Isolation mechanism used by valve V3 to remove an aliquot of reaction from the flow reaction stream. Red arrows indicate flow of reaction stream. Black lines indicate connected ports. Blue line is tubing



To test the general ability of the software to run the pumps and valves accurately, a basic setup of one reagent delivery syringe pump (P1) and one valve (V3) was created. P1 is programmed to flow solutions at a certain flow rate through V3, a 6-port 2-position sample isolation valve. V3 has two operating positions (Figure 5). In one position (normal operation) the valve sits such that the flow coming from P1 (the reactor) enters the valve and leaves very quickly to collection. In position 2 (sample isolation) the valve changes positions which coincides with changing which ports of the valve are connected. When in position 2, the stream from P1 is diverted into a very small length of tubing known as a sample loop. This loop is used to isolate an aliquot of the reaction stream so that a small portion of the reaction can be analyzed; everything isolated in V3 will be analyzed by the GC/MS. After sample isolation and after V3 has switched back to position 1, a different pump, P3, under the control of the same software as P1 and V3, pushes solvent through the V3 loop. P3 pushes the sample out of the loop and into an awaiting vial used to collect the sample. These samples are then brought to the GC/MS to be injected and analyzed.

1.2.2.2 Testing the control of the pumps and valves using MACOS software

The volume of solvent isolated in the loop is extremely important for quantification purposes because calibrations that are made to monitor the progression of a reaction rely on the V1 loop volume to create the calibration solutions. Following equation 1:

$$C_{rxn} \cdot V_{loop} = C_{dil.rxn} \cdot V_{dil} \qquad \text{(eqn 1)}$$

where C_{rxn} = concentration of the synthetic reaction (e.g. 0.1 mol/L APE), V_{loop} = isolated loop volume (from V3), C_{dilrxn} = the concentration of the reaction sample after dilution (e.g. isolated sample pushed with P3 to vial) and V_{dil} is the dilution volume from P3 pushing the sample.

$C_{dil.rxn}$ cannot be calculated accurately without knowing the volume or fill volume of the loop and since $C_{dil.rxn}$ is also equal to the concentration of the calibration solutions that are made to monitor reactions, quantification is not accurate if the volume isolated in the loop is not always (as close as possible to) V_{loop} There are two broad methodologies that can be adopted to fill the sample loop: partial fill and overfill. During overfill, a fluid volume greater than the loop capacity is passed through the loop, and the loop itself is used to cut the sample. While this strategy should lead to high run-to-run reproducibility, partial filling, isolating a volume smaller than the loop capacity, offers the advantage of sample size variability without the need to change the loop. Both methodologies were tested looking to determine which has better accuracy and precision so that methodology can be employed when isolating a real reaction sample.

1.2.2.2.1 Partial Loop Fill Experiments

In order to test the control of P1, V3 and P3, an experiment was completed such that P1 continuously delivered a solution of 1000 ppm 1,2-dimethoxybenzene (veratrole) in isooctane through tubing and into a 100 μL loop installed on V3. When ready, the software instructed V3 to switch from position 1 to position 2 so that sample isolation could occur. V3 is programmed to switch back to its original position and stop the collection once the software indicates that P1 has flowed the desired amount. Then, P3 is used to push the isolated sample into a vial. Each sample isolated was analyzed against a calibration curve to determine how well the setup performed. This test is not only to determine the ability of P1 to deliver the volume the software states it has, but also to verify that the valve switch is quick enough, with no leaking, such that only the desired amount of reaction solution is captured. The protocol of using V3 to switch back and forth instead of having P1 stop flowing solution is done so that P1 can maintain forward motion and preserve the continuous operation needed (Table 1).

Table 1 – Results of partial loop fill experiments

Entry	Isolated Sample Calibration Reading (ppm)[a]	Theoretical Reading (ppm)	Difference (ppm)	Difference Actual - Theoretical (average, ppm) / % Difference / Standard Deviation
5 μL isolation				
1	3.44	3.33	0.11	0.40
2	3.70		0.37	
3	3.77		0.44	11.89
4	3.80		0.47	
5	3.80		0.47	0.14
6	3.85		0.52	
10 μL isolation				
1	7.13	6.67	0.47	0.67
2	7.35		0.68	
3	7.12		0.45	10.08
4	7.48		0.82	
5	7.52		0.85	0.16
6	7.43		0.77	
15 μL isolation				
1	10.74	10.00	0.74	0.51
2	10.74		0.74	
3	10.66		0.66	5.07
4	10.03		0.03	
5	10.51		0.51	0.25
6	10.36		0.36	
25 μL isolation				
1	16.45	16.67	-0.22	0.09
2	16.55		-0.12	
3	16.67		0.00	0.24
4	16.60		-0.07	
5	16.79		0.12	0.11
6	16.70		0.03	

The largest variation for the partial fill experiment was seen at low isolation volumes (Table 1, 5 μL isolation, ~12% off from expected value). The variation was anticipated however; as

partial filling of sample loops has already been documented variable in many HPLC applications[19] where typically no less than 10% of the loop is ever isolated for analysis.[20] Fortunately it is noticed that as the percent volume of the loop being filled is increased, there is a dramatic decrease in average difference between the sample reading and the theoretical reading (Table 1, 5 μL vs 25 μL isolations). The standard deviation between the 6 injections of the same isolated volume also decreases with increasing sample volume (Table 1, 5 μL vs 25 μL isolations). These results indicated that the pumps and valves were functioning properly, that the software was controlling them as expected and that the errors were being introduced through the partial filling of the loop. It can be hypothesized that if one were to attempt higher volume loop fills a "sweet spot" could be mapped out in order to get quantitative sampling, but larger sampling volume means longer time spent sampling and more material wasted just for analysis. Instead, the loop overfill methodology was tested in order to determine if it would give more precise sampling values compared to the partial fill method.

1.2.2.2.2 Overfill Loop Experiments

To minimize sampling volume and time, the 100 μL loop on V3 was replaced with a 5 μL loop. However, given a quote taken directly from the maker of the valve and loop, Valco, website:

"With small volume loops, the tolerance on the ID of the tubing (±0.001") can have a significant effect on the volume. Therefore loop volume and loop appearance may differ from batch to batch".

This statement indicates that even though this loop is labeled as 5 μL, the exact volume may be much different than that. In the overfill experiments there was no theoretical value that

the isolated samples should read. Therefore, the overfill methodology was used not only for comparison to the partial fill method but also to determine the exact loop volume.

For the overfill experiments, instead of using V3 to switch positions after a certain amount has been delivered via P1, the isolated volume is independent of the valve switch timing and the loop itself cuts the sample from the reaction. The overfill isolation experiments were completed with allyl phenyl ether (APE). A 0.1 mol/L solution APE was loaded into P1, isolated in V3 and collected with P3. Here the higher concentration of analyte is used as it is more representative of a real reaction solution. 45 overfill samples were taken and analyzed against a calibration curve. The calibrated volume of the loop after the overfill experiments were complete was found to be 6.85 μL with a pooled standard deviation of 0.059 μL (Table 2). This value is much smaller than the standard deviations of the partial fill method.

Table 2 – Results of loop overfill sample isolation experiments

Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Isolated Volume (μL)[a]				
6.746	6.688	7.157	6.997	6.890
6.769	6.595	7.237	7.028	6.848
6.712	6.587	7.204	6.887	6.855
6.771	6.659	6.963	6.936	6.845
6.694	6.736	7.015	6.930	6.852
6.675	6.641	7.042	7.002	6.796
6.690	6.633	7.087	6.842	6.814
6.696	6.712	7.108	6.916	[c]
6.604	6.641	7.146	6.918	[c]
6.683	[b]	7.237	[b]	[c]
Individual Averages (μL)				
6.704	6.655	7.120	6.940	6.843
Overall Average (μL)				
6.852				
Standard Deviation				
0.047	0.047	0.089	0.056	0.028
Pooled Standard Deviation				
0.059				

[a] – As per reading from GC/MS calibration curve with undecane internal standard

[b]- At this point in time, some troubles with the communication between pumps and software occurred intermittently. These 2 isolations were omitted as there was a known malfunction in the sampling procedure.

[C] - Only 7 samples were taken

Unfortunately using the overfill methodology would have meant that the sample volume would always remain at 6.85 μL. One may want to change the sampling volume for a variety of reasons, for example if the current reaction concentration is low and more than 6.85 μL sample is required for analysis. Sample size variability would not be possible with the single overfill methodology described above. Fortunately, one can exploit the precision of the overfill method to create a serial-overfill method. Here, the V3 loop was repeatedly filled with sample then

incremental flows of carrier solvent were pushed in between successive isolations. In other words, a number of plugs of sample were pushed into the beginning region of the transfer tube, separated by small volumes of ethyl acetate. The total sample volume of all isolated aliquots was pushed into a vial by P3. The isolated samples were analyzed against the same calibration curve used for testing the single overfill. For 45 two-fill isolations, the average amount collected as per the GC/MS data was 13.507 μL with an average standard deviation of 0.144 μL. This number is 0.2 μL less than expected, given the calibrated average of 6.85 μL for single-fill isolations (i.e., $6.85 \times 2 = 13.7\mu L$). This discrepancy, while small, is consistent throughout measurement and is thought to be a result of insufficient flushing of the sample loop with carrier solvent between isolations (200 μL flush between each isolated sample in the serial fill method versus 8 mL in the single overfill method). It is expected that, due to the smaller flush volume following the first isolation, 1.5% of the sample was not effectively displaced from the loop. Future experiments could be completed in order to determine how much volume it would take to flush the entire sample loop between isolated samples, however it was enough at this point to note the serial overfill methodology a feasible and relatively reproducible method that can be used in the future for changing the total volume of reaction sample isolated without a partial fill methodology employed. With these results, it was now determined that the pumps and valves functioned as desired using the MACOS software and the sampling technique to be used to help monitor a reaction is the overfill methodology. Without further information needed, it is to be noted that concurrently with the pump and valve testing it was also determined that the microwave heated and cooled according to the MACOS software commands. Being able to control the microwave meant that the GC/MS was the next focus of research.

1.2.3 In-Line Analytics

Up until this point, the vial that housed the sample after it was isolated in V3 was placed by hand at the end of the tube. Then, after a sample was collected, the vial was taken by hand to be placed onto the GC/MS vial holder so that it could be injected and analyzed. Of course, in an automated system there will be no human transport of the vials that is completed, and there needs to be a way to seamlessly transport a sample to a clean, dry vial on the GC/MS deck. Fortunately, the GC/MS is outfitted with a CTC autosampler that is already capable of automated movements that will help deliver the sample from V3 into a vial. However, in-house made upgrades to the autosampler's syringe plate hardware were required in order to facilitate this sample transfer through automation.

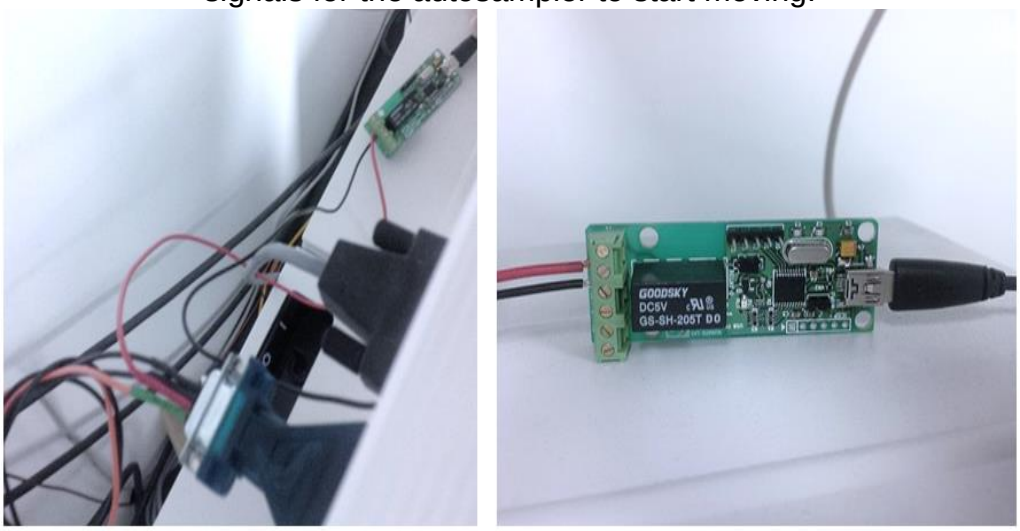
1.2.3.1 Autosampler

Autosamplers are increasingly being used for complete automated sample preparation.[21] One and two arm instruments have become available with advantages and disadvantages to each. One arm instruments have the free movement of the entire area; however only one needle is available and only one action can be completed at once. One arm can increase sample preparation time significantly and decrease productivity if complex steps are required. A two arm system, which can have a combination of needle sizes and is capable of multitasking, can also impede itself with trying to program both arms to move in the same area without hitting each other. A single-arm instrument, a CTC CombiPAL instrument with two vial trays and heater, is the autosampler used here. A single arm was selected to simplify the synchronisation between the autosampler and sampling events at V3.

The CTC software is far more advanced than the pump and valve software, it would be unwise to try and create an entirely new software that was capable of controlling all movements

of the autosampler; not to mention issues with CTC about being able to access the required codes. Instead of brand new software to control the CTC, the introduction of some relays and wired connections (Figure 6) allowed MACOS software to communicate with the CTC hardware on a limited basis but enough for automation purposes.

Figure 6- USB connecting device between the autosampler and MACOS computer. Attached to back of CombiPAL (left) and shown in detail (right), the MACOS software sends a signal via this relay which signals for the autosampler to start moving.



For all automated experiments using the autosampler, programming of the CTC arm's major functions was completed through CTC proprietary software and throughout the course of this research the main control of the arm has always remained with this software. However, the MACOS software, through the wired connections in Figure 6, can control the autosampler's start signal. The start signal gives MACOS software control over *when* the autosampler arm moves (but not how it moves). The proprietary software takes over all arm functions after the initial start signal is sent through the wired relay and it is this software that is responsible for completing the rest of the autosampler's movements. In order to use the autosampler and the wired relay connections to facilitate sample transfer, in-house applied upgrades were made to the

autosampler's syringe plate hardware as the second arm was necessary for the complete automation of the system to occur.

1.2.3.1.1 Side-arm Needle

There are commercial examples of autosamplers that use needles capable of what is called "sideport" entry to aid in solvent/sample delivery (Figure 7). Here, the beige screw indicates the entry path of solution to the syringe. The syringe plunger raises up past the hole and liquid flows through the GC injection needle (not pictured, flows out bottom of needle)

This sideport method of sample delivery is beneficial when low concentrations are used. However, with low detection limits of the GC and high reaction concentrations coming from MACOS, this type of needle was not suitable for use with the current set up. Carry over would have been a large issue if the GC injection needle came into contact with the crude reaction mixture. Instead, several iterations of a side-arm needle were created (Figure 8).

Figure 7 – Commercially available sideport entry needle.

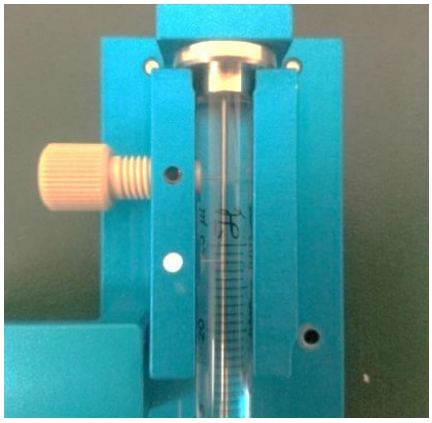
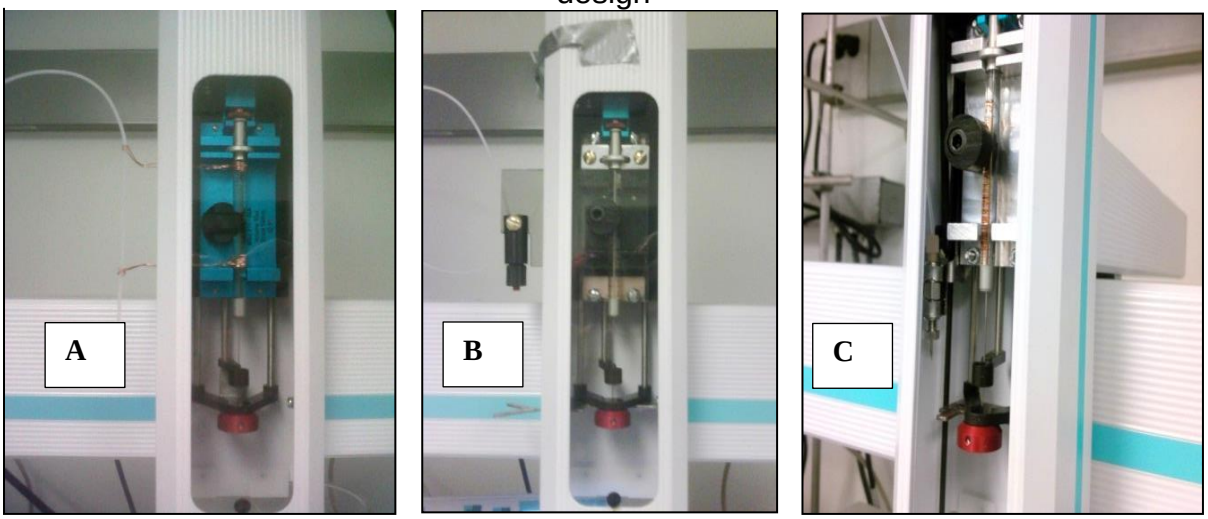
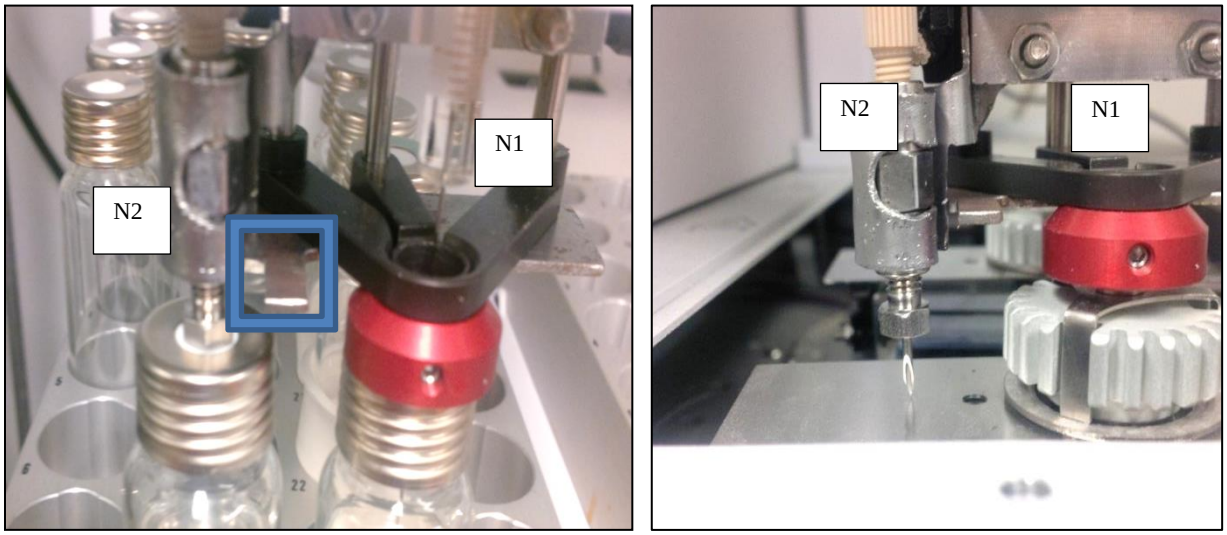


Figure 8 - Three iterations of the side-arm delivery system used to transfer sample from reactor to analytics. A) Proof of concept design B) Initial prototype c) Final design



The side-arm needle, N2, used for the final connection of the analytics to V3 and can be seen in Figure 8 picture c, as well up-close as in Figure 9. Figure 8 A) shows initial proof of concept "needle"; copper wire holds tubing in place over a septumless vial (since no actual needle is attached, the tubing cannot pierce a septum). B) Shows a side needle that is directly attached to the autosampler syringe plate and C) shows the final needle design. Hidden behind the autosampler shell, this side-arm needle has been used for all of the automated work in this thesis.

Figure 9- Side-arm (N2) position during sample delivery (left) and GC injection (right). N1 is GC injection syringe.



In Figure 9, the left picture shows the side needle (N2) when reaction sample is being delivered. In the blue box in Figure 9 is a small metal piece used to prevent the vial from traveling with the autosampler. In the right picture, the position of side needle when a sample is being injected into the GC/MS. The placement of this needle was very important as the autosampler used for these experiments is a very precisely controlled instrument. The movements that it makes are extremely exact in order for it to be able to access the proper spots on top of the GC/MS. It was feared that the added weight of the side-arm needle would cause disruptions to the normal operations of the autosampler. It is seen from Figure 9 that the side needle has been engineered so that it does not cause any damage to the GC injector when the autosampler is injecting a sample. The injection port can be one of the most expensive pieces of a GC instrument to replace, so care was taken to make sure the fabricated needle did not damage it. Even though it was apparent the needle did not impede the normal operations, it needed to be confirmed that when the GC injection syringe enters the injection port, the results of that injection are exactly the same compared to if N2 was not attached. To do so, calibration solutions were created of

APE and 2-allyl phenol (2AP), a pair of starting material and product from a Claisen rearrangement reaction (see Scheme 1). These calibration solutions were created such that they mimicked those that would be used to monitor a reaction of APE to 2AP at 0.1 mol/L. Each calibration solution was subsequently injected into the GC using both the original CTC syringe plate and the fabricated one with N2. As long as the calibration curves are similar and there is no change in peak shape or height in the side-arm injections, it is determined that the addition of the side-arm has no bearing on the autosampler's ability to do it's intended job.

The R^2 values obtained for APE and 2AP using the fabricated syringe plate were 0.997 and 0.995, respectively (Figure 11- a and c). Similar calibration curves were generated using the CTC syringe plate and the performance gave R^2 for APE and 2AP of 0.996 and 0.991 (Figure 11 – b and d). The similarities between calibrations indicate that functions of the autosampler arm are not affected by modifications to the syringe plate. Moreover, there was no observed change in peak shape or retention time between the resultant chromatogram of the fabricated plate and the original one (Figure 10).

Figure 10 - Overlaid chromatogram of 100% calibration solution of allyl phenyl ether and 2-allyl phenol, injected using fabricated side-arm plate (red) and the original plate (green).

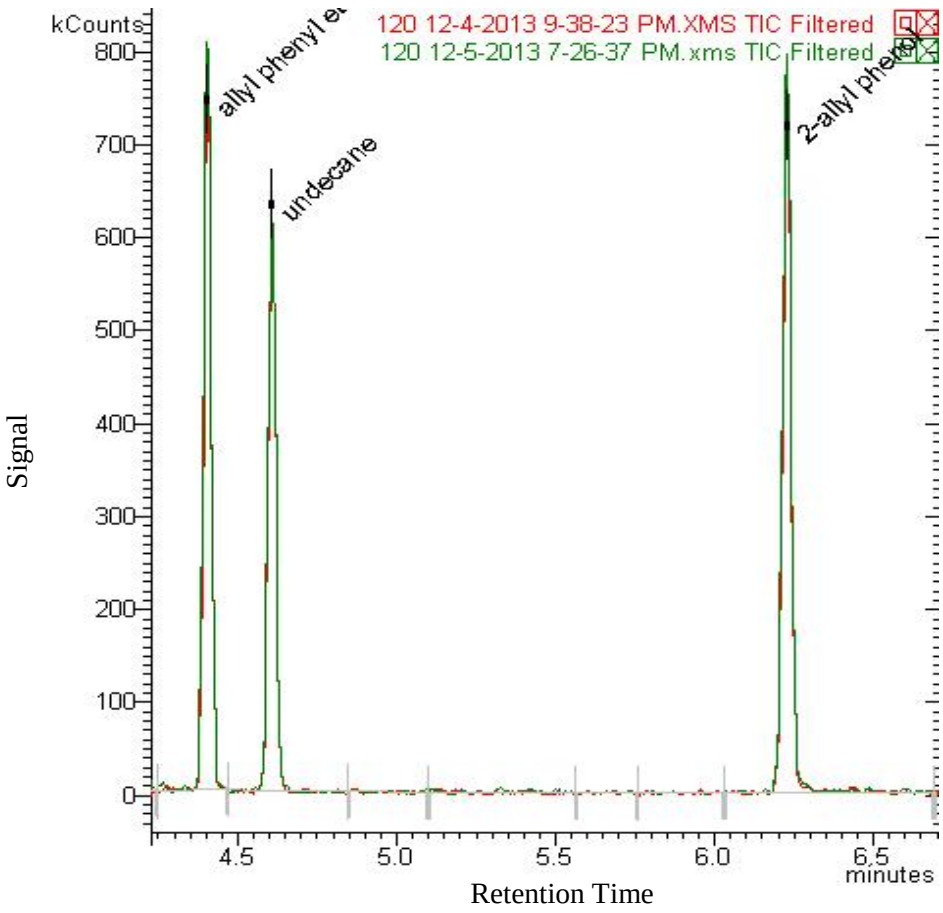
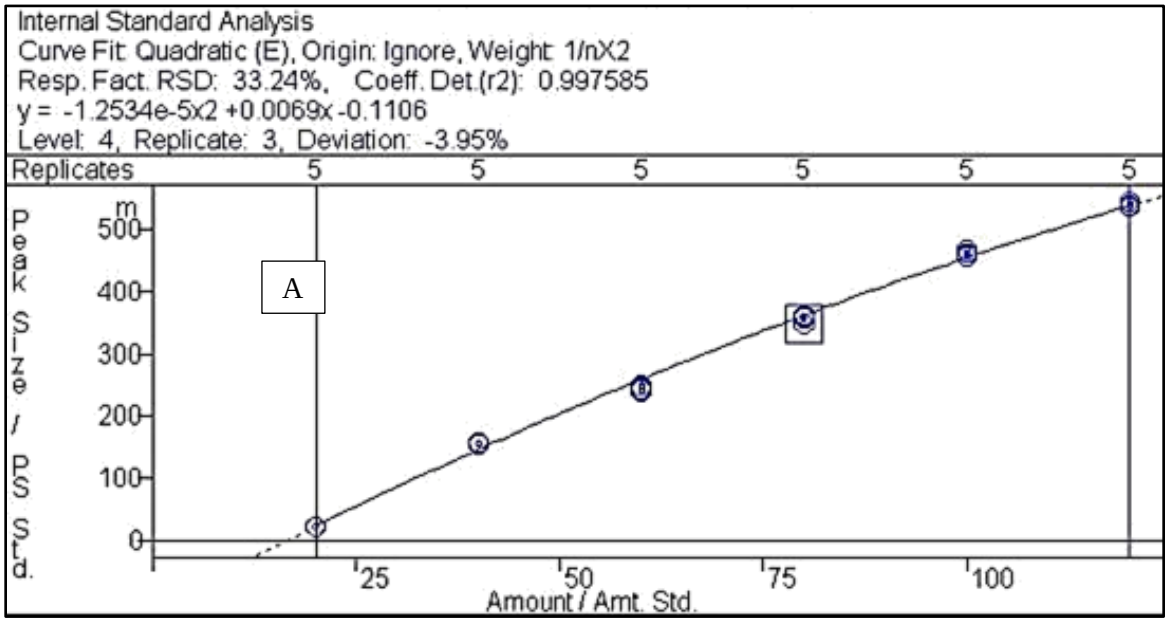
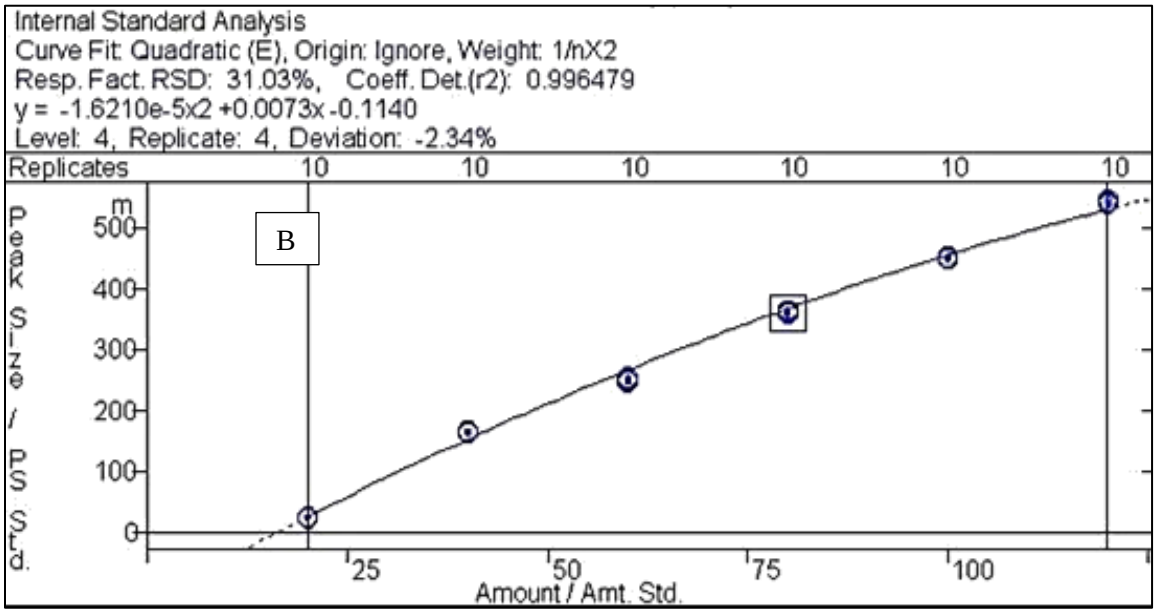


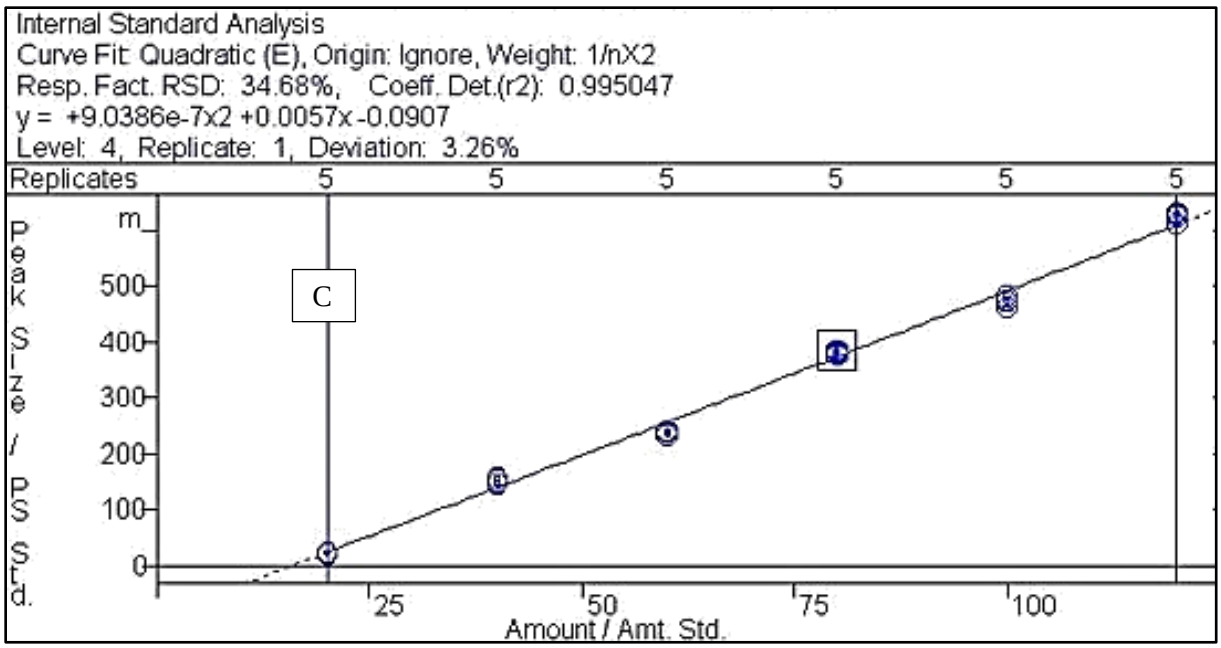
Figure 11 – Comparison of the calibration curves created with fabricated side-arm syringe plate (a and c) and the stock blue syringe plate (b and d).

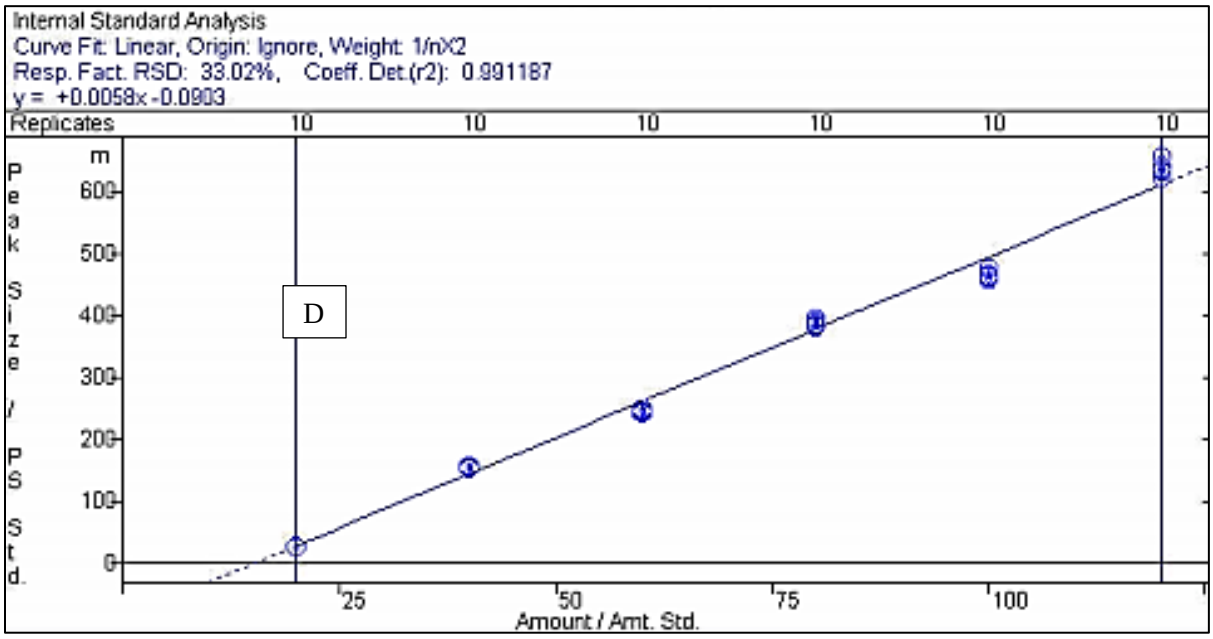
Allyl Phenyl Ether





2- Allyl Phenol





In order for the new side-arm needle attachment to be used, a sequence of autosampler commands was required (Figure 12). This sequence of commands is used to complete the sample preparation steps that are required after a sample is collected during reaction monitoring. This sequence of commands is initiated by the start signal relay mechanism that was discussed above.

Figure 12 - Sequence of commands used to program the autosamplers movements during automated reaction monitoring.

01 Wait Signal
02 Aspirate
03 Wait Signal
04 Transport Vial
05 Home
06 Start Agitator
07 Clean Syringe
08 Wait Agitator
09 Transport Vial
10 GC Inject
11 Clean Syringe

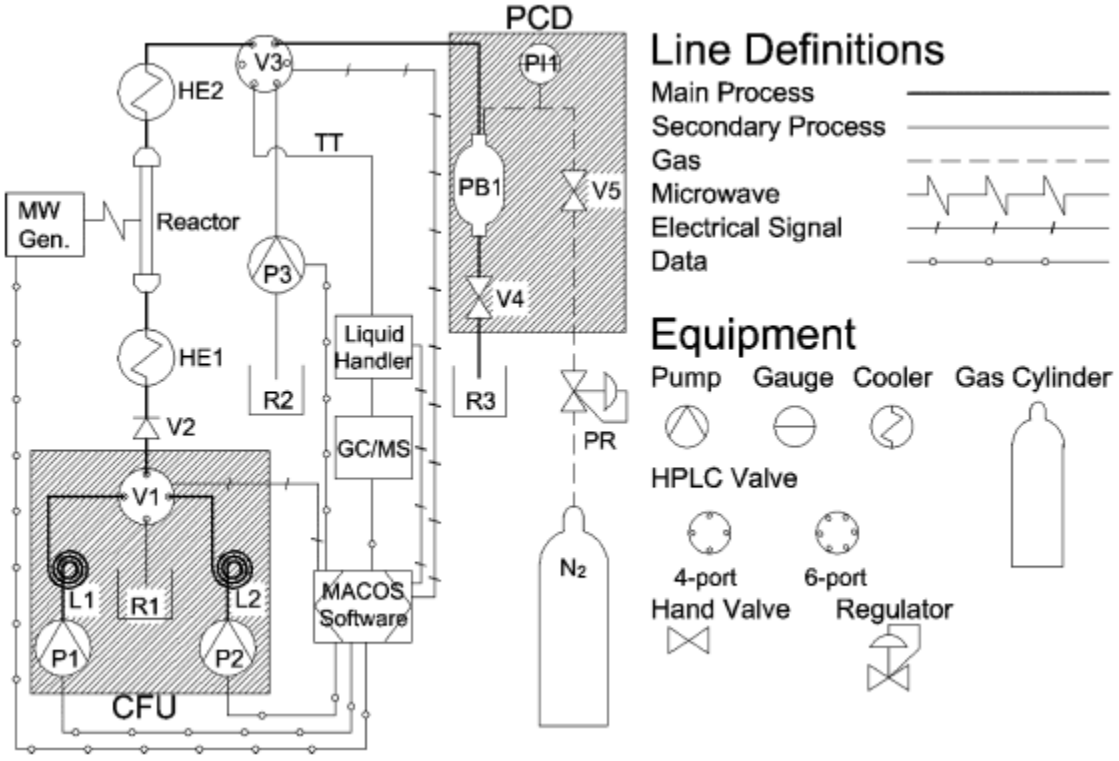
The sequence of directions in Figure 12 starts with a wait signal command (01). This command sets the autosampler into a state of waiting and the only thing that will make the autosampler proceed with the rest of the sequence is receiving the start signal from the MACOS software. When the software sends the signal to the autosampler it is an indicator that a sample is being isolated in V3. Once the autosampler receives the start signal, the aspirate command (02) that is used to line up the side-arm needle to a vial so that a sample can be delivered is initiated. The wait signal (03) is then used again so that the side-arm needle remains in the vial for as long as is necessary to collect the entire sample. The autosampler receives this wait signal from the MACOS software after P3 completes the entire sample push into the vial. After the sample is collected, the autosampler uses a magnet to pick up and deliver the metal-topped vial (04) to an agitator and then the autosampler returns to the home position (05) while the sample is homogenized via shaking/stirring (06). While the sample is being homogenized, the autosampler arm is free to undergo the typical syringe cleaning routines (07) that would be employed for every GC injection. This multitasking decreases the sample preparation time by at least one minute as that is how long the cleaning sequence takes. After the syringe is cleaned, it waits for the agitator (08) to finish with the sample before the autosampler transports the vial back to its

original position in the vial tray (09). A GC injection sequence is initiated and the sample is injected into the GC (10); after which the GC injection syringe goes through cleaning sequence while the sample is analyzed in the GC/MS (11). Once the cleaning sequence is complete, the autosampler starts the next sequence which also begins with the wait signal command (back at 01). The autosampler is always ready to collect a sample while command 01 is initiated.

The ability of the autosampler to remain in a state of waiting so that a sample could be delivered whenever necessary was the last step in combining all aspects of the flow reaction and analysis system. During the time of creating, validating and programming the autosampler, the reactor system was completed by combining the microwave, pumps, valves, and various stainless steel tubing and the entire reactor-analysis system was assembled.

1.3 The Complete Automated Microwave Assisted Continuous Organic Synthesis (MACOS) Reactor and Analysis System

Figure 13 - Complete MACOS System with In-Line GC/MS Analytics. P1 and P2: reagent delivery pump, P3: carrier solvent pump L: holding loop, R1: reactant reservoir, R2: carrier solvent reservoir, R3: product collection reservoir, V: valve, V3: sampling valve, HE: heat exchanger, PB: pressure ballast, PI: pressure indicator, PR: pressure regulator, TT: transfer tube.

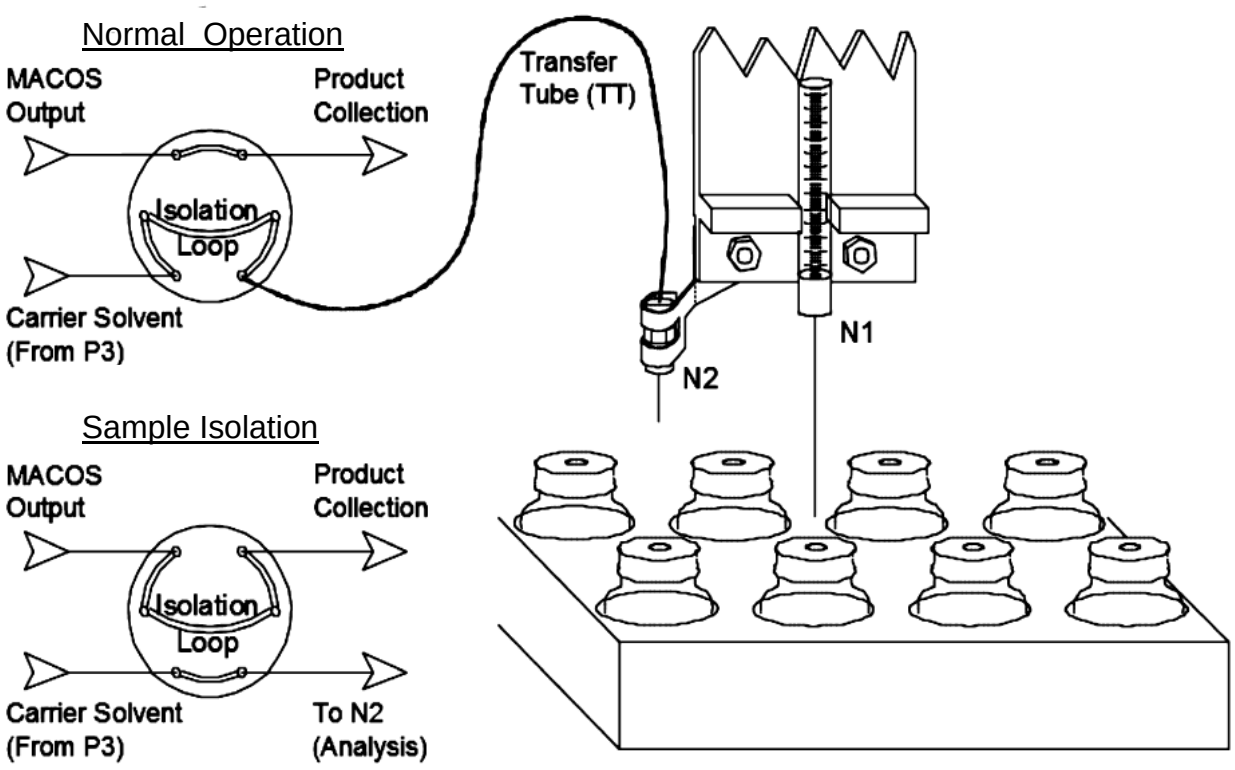


The sample delivery pumps P1, P2 have syringes that are connected to holding loops L1 and L2; both P1 and P2 are the same type of high force syringe pump. L1 and L2 loops are coils of stainless steel tubing used to house the reaction starting materials while a buffering agent, FC-40, a mixture of completely fluorinated compounds, is held within the P1/P2 syringes. This is done to prevent corrosion that may occur overtime to the P1/P2 stainless steel syringes if they were to come in direct contact with the reaction reagents. The holding loops are connected to a 4-port valve V1, which switches the connection of the syringes between the process line to deliver

reagent to the reactor, and the reactant reservoir, R1, used to house reaction reagents for syringe refilling. Each reagent that is being pumped into a flow reactor requires 2 pumps, 2 holding loops and 1 valve in order to maintain continuous operation. This pump, loop and valve combination is referred to as a continuous flow unit (CFU).

A 2-position 6-port isolation valve (V3) is located just downstream of the reactor and houses a small volume stainless steel loop (6.85 μL) that is used to isolate an aliquot of the reaction from the main process. The isolated sample from the V3 loop is transferred to analytics via a positive displacement pump, P3, through flexible plastic tubing. This tubing is connected between V3 and a side needle on the CTC autosampler, N2 in Figure 14, that was created for this purpose. After a sample is collected, the autosampler uses the onboard agitator to homogenize the sample and then a GC/MS injection sequence is completed. After injection, the MACOS software collects data for each sample from the GC/MS calibration and changes reaction conditions accordingly until the GC/MS calibration reads optimized conditions are reached.

Figure 14 - Sample transfer mechanism between the isolation loop V3 and N2[1]

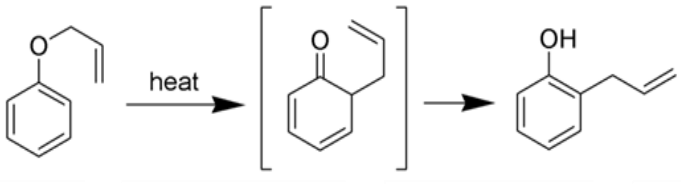


1.4 Automated Reaction

To demonstrate the abilities of the completed system to run, sample, prepare, inject, analyze and optimize a reaction through completely automated means, a model reaction was run on the system.

1.4.1 Model Reaction

Scheme 1- Claisen Rearrangement of Allyl Phenyl Ether (APE) to 2-Allyl Phenol (2AP).



APE 2AP

A model reaction was run after all calibration and validation of the system was complete (Scheme 1). Using a model reaction with the MACOS system is done so that system performance can be evaluated. This reaction has previously been studied by groups as a model reaction, therefore a benchmark performance to meet with the system is easy to find.[22] Achieving 95% yield of product is required in order to determine the synthesis a success. To demonstrate that the entire system is a success—and not just the synthetic portion—the reaction needs to be able to be optimized through automated means using GC/MS calibration data.

1.4.2 Automated Reaction Monitoring and Optimization

To start the automated experiments, the process lines between P1/P2 and V3 required priming. This is completed by flowing the starting material solution through the lines for an extended period to ensure no air bubbles are present in the system. In these reactions, a 0.1 mol/L solution of APE in 1-butanol was used.

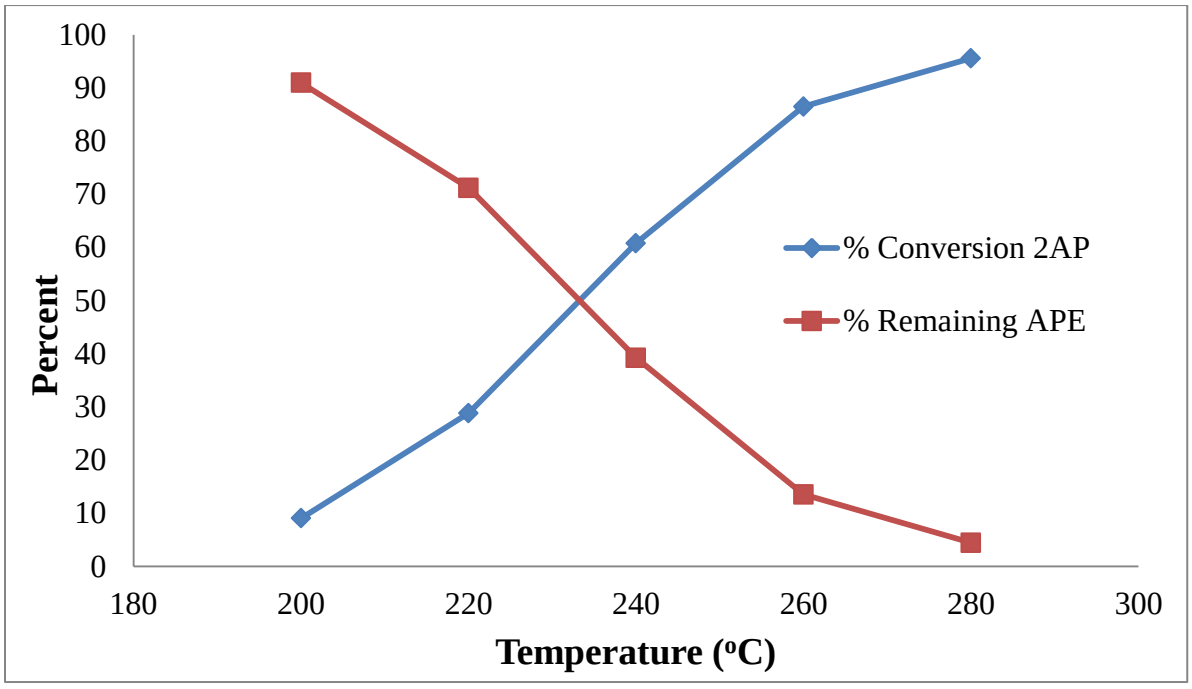
The analytical transfer tubing that is connected from V3 to N2 also required priming, therefore the entire pathway was saturated with the transfer solvent; ethyl acetate with undecane internal standard. Once all lines were appropriately primed, the automated reaction sequence was initiated. Before there is any heat applied to the starting materials, a sample is taken in V3 of the unreacted solution and analyzed to give a background level of APE in the system. This value was used to normalize the collected data – due to slight variations in internal standard or APE concentration from trial to trial, the calibration being used may be slightly off - if APE read 106% (instead of the expected 100%), then data from that reaction was normalized to equal that number instead of 100%.

To begin the optimization process, the initial reaction temperature was set at 200 °C (as little reaction takes place until this temperature is reached). The flow rate of P1 and P2 delivering

APE was set at 175 µL/min giving a residence time of 4 minutes inside the microwave reactor. To produce optimized conditions through analysis of reaction samples by GC/MS calibration, the data that is collected from the GC/MS calibration curve after each sample is injected is accessed by the MACOS software. The information available in the file created after a sample is analyzed that is important to the MACOS software is the percent conversion of desired product (here 2AP). If the calibration analyzes a sample at less than the optimized conversion (95%), the MACOS software then increases the temperature of the reactor by 20°C until an analyzed sample reads over 95% 2AP on the calibration.

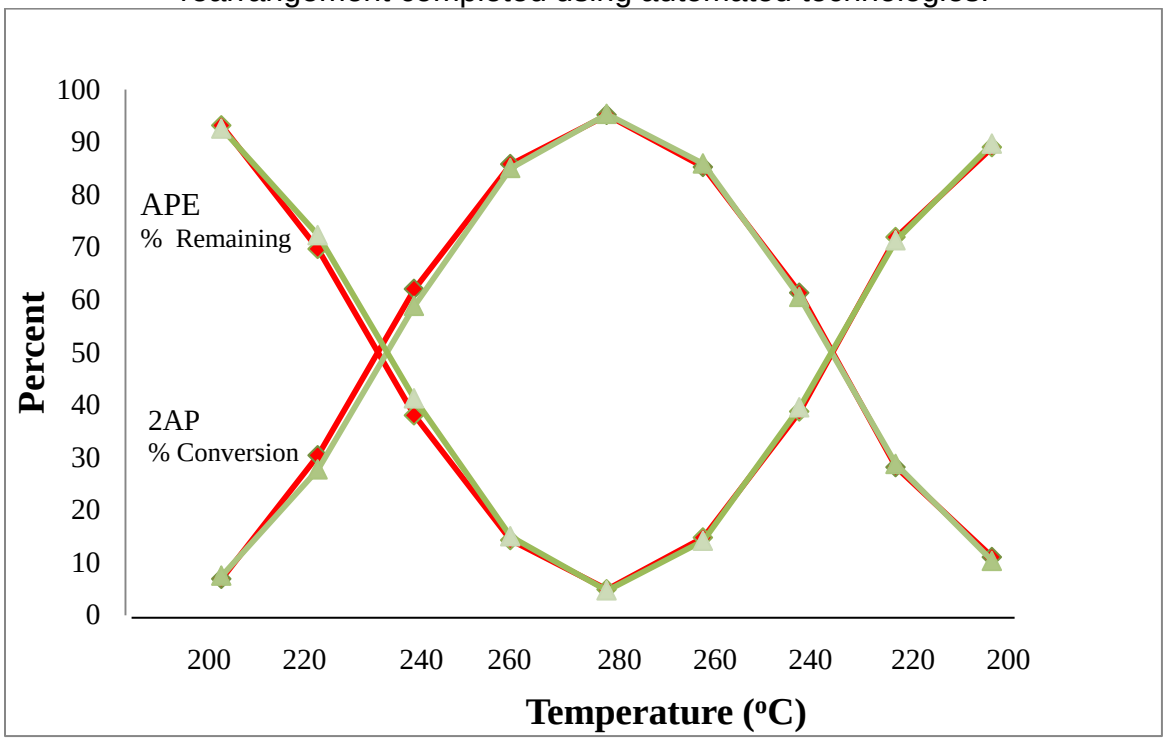
The results of an automated reaction optimization can be seen in Figure 15.[1] Here it was demonstrated that the conversion of 95% was reached through automated reaction monitoring at 280°C, which corresponds with the same temperature in which optimized conditions were reached with the benchmark system.

Figure 15 – Effect of temperature on the Claisen rearrangement optimized using completely automated reaction process control.



To ensure that there was no carry over in the system as well as showing that the entire mechanism is reproducible, a similar experiment was completed with the automated system, but instead of stopping production after the optimized conditions were reached, the experiment was continued. However, instead of continuing to increase the temperature after 95% conversion was reached, the temperature of the reaction was decreased by 20^{o}C temperature steps. During this experiment, once P1 and P2 flowed enough APE solution through the lines such that a sample could be taken that was representative of the new temperature condition (e.g. reactor and lines connecting syringes and V3 are approximately 1 mL, therefore at least 1 mL needs to be flowed at the new conditions before a sample is taken, approximately every 6 minutes at reaction flow rate), V3 was used to isolate a sample for analysis. The MACOS software was instructed to lower the reaction temperature after every sample was isolated until 200^{o}C was reached. Carry-over in the reactor would be evident by an increased amount of analytes being present in the samples during the decreasing temperature runs; I.e. there should be the same percent yield reported for the 240^{o}C sample of both the temperature increase and decrease experiments (Figure 16).[1]

Figure 16 – Effect of temperature cycling on the conversion of the Claisen rearrangement completed using automated technologies.



Green and red lines represent identical separate experiments.

The results of the increasing them subsequent decreasing of temperature experiment are shown in figure 16. In this chart there are four lines with 2 colours. Each set of lines that are of the same colour represent the results from one experiment. The lines that represent percent conversion of 2AP increase then decrease from left to right, where the percent remaining of APE decrease then increases from left to right. The other coloured lines are from another identically run experiment. Combining the two experiments onto one graph shows that the system is capable of producing reproducible results, as the two experiments are almost indistinguishable when each ones data is placed on top of each other. The samples analyzed at similar temperatures on both the increase and decrease experiment showed similar 2AP conversion readings, where the biggest difference was between the lowest temperature (200^{o}C) samples, showing a 2.85% difference in 2AP conversion. This value by itself is not very large, but when compared to the

difference between the higher temperature samples, where the difference between the increase and decrease runs were only 0.85% at 260^{o}C, it is significant. There was worry that the system may be collecting reagent materials in the reactor system over time. Therefore, a scale out protocol was completed to determine the reproducibility of the reactor system while in production mode.

1.4.3 Optimization and Scale Out

In the first scale out experiment, 150 mL of 0.1 mol/L APE was delivered over 24 hours at a flow rate of 100 μL/min. A second run delivered the same volume of material over 72 hours at 33 μL/min. In both cases the reaction was optimized, and these conditions were maintained for the remainder of the run. The average optimized conversion was 96.7% with an optimized standard deviation of 0.40 for the 24 hour run and 97.7% with optimized standard deviation of 0.17 for the 72 hour run. Since the scale out experiments provided good results, it was confirmed that the MACOS reactor and analysis system can be used to optimize and reproducibly scale out a reaction for up to 72 hours without operator intervention with the system.

1.5 Conclusions

The MACOS system has been shown to be able to run and optimize a reaction using completely automated technologies.[1] Chemical reagents are flowed through a system heated by microwave irradiation and aliquots of the reaction are isolated from the main process line without interrupting continuous flow conditions. The isolated sample is then sent to analytics for work up and analysis via GC/MS calibration. Reaction conditions (temperature) can be changed in the reactor after MACOS software reads the output file created by the GC/MS which includes the percent conversion of product in it. Reaction conditions are changed when the user-

determined optimized conversion selected before each reaction is not achieved. Once this optimized value is met, reactions can be scaled out to reproducibly produce a target compound at any given scale.

1.6 Experimental

All chemicals were purchased from Sigma-Aldrich Chemical Company and were used without further purifications. All chromatographic separations were performed using a Bruker 450 GC outfitted with a VF-5ms 30 m x 0.25 mm x 0.2 μm column. Detection was performed on a Bruker 300 triple quadrupole electron ionization mass spectrometer (EI) with selected-ion monitoring (SIM) and full scan methodologies employed. All injections into the GC were 1 μL.

1.6.1 Partial Fill Experiments

Veratrole partial loop fill experiments were conducted using one pump (P1), a 6-port isolation valve (V3) with a 100 μL loop installed, and M50 pump (P3).

1.6.1.1 GC/MS Method and Calibration

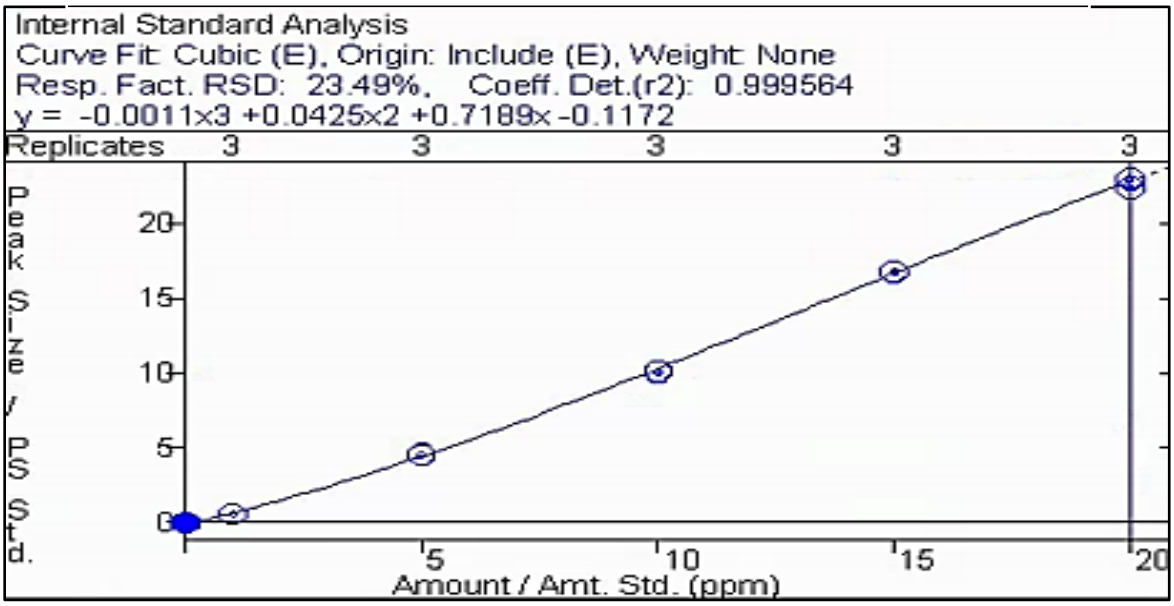
Pre-dried helium gas was used as the carrier gas at 36 cm/s of linear velocity to produce a chromatographic flow-rate 1.0 mL/min. GC injections were made using a 1:10 split protocol at an injector temperature of 250 °C. The GC oven temperature was initially set at 50 °C and was increased by 60 °C/min until 120 °C was reached. The oven temperature was kept at 120°C for additional 4 min. The transfer line and the EI source temperatures were set at 200 °C and 250 °C, respectively. The MS was programmed to monitor between 50-300 m/z, a SIM (selected ion monitoring) channel at 138 m/z (for veratrole) and an additional SIM channel at 156 m/z for undecane (internal standard) using an ionization potential of 70 eV.

The calibration curve was created (Figure 17) from the same 1000 ppm veratrole stock solution that was flowed through the system for the experiment (see page 43). The 1 ppm calibration solution was created by taking 1 μL of the stock solution, with 10 μL of a 1000 ppm stock solution of undecane (see page 43) and diluting with isooctane to make the solution to 1 mL. The 5 ppm solution was created using 5 μL of the stock solution, 10 ppm with 10 μL etc.

1.6.1.2 Sample Isolation and Analysis

A 1000 ppm stock solution of veratrole (46 μL, 0.36 mmol) in isooctane was made in a 50 mL volumetric flask. This experiment saw P1 flowing and V3 isolating small percentages of the overall loop volume of V3 with the 100 ppm solution, and then P3 was used to push and dilute these samples with 1.5 mL of pure isooctane. After internal standard spike (10 μL undecane solution from a 1000 ppm stock - 27 μL, 0.12 mmol in 20 mL isooctane), reaction samples were analyzed via GC/MS calibration curve (Figure 17).

Figure 17 - Veratrole calibration curve used to analyze samples for V3 partial fill sample isolation experiments.



1.6.2 Overfill Isolation Experiments

Allyl phenyl ether overfill experiments were conducted using P1, V3 with 5 μL loop installed, and P3.

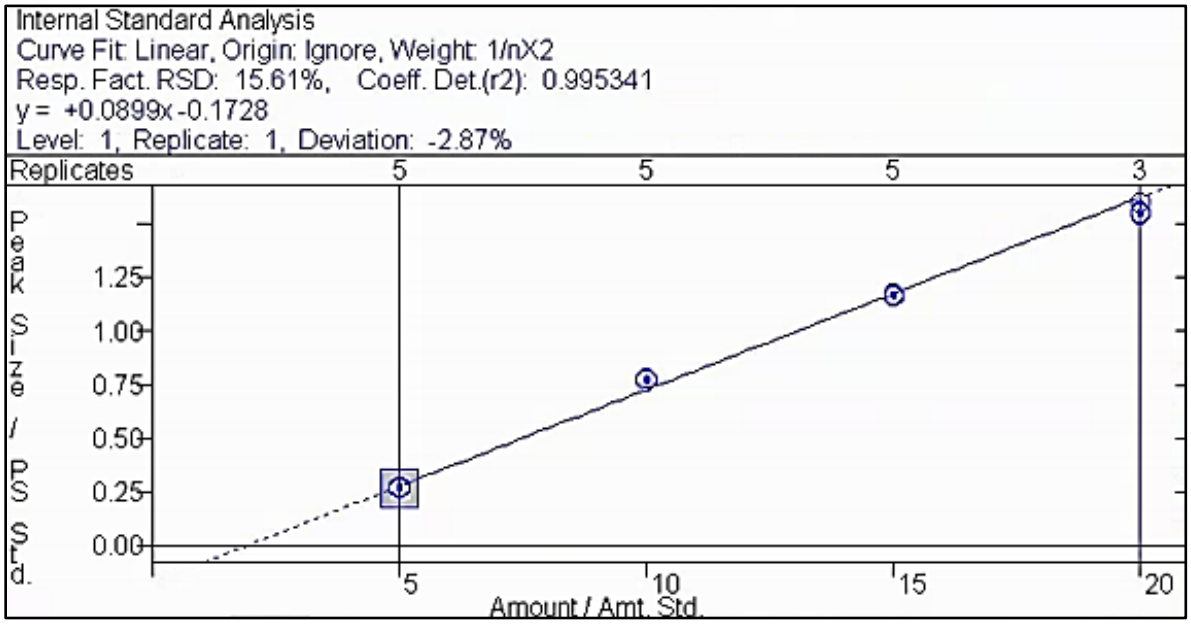
1.6.2.1 GC/MS Method and Calibration

Pre-dried helium gas was used as the carrier gas at 41 cm/s of linear velocity to produce a chromatographic flow-rate 1.3 mL/min. GC injections were made using a 1:20 split protocol at the injector temperature of 260°C. The GC oven temperature was initially set at 50 °C and was increased at injection by 30 °C/min until 100 °C was reached. The oven temperature was kept at 100 °C for additional 1 min. Then, a 5 °C/min ramp was applied until the oven was heated to 120 °C. The transfer line and the EI source temperatures were set at 220 °C and 260 °C, respectively. The MS was programmed to monitor at three separate scan channels (a full scan between 50-300 m/z, a SIM (single-ion monitoring) scan at 134 m/z for APE (and 2AP when method used for

reaction monitoring) and an additional SIM channel at 57 m/z for undecane (internal standard) using a ionization potential of 70 eV.

The calibration solutions were created using the same 0.1 mol/L solution that was flowed through the system (see page 45). 5, 10, 15 and 20 μL aliquots of this solution were taken via Eppendorf pipette and diluted with 8 mL ethyl acetate with internal standard from a stock solution (200 mL ethyl acetate with 2 μL internal standard spike from 1000 ppm stock - 27 μL, 0.13 mmol undecane in 20 mL ethyl acetate).

Figure 18 – Calibration curve used for analysis in APE sample overfill isolation experiments



1.6.2.2 Sample Isolation and Analysis

To a 100 mL volumetric flask, 1372 μL of APE (10 mmol) was diluted to the mark with 1-butanol and the solution was stirred until homogenous (0.1mol/L). P1 was used to flow the 0.1 M solution of APE through the lines to prime the system, while P3 flowed ethyl acetate through the collection lines and V3's 5 μL loop to ensure no air bubbles were present in the system. Once primed, P1 continued to flow the 0.1 mol/L solution through the system. When ready to sample,

V3 switched to the load position and collected a sample in the loop. A volume much larger than 5 μL was flowed through V3 in order to ensure that the entire loop is saturated with the 0.1 mol/L solution. V3 then switches back to its original position and P3 pumps 8 mL ethyl acetate with undecane solution (200 mL ethyl acetate with 2 μL internal standard spike from 1000 ppm stock - 27 μL, 0.13 mmol undecane in 20 mL ethyl acetate) through the loop to isolate the sample. The solution is then injected into the GC/MS for quantitation against a calibration curve (Figure 18).

1.6.3 Claisen Rearrangement Automated Reaction

These experiments were completed using the entire MACOS set up as seen in Figure 13**.**

1.6.3.1 GC/MS Method and Calibration

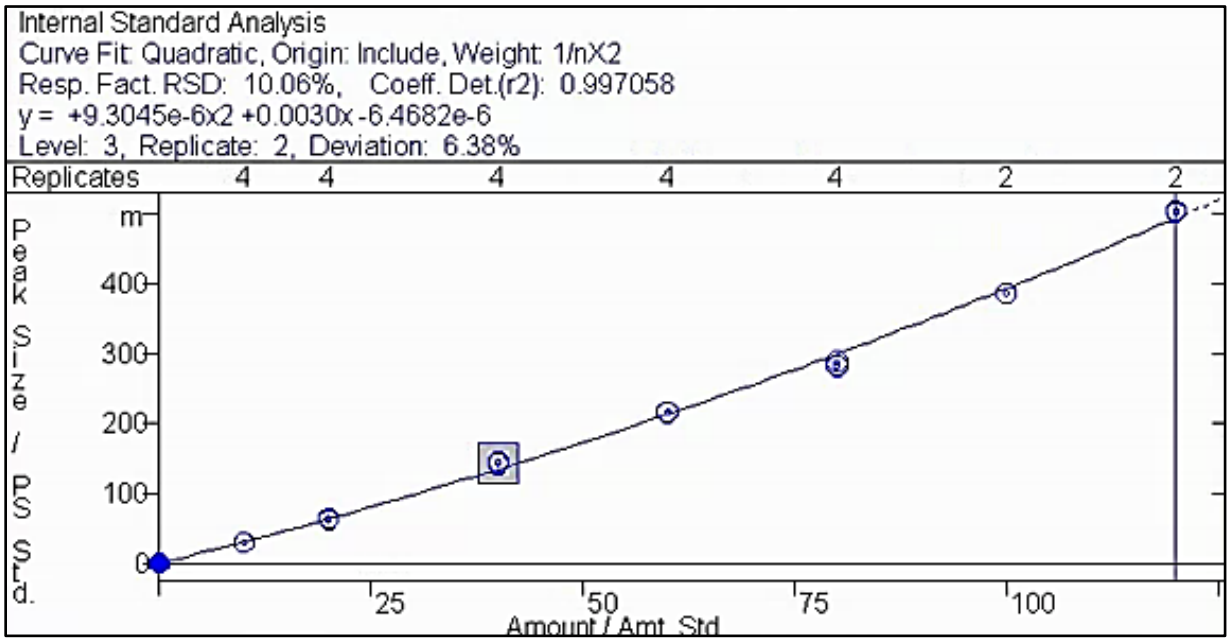
The GC/MS method used for all automated experiments is the same that was used for the APE overfill sample isolation trials.

The calibration stock solutions were created using an initial solution of APE and 2AP at 0.12 mol/L (120%) (823 and 783 μL [0.6 mmol each] of APE and 2AP respectively in a 50 mL volumetric flask made up with 1-butanol). The 100 % solution was made by taking 833 μL of the stock solution and diluting to 1 mL with butanol. The 80 % solution was created taking 666 μL of stock and diluting to 1 mL etc. Seven solutions were created - 10, 20, 40, 60, 80, 100 and 120% of the reaction concentration. To create the final solutions that were injected into the GC/MS to obtain a calibration curve (Figure 19), a 6.85 μL of calibration stock was taken via Eppendorf pipette from each solution and was diluted in 8 mL of GC grade ethyl acetate with undecane as internal standard (see 1.6.2.2).

1.6.3.2 Automated Claisen Experiments

A 0.1 mol/L solution was made in the same way as the APE isolation experiments (see page 44). The MACOS process lines were thoroughly primed first using 1-butanol and then with the APE solution. After priming was complete, the APE solution flowed through the reactor at 175 µL/min while the temperature of the reactor was increased until optimized conditions were met. Samples were collected with the autosampler side arm attachment and homogenized before GC/MS injection and analysis. A calibration curve was created for both the starting material APE (Figure 19) as well as product 2AP to monitor the reaction.

Figure 19 – Calibration curve of APE for reaction monitoring purposes.



Chapter Two

In-Line Derivatization of Protic Compounds for GC/MS Reaction Monitoring

2 Chapter Two

2.1 Introduction

Chemical synthesis is used across a variety of industries to create products for everyday and industrial use. Without some type of analytical method to determine what products have been formed in a reaction, chemical synthesis is useless as there would be no way to determine what had been created. Analytical instrumentation and improving analytical performance have increasingly become the focus of research. Widely used in an everyday chemistry laboratory is the use of analytical instruments for some type of synthetic reaction monitoring. As the technology used to run these synthetic reactions is increasingly being completed via automated technologies, many analytical instruments have also been designed to be able to perform automated sample preparation steps and analysis sequences.[21] These analytical instruments can also be combined in-line with a synthetic reactor system in order to decrease sample analysis time. Here the reactor is capable of quick, automated small molecule synthesis and the analytical instrumentation is used to perform quantitative sample preparation, separation, and analysis.[1]

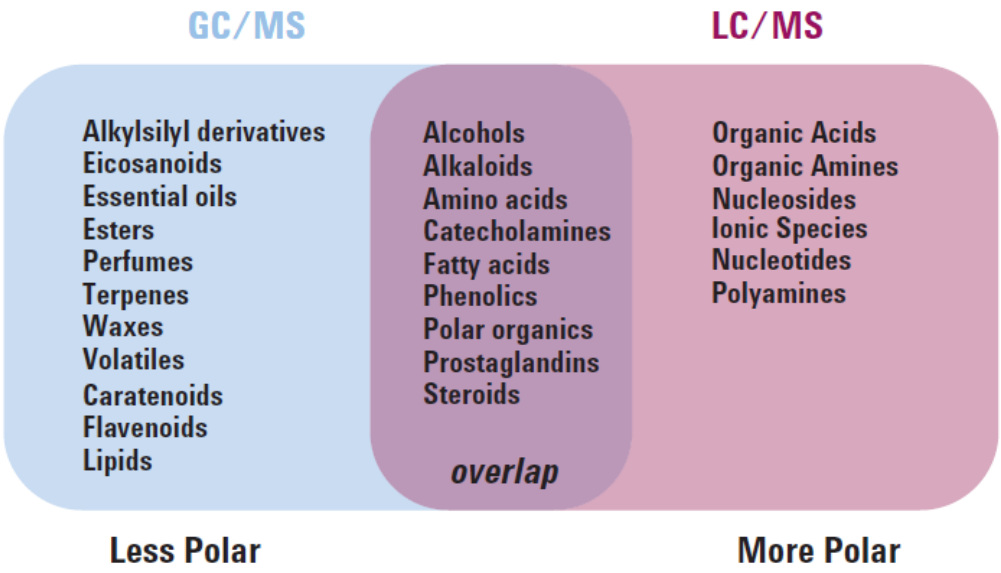
The choice of analytical instrument for separation and detection in these integrated reactor-analysis systems is crucial for this combination to ultimately be successful. The selection of the analytical method should be based on what type of compounds are being analyzed, how much is being analyzed, as well as the cost and efficiency of the technique. As a first choice, one might be tempted to choose high pressure liquid chromatography (HPLC) to use for mixture separation after a reactor. HPLC, with different variables such as solvent polarity, solvent gradients, and mobile phase additives, can be an effective technique to help with separation of complex mixtures to help with analysis of them. HPLC (like all chromatography methods) is a separation technique only; an attached detector is required in order for compounds eluting from

the column to be analyzed. Combined most often with HPLC is an ultra violet (UV) spectrometer which measures the absorbance of UV light of compounds passing through the detector. A peak is obtained whenever a compound with a UV active functional group elutes from the column. If one uses the HPLC/UV combination for separation and analysis and the compound does not have a UV active functional group, no detection of that compound will occur. UV detection is also unable to give any structural information on the eluting compounds. This makes using HPLC with only a UV detector unsuitable for most reaction discovery purposes or for structural confirmation of compounds. Using HPLC with a mass spectrometer (MS) as a combined separation and detection technique will give structural information of compounds eluting, however HPLC/MS can often suffer from ion suppression, where one of two co-eluting compounds supresses the ionization of the other,[23] and volatile organic compounds (VOC's) are often not amenable to HPLC/MS because they are often lost during ionization in the mass spectrometer by electrospray ionization (ESI); ESI is designed to eliminate solvent from the sample and will therefore eliminate many volatiles as well. Both the ion suppression and ionization issues make quantification difficult with HPLC/MS and combined with the high initial costs, long term maintenance, required user capabilities and complexity of separation of HPLC/MS, it can be an unsuitable technique for quick reaction monitoring.

An alternative but related approach, gas chromatography mass spectrometry (GC/MS) is one of the most common techniques used to detect small molecules such as illegal drugs and pharmaceuticals in biological[24] or environmental samples.[25] The outstanding resolving power of GC separation leads to exceptional separations compared to other chromatography techniques and since GC separations are done in the gas phase, they are inherently easier to do than liquid separations.

GC/MS can be used to determine molecular fragments of compounds analyzed and can quantify the amount of volatile and semi-volatile organic molecules in a mixture. A single gaseous mobile phase is used in GC separation, and temperature programming of the oven that houses the GC column is done to help elute compounds injected into the instrument. GC/MS is a simpler separation technique and often requires less sample compared to HPLC/MS. GC/MS instruments are also noted to be much less expensive to purchase and maintain and GC is often chosen as the technique to use for everyday analysis by non-expert chemists because of its ease of use. However, even with all these positive attributes, like LC/MS, certain compounds cannot be analyzed directly via GC/MS methods. Although there is some overlap in the types of compounds that can be analyzed by both LC and GC/MS (Figure 20) there are some stark differences between why compounds are suitable to each technique.

Figure 20 – Suitable compounds for analysis by GC/MS and LC/MS.[23]



GC requires gas phase compounds for separation whereas LC uses liquid phase only. Analytes introduced onto the GC column must be vaporized in order to be separated, but also because vaporization of injected compounds is important for maintaining a clean and properly

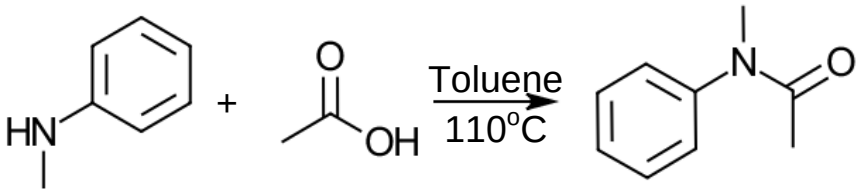
functioning analytical instrument. If a compound is introduced into the GC injector and is not vaporized, it may remain in the injector causing contamination issues. If the high-boiling organics are able to reach the column, they can remain bound to it, which leads to column damage, loss of analytical performance, and instrument down time for maintenance. To try and help with volatility issues, one can increase the temperature of the GC injector to try and help with vaporization, but if other compounds in the sample are temperature sensitive there could be more degradation than there is help for volatilization. These high boiling or vapour pressure compounds will most likely not elute from a GC column at any reasonable temperature, and even if compounds are able to elute, the peaks of non-volatile compounds often exhibit unacceptable levels of peak tailing.

Alternatively, if compounds being analyzed via GC/MS are too volatile, the analytes can be lost in the MS collection delay that is required to prevent detector overload when the large quantity of solvent present in all samples elutes from the system. Compounds that include protic functionalities, such as hydroxyl, amine, or carboxylic acid moieties also cause issues in GC analysis as these active groups can cause irreversible damage to typical GC columns by reacting with the stationary phase. When the stationary phase of a GC column connected to a MS becomes damaged, the chromatogram will often contain random peaks, unacceptable baseline increases and it can also decrease signal-to-noise ratios for all compounds analyzed. With these limitations, it would seem as though monitoring the profile of many commonly-encountered reactions would be very difficult to do using GC/MS instrumentation. However, unlike the problems with LC/MS analyses that sometimes are not easily solved, the biggest issues compounds have in relation to GC/MS analysis can be solved by a simple sample preparation step known as derivatization.

2.1.1 Derivatization

Derivatization is a sample preparation technique where compounds that are not amenable to a certain technique react with a derivatization reagent such that these once unanalyzable compounds become available for analysis after reaction. The technique can be used for a variety of different reasons and for different analytical methods (for chiral NMR as an example)[26], but most compounds that are not readily analyzed via GC/MS are as such because they contain an undesired functional group that is easily modified. During derivatization, compounds undergo a chemical transformation with a derivatization reagent to remove the unwanted functionality (normally an acidic proton) and the resulting derived compound possesses a functional group more amenable to GC/MS analysis. Derivatization has many benefits and advantages over other sample preparation techniques and can be used as a sample preparation step to help in reaction monitoring.

Reaction monitoring is often completed qualitatively, monitoring the production of compounds without knowing their exact conversion percentages. If one is not interested in the exact quantities of a compound in a mixture, any technique that is capable of detecting (but not necessarily quantifying) a compound is acceptable to use. However, if one wants to quantitatively monitor a reaction (or other mixture) to determine the exact quantities of each compound involved, it is necessary to have an analytical technique that is capable of reliably, reproducibly and accurately measuring the amount of each compound in a sample. If a reaction contains a compound at any stage of the reaction that is not amenable to GC/MS analysis, monitoring and quantification of the entire reaction can be difficult using that method.



Scheme 2 - Example of a reaction with both derivable (starting materials) and non-derivable (product) compounds.

Alternatively, if a compound is already amenable to GC/MS analysis and does not possess any derivable functionality, there should be no effect on that compound if it is present during a derivatization procedure. The derivable compounds will be chemically changed and then quantified; the underived compounds remain unreacted and can also be quantitatively analyzed. This is important as many synthetic reactions will contain both derivable and nonderivable compounds (Scheme 2). This makes derivatization an ideal tool for reaction monitoring.

Optimized derivatization procedures produce the derived compound in 95% or greater conversion and do not induce structural rearrangements in the compound. Besides improving volatility and removing troublesome active functional groups, derivatization can also help resolve overlapping peaks in chromatograms,[27] and can assist in distinguishing between isomers of organic compounds.

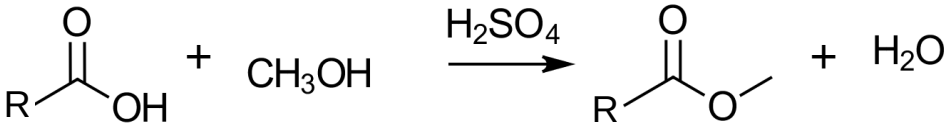
In order to assure breadth of scope and compatibility with as many compounds as possible, three main types of derivatization reaction exists: alkylation/esterification, acylation and silylation.

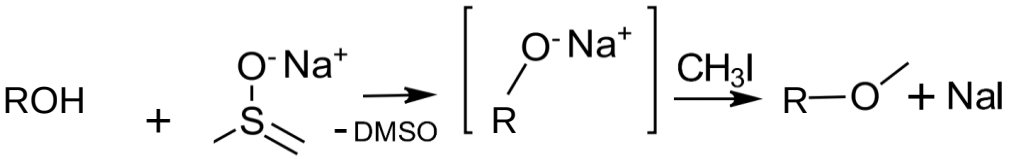
2.1.1.1 Alkylation/ Esterification

Alkylation is a derivatization procedure that replaces active hydrogens with an alkyl group. Ethers, thioethers and thioesters, N-alkylamines/amides can be produced by the alkylation of alcohols,

thiols, amines and amides, respectively.[28] Seen in Scheme 3 are two schematics of alkylation reactions that have been employed to help with separation and volatility issues for certain compounds.

Scheme 3 – Alkylation derivatization reaction examples run under acidic (top) and basic (bottom) conditions.





Under acidic catalysis, the esterification of carboxylic acids is completed using methanol. This reaction is reversible and creates water *in situ*; therefore in order to get quantitative derivatization water needs to be removed, chemically or physically, from the reaction. Under basic conditions, methylsulfinyl carbanion followed by methyl iodide esterifies alcohols into methyl esters.[29] The primary chromatographic use of alkylation is the conversion of organic acids into esters, and most commonly methyl esters. These esters are often much more likely to produce chromatograms of proper resolution compared to the free acids. Several electrophiles are readily available to produce such alkyl derivatives, but there are some downsides to the alkylation procedure. When derivatizing acids, as the acidity of the active hydrogen decreases, a stronger alkylating reagent must be used. As the reagents and conditions become harsher, the selectivity and applicability of the method become more limited. The deriving reagents themselves can also be highly toxic, such as strong electrophiles like methyl iodide.[30]

2.1.1.1.1 Dialkylacetal Alkylating Reagents

Figure 21 shows the alkylating reagent N,N-dimethylformamide di-*tert*-butyl acetal (DtBA). This particular reagent been used for decades as a derivatization reagent for many protic compounds. In one example, this reagent was used as an on-column derivatizing reagent for both cocaine and benzoyl ecgonine (the main cocaine metabolite, see Figure 22).[34] At the time of this publication (1977) there was little precedent to quantitatively measure the amount of cocaine (Figure 22, left) in a biological sample due to its quick metabolism to bezoylecgonine (Figure 22, right), and it was also difficult to analyze the metabolite because it is highly soluble in water and problematic to extract. Using alkylation with DtBA (and various other alkylation reagents), cocaine and the metabolite were capable of being positively identified in the same sample, even when other illicit drugs and metabolites were present in the solution that underwent derivatization.[31]

Figure 21 N,N-dimethylformamide di-*tert*-butyl acetal (DtBA) alkylating reagent.

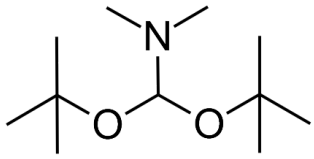
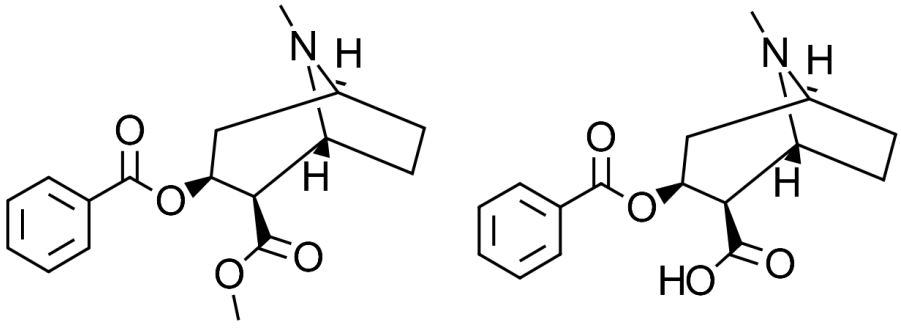


Figure 22 –Structure of cocaine (left) and its main metabolite benzoylecgonine (right).



In other work, after optimization of the derivatization procedure, DtBA was chosen as the most suitable dialkyl acetal for the analysis of a meat extract's heterocyclic amines (HAs), a class of compounds known to be carcinogenic in rodents and present in many cooked foods. Here, the derivatization step was required to increase the volatility of the HAs for GC/MS analysis (Scheme 4).[32] Noted in this work was the required use of dry reagents, inert gas and molecular sieves to ensure no moisture is present, which will hydrolyze the deriving reagent.

Scheme 4 - Reaction between heterocyclic amines (HA) and a N,N-dimethylformamide derivatizing reagents.[32]

R' = CH_3 1,1-dimethoxy-N,N-dimethylmethanamine
CH_2CH_3 1,1-diethoxy-N,N-dimethylmethanamine
$CH(CH_3)_2$ 1,1-diisopropyoxy-N,N-dimethylmethanamine
$C(CH_3)_3$ 1,1-di-tert-butoxy-N,N-dimethylmethanamine

2.1.1.1.2 Boron Trifluoride in Methanol Alkylating Reagent

Much like the esterification reaction seen in (acidic conditions), boron trifluoride (BF_3) catalyses an esterification reaction of a carboxylic acid to form a methyl ester using methanol. BF_3 acts as a Lewis acid in the reaction to catalyze it. The use of BF_3 in methanol as a derivatization reagent has the added bonus that the reaction is usually clean, with no side products formed and the by-products of the reaction are volatile and do not need to be removed from the sample before analysis.

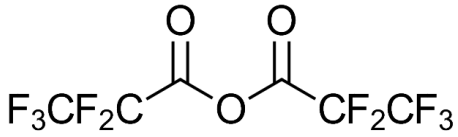
2.1.1.2 Acylation

Derivatization by acylation results in the conversion of compounds possessing hydroxyl, amino, and thiolate functionality into esters, amides and thioesters, respectively. Acylation can

produce highly volatile derivatives and can improve the thermal stability of compounds with highly reactive functionality. Furthermore, the signal of low concentration analytes can be increased by the use of highly fluorinated acylating reagents in conjunction with use of an electron capture detector.[30] Acylation reactions are often catalyzed and form acidic side products that must be removed before the sample can be injected into the GC for analysis.

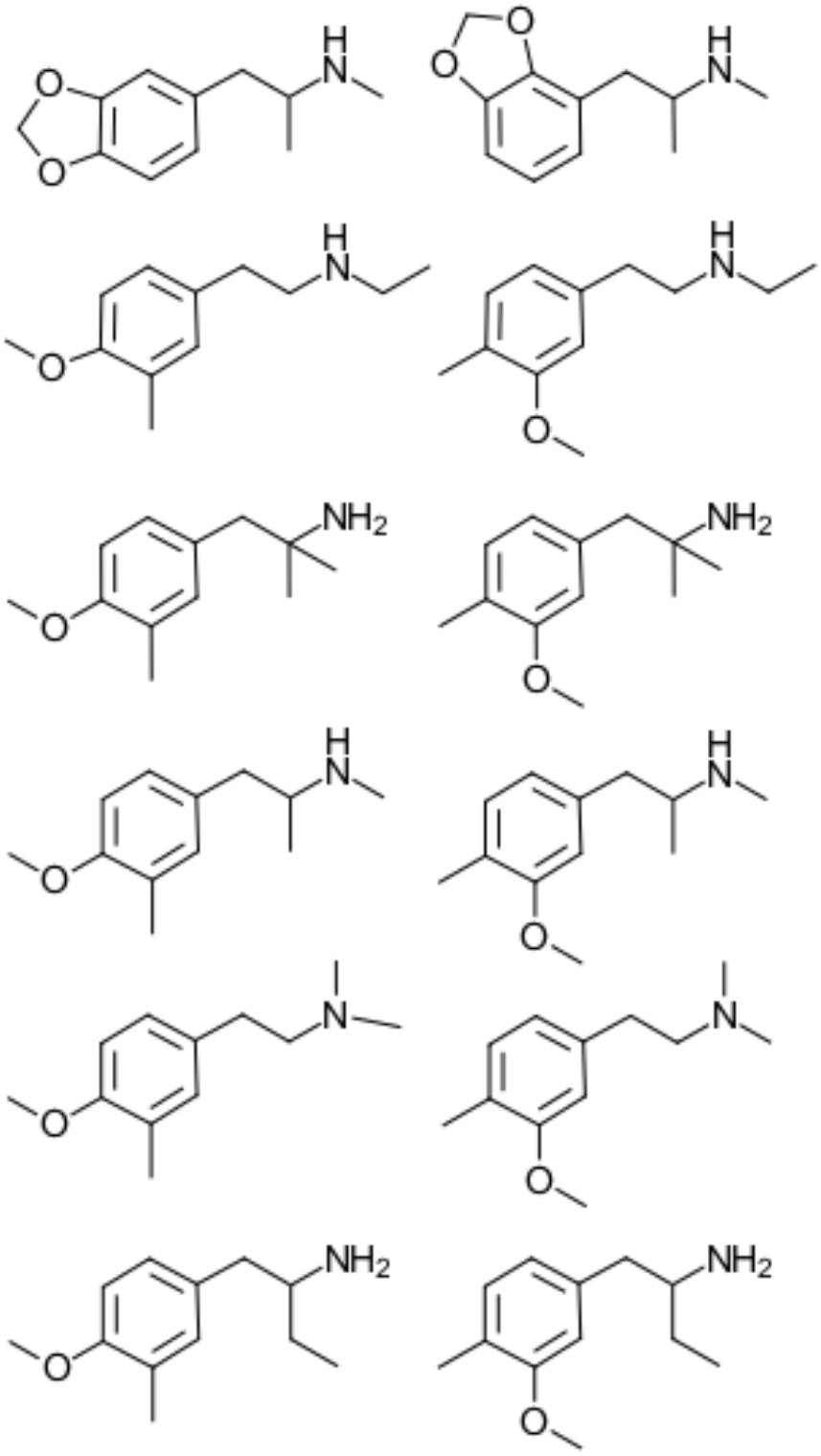
Acylation derivatization has also been used to resolve legal isomers of illegal substances (Figure 24).[33] Here, pentafluoropropionic anhydride (Figure 24) derivatization is used to help distinguish between regioisomers of 3,4-methylenedioxymethamphetamine (MDMA), a controlled substance that is used as a recreational drug. The derivatization of MDMA regioisomers was done in order to help forensic analysis identify the real controlled substance from the compounds of no legal interest.

Figure 23 - Pentafluoropropionic anhydride (PFPA) acylating derivatization reagent.



Here the derivatization is especially important because all of the regioisomers and MDMA give very similar mass spectra and retention times without derivatization and it can be difficult to determine if the banned substance is actually present in the sample or if it is a false positive from related compounds. After derivatization, retention times shift and mass spectra are individualized such that each regioisomers can be identified and the illegal substance can also be positively determined even with other regioisomers in the sample.

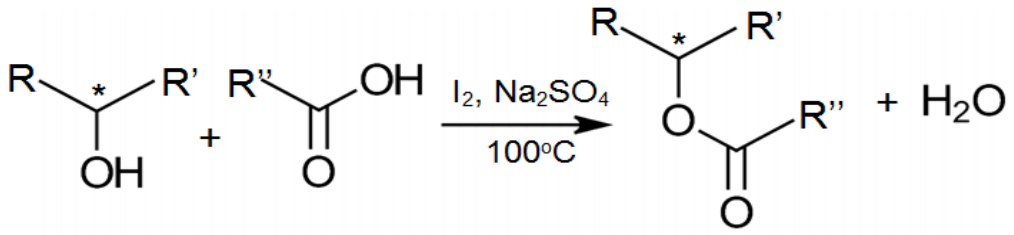
Figure 24- Regioisomers of MDMA (MDMA top left). Compounds drawn that have derivable functionality require derivatization with PFPA to aid in regioisomers/isobaric identification.



2.1.1.2.1 Carboxylic Acid Acylating Reagents

A procedure to help with resolution of chiral alcohols has been developed and is a good example of how acylation could be used as a diagnostic technique for monitoring reactions.[27] Scheme 4 shows the general reaction scheme for an iodine catalyzed reaction where either acetic acid or trifluoroacetic acid is used to derivatize chiral alcohols.

Scheme 5 - Acylation of chiral alcohols with acetic or trifluoroacetic acid.



R, R' = alkyl, aryl R''= CF_3, CH_3

The reaction in scheme 5 is completed and the resolution of the resultant enantiomeric esters and the free chiral alcohols was measured and compared using chiral GC/MS columns. It was found that the esters usually had a much better separation factor (α) than the alcohols, which included some esters being baseline resolved while the corresponding alcohols were partially or completely overlapped. This procedure could be adopted to monitor the ratio of chiral compounds being created in particular reactions used in the creation of active pharmaceutical ingredients (APIs) that often contain chiral centres and very different chemical or physiological properties based on those configurations.

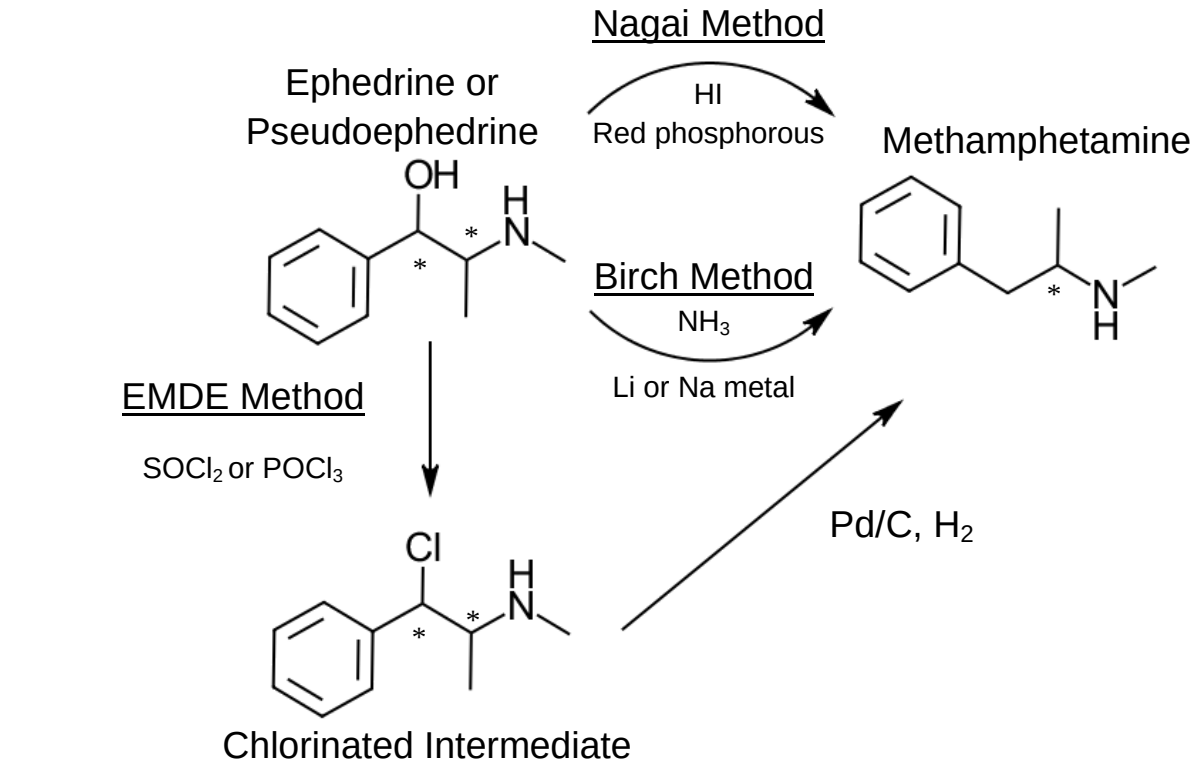
2.1.1.2.2 Fluorinated Anhydride Acylating Reagents

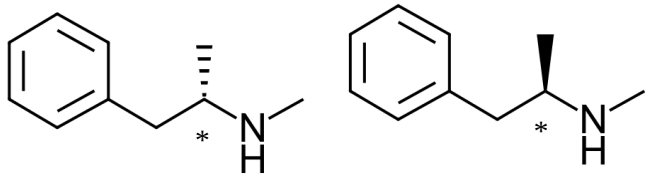
In many countries, the possession or ingestion of the (+)(S) enantiomer of methamphetamine has a higher criminal sentence than that of the (-)(R) enantiomer due to the

stark difference in the drugs effect on the human central nervous system. Thus, determining which enantiomer is in blood and urine sample is especially important, and derivatization can determine whether the (-)(R) enantiomer is present, which is used in non-prescription decongestants, or if the (+)(S) enantiomer, the a central nervous system stimulant, is present (Figure 25).[34]

Fluorinated anhydrides are a class of compounds used to introduce acyl groups onto alcohols, amines, and phenols. In one example, stock solutions of racemic ephedrine, pseudoephedrine, chlorinated intermediates and methamphetamine in methanol were derivatized to show that fluorinated anhydride derivatization can help separate unresolved enantiomers. Derivatization can also be used to monitor the source of the drug and not just which isomer is present. Pentafluoropropionic anhydride (PFPA, Figure 23) has been used to determine the manufacturing process in which the illegal drug was created. Even though there are three known (main) manufacturing processes – the Nagai, Birch and Emde methods[35] (Figure 25) – only one, Emde, proceeds through chiral chlorinated intermediates (often not fully purified away from the final product). Derivatizing and analyzing samples and finding the ratio of these chlorinated intermediates can lead law enforcement officials to match batches of seized chemicals through their analytical profiles. [36]

Figure 25 – (top) Schematic of 3 different methods for producing methamphetamine. (bottom) Legal and illegal isomers of methamphetamine.





(+)(S) illegal isomer (-)(R) legal isomer

2.1.1.3 Silylation

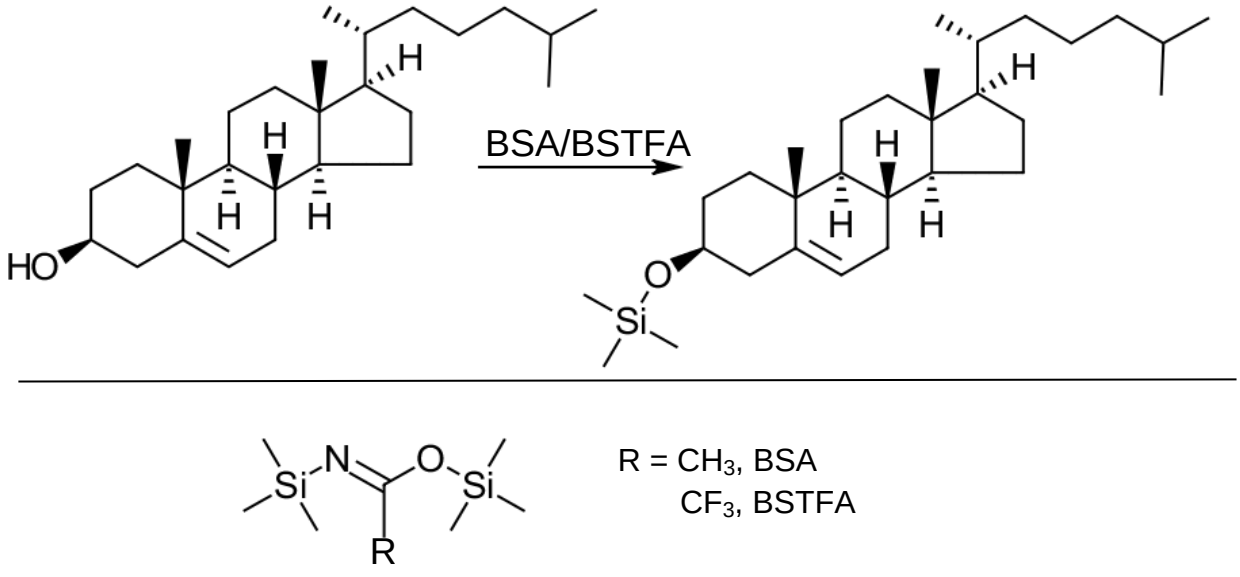
The most widely used GC derivatization technique is silylation, where a silyl group is introduced onto a compound, resulting in the replacement of an active hydrogen in the process. To increase volatility and enhance the thermal stability of compounds at the high temperatures required for GC analysis, –OH, -COOH, -NH, -NH_2 and -SH functional groups can all be derivatized using silylation. There are many different silylating reagents commercially available,

and the reactions between silylating reagents and active hydrogens often proceed at moderate temperatures and with short reaction times. Advantageously, almost all by-products of each silylating reagent can be directly injected into the GC after reaction.

2.1.1.3.1 Trimethylsilyl (TMS) Silylating Reagents

Trimethylsilyl-based derivatizing reagents introduce the trimethylsilyl (TMS) group onto the compound of interest and, while these reagents can be moisture sensitive, the TMS-ethers formed are quite stable. Bis(trimethylsilyl)acetamide (BSA) (Scheme 6), one of the first GC derivatization reagents to be developed, requires mild reaction conditions, but the by-product trimethylsilylacetamide (TMSA) often overlaps with the peak of the desired derived compound. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Scheme 6), is similar to BSA but its reaction by-products are more volatile, thereby eliminating any peak overlap as they elute much earlier than the derivatives of interest.

Scheme 6- Derivatization of cholesterol, a steroid, with BSA and BSTFA.



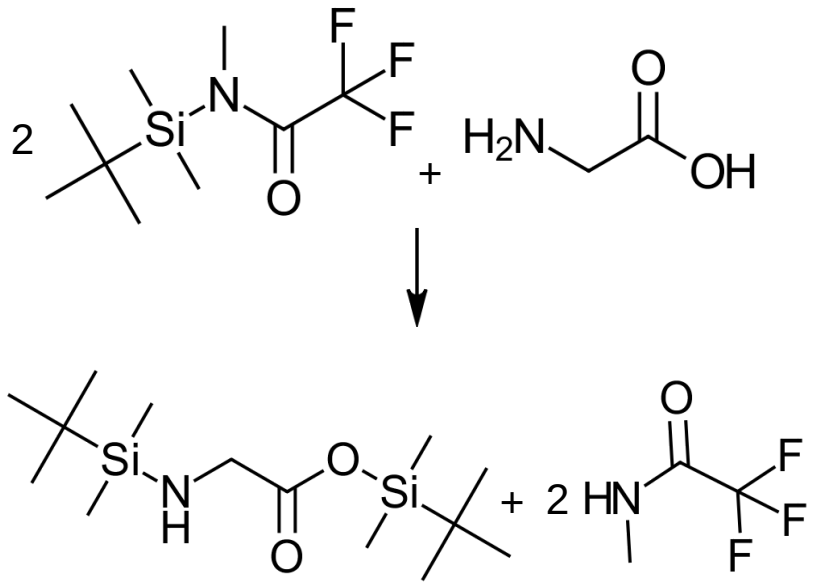
In an example, both BSA and BSTFA were used to derivatize steroids for enhanced analysis using GC/MS.[37] Here microwave irradiation was being investigated for its apparent effect on the derivatization process and both reagents underwent optimization reactions to determine the best method for preparation of the derivatives. Both compounds derivatized analytes to completion; however the BSTFA reaction was a much higher temperature due to microwave heating the polar BSTFA molecules more thoroughly. Both deriving reagents had 1 minute reaction times and 95^{o}C vs 56^{o}C for BSTFA and BSA respectively.

It has also been documented that when using either BSA or BSTFA to derivatize both hydrogens on a primary amine, both reagents require the use of trimethylchlorosilane (TMCS) as a catalyst and more forcing reaction conditions (e.g., 80^{o}C for 2 hours).[38]

2.1.1.3.2 *tert*-Butyldimethylsilyl (*t*BDMS) Silylating Reagents

t*ert*-Butyldimethylsilyl silylating derivatization reagents replace active hydrogens with a *tert*-butyldimethylsilyl (tBDMS) group. These derivatives have been shown to be much more stable than other silylating reagents, with reports of thermal stabilities much greater than similar TMS derivatives. As an example, *t*BDMS derivatization reagents are preferred over TMS for long chain fatty acids that require injector and column temperatures for separation that are higher than typical runs.[39] While the larger *t*BDMS groups prevent hydrolysis of the derivatization reagent as well as the formed derivatives, it can also prevent derivatization from occurring when the compound of interest is sterically hindered.

Scheme 7 – Reaction of *t*BDMS silylating reagent MTBSTFA with amino acid glycine.



N-methyl-N-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) is shown in Scheme 7. Here, 2 molecules of MTBSTFA undergo reaction with an amino acid to form the di-substituted derived compound. MTBSTFA can derivatize most active hydrogens but to do so it often uses *tert*-butyldimethylchlorosilane (*t*BDMCS) as a catalyst. This catalyst and its products are amenable to direct GC injection and do not need to be removed after the reaction is complete. MTBSTFA is sensitive enough to derivatize a wide variety of functional groups and is the derivatization reagent of choice for space exploration studies due to its simplicity of use and substrate versatility.[40] In one example, the use of MTBSTFA in DMF is done to analyze samples of Martian soil to determine if there are any organic acids, amino acids or other compounds that would indicate that life once existed or could exist on the planet. Using MTBSTFA derivatization, NASA was able to detect some derivatized compounds in a Martian sample that may indicate that organic acids are indeed present on the planet. However, the mars

derivatization is preliminary work and further analysis of the received data is necessary to make concrete analyses.[41]

Discussed below is the design and implementation of a flow derivatization sample preparation protocol where derivatization is performed on the output of a flow chemical reactor to help quantify and monitor reactor output.

2.2 Results and Discussion

The advantageous outcomes associated with derivatization procedures significantly improve the robustness of GC/MS based monitoring techniques; and as such, it has become a valuable analytical sample preparation tool. In light of these positive attributes, an in-line GC/MS based sampling technique utilizing in-line derivatization was developed for the analysis of continuous flow products synthesized under microwave irradiation. This combination of derivatization with a reaction system will increases the scope of reactions that can be analyzed using GC/MS.

2.2.1 Deriving Reagent Selection

The first step in creating the flow derivatization setup was selecting a model derivatization reagent to be used in the system. After considering various derivatization reagents and taking into account typical reaction times, temperatures, by-products and type of derivative formed, silylation was chosen as the reaction type with N-methyl-N—(*tert*—butyldimethylsilyl)trifluoroacetamide (MTBSFTA) selected as the deriving reagent. MTBSTFA and its derivatives have been noted to be much more stable to hydrolysis compared to other silylating reagents;[42] furthermore, the derivatives give signature fragmentation patterns for mass spectral analysis (often $(M+57)^+$), and after the derivatization reaction is complete there are no

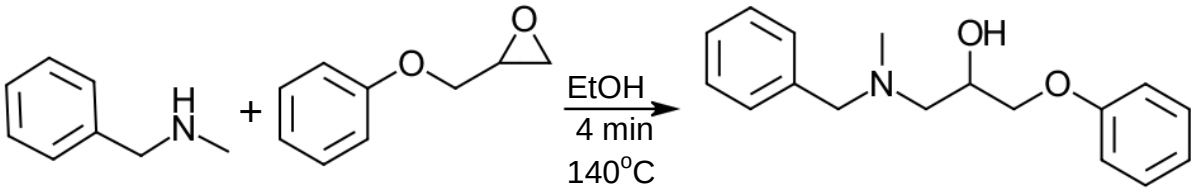
clean up steps necessary as all by-products are neutral and volatile. This reagent was used with a 1% additive of *tert*-butyldimethylchlorosilane (tBDMCS) as the derivatization catalyst.

2.2.2 Derivatization Targets - Reaction Selection and Optimization

The derivatization procedure is being designed for monitoring the output of a reactor system; therefore the transformations being considered as derivatization targets must involve reactants and products possessing suitable functionality for silylation. However, as many reactions include diverse compounds with various functionalities, reaction samples often contain compounds that are both derivable and nonderivable (Scheme 2). Ideally, after derivatization is complete on a reaction sample of containing both types of compounds, both unreacted compounds and compounds that undergo the derivatization reaction should be able to be quantified. Therefore, the ideal reaction to study with derivatization should have compounds that are both derivable and nonderivable.

The general scheme for the reaction selected to be used to optimize the derivatization procedure is shown in Scheme 8**.** One starting material (amine) as well as the expected final product (alcohol) have derivable functionalities on them that can be used to help validate the derivatization procedure and the other starting material should be unaffected by the process, allowing the nonderived compound to act as a procedure internal standard; nonderivable compounds can be analyzed with and without undergoing the derivatization procedure. Therefore, to ascertain that no sample is lost during the derivatization procedure, the nonderived compound can be compared with and without derivatization and the values should be the same.

Scheme 8 – Schematic of an epoxide ring opening reaction first used as derivatization targets.[43]



The epoxide ring opening using a secondary amine to form 1-aminopropan-2-ol derivatives was chosen as the model reaction for these studies. N-benzylmethylamine (BMA, 1.5 equiv.) reacts with 1,2-epoxyphenoxypropane (EPP, 1 equiv.) in ethanol to form 1-[Benzyl(methyl)amino]-3-phenoxypropan-2-ol (BMAPP) with 99% conversion.

A modification to the reaction conditions was required; ethanol used as the reaction solvent is an alcohol and it will be derivatized when MTBSTFA is added to the sample. Since the molecules of solvent vastly outnumber the reactants and products, MTBSTFA is wasted on derivatizing the solvent instead of the analytes of interest. To determine the best solvent for this procedure, a solvent screen for the epoxide ring opening reactions was conducted using similar conditions (0.4mol/L EPP concentration, 1.5 equiv. of BMA, 140^{o}C, 4 minutes) and these reactions were run on a batch Biotage microwave. In order to demonstrate the applicability of the derivatization approach for reaction monitoring purposes, it was stipulated that a suitable solvent should facilitate at least a 60% yield of products.

Table 3- Results of solvent screen on the epoxide ring-opening reaction.

Entry	Solvent	Conc. Epox (mol/L)	Temp (^{o}C)	Time (min)	Conv. %[a]
1	EA	0.4	140	4	16
2	THF	0.4	140	4	15
3	DME	0.4	140	4	DC[b]
4	MeOH	0.4	140	4	100
5	neat	-	140	4	83

[a] - ^{1}H NMR conversions in $CDCl_3$ [b] - decomposed

The original attempts at the epoxide ring opening reaction with ethyl acetate (EA), tetrahydrofuran (THF) and dimethylethoxide (DME) were unsuccessful, with little or no product being found by ^{1}H NMR spectrum of the crude reaction mixture (Table 3). When methanol (MeOH - as a control) was used under the same conditions it was found that the reaction went to completion (as expected). A neat reaction was completed with good results, however running pure reagents through a flow system would be difficult and should be avoided unless absolutely necessary.

EA was further used for testing as it is often used as a GC injection solvent and can be used in derivatization reactions as well, streamlining the process by eliminating the need to switch solvents after the reaction and before derivatization.

Table 4 – Effects of increased concentration and elevated temperature on the epoxide ring opening reaction.

Entry	Solvent	Conc. Epox (mol/L)	Temp (^{o}C)	Time (min)	Conv. %[a]
1	EA	0.4	200	4	39
2	EA	1	140	4	56
3	EA	1	200	4	80

[a] - ^{1}H NMR conversions in $CDCl_3$

A 4 minute reaction at the same concentration but with elevated reaction temperature Table 4, entry 1 vs. Table 3, entry 1) showed some increase in yield. However, increasing the concentration of the reaction significantly improved the reaction conversion and the 60% conversion target was met (Table 4, entry 3). So, after optimization, 1 mol/L of EPP (1.5 mol/L BMA) was chosen as the reaction concentration and at least a 4 minute reaction time for the epoxide ring opening.

2.2.3 Derivatization – Batch vs. Flow

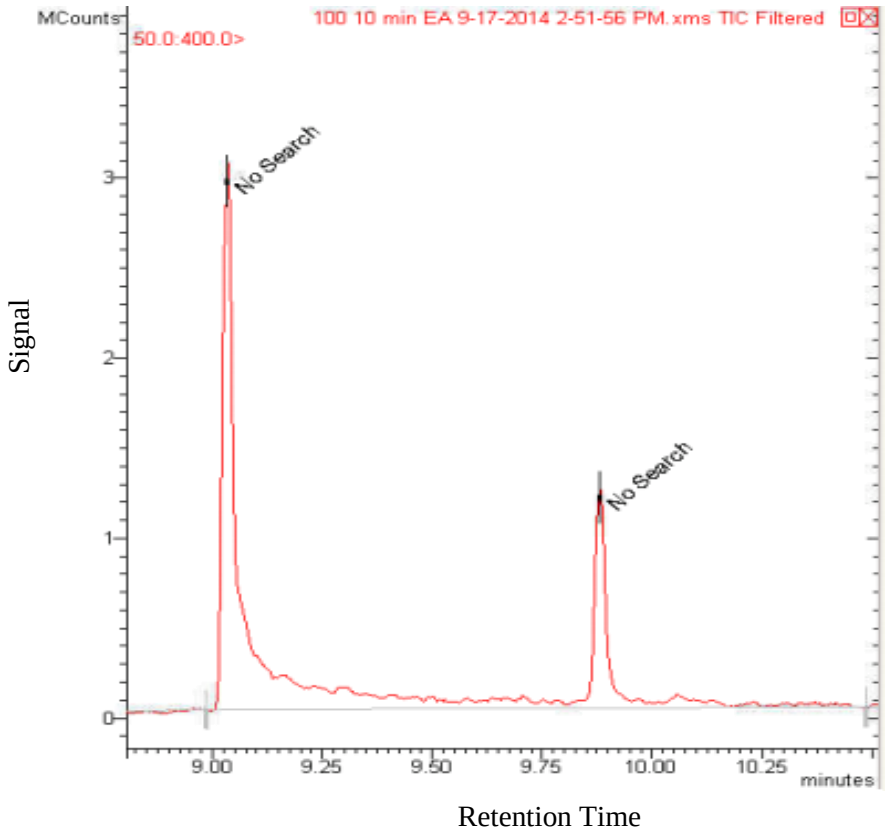
Derivatization is performed in batch reactions in most literature protocols. In such instances, the standard method of preparing derivatives is accomplished by a worker in the field manually taking a sample of the analytes of interest, placing them in a vial and then adding solvents and deriving reagents by syringe or pipette. Following this solution addition, some sort of heater or agitator is used to perform the reaction and when completed, the sample is again manipulated by hand (dilution, extraction) in order to create a suitably injectable GC solution. These sample preparation methods can have inherent issues with reproducibility as different chemists repeat the same procedure. The goal of this work is the development of a highly reproducible automated flow derivatization protocol that can be used to help monitor the output from a reactor using GC/MS analysis.

Automated technologies are thought to be more precise that manual labour and theoretically should be able to match or improve on batch methodology results. Therefore, the first derivatization experiments that were completed were batch experiments to create a benchmark performance to meet or exceed with the flow system.

2.2.4 Batch Reaction Optimization

To optimize the derivatization process, several experimental parameters including volume of deriving reagent, solvent type, solvent volume, temperature, and length of reaction were tested for their apparent effect on the derivatization reaction. Using EA as a solvent[44], batch derivatizations of the aminoalcohol were performed. Initially, a 50 µL volume of MTBSTFA was used (0.2 mmol), which is in large excess of the analytes of interest (7 µL samples of 1 mol/L = 0.07 mmol compound being analyzed). Such a derivatization protocol should be applicable to a wide variety of compounds; an excess of MTBSTFA leaves room for the protocol to be used for higher analyte concentrations that may be encountered while running reactions.

Figure 26 – Comparison of underived BMAPP peak (left) derived BMAPP peak (right).



Initial experiments designed to explore the effect that time, MTBSTFA volume and solvent volume had on the derivative percent conversion were conducted (Table 5).

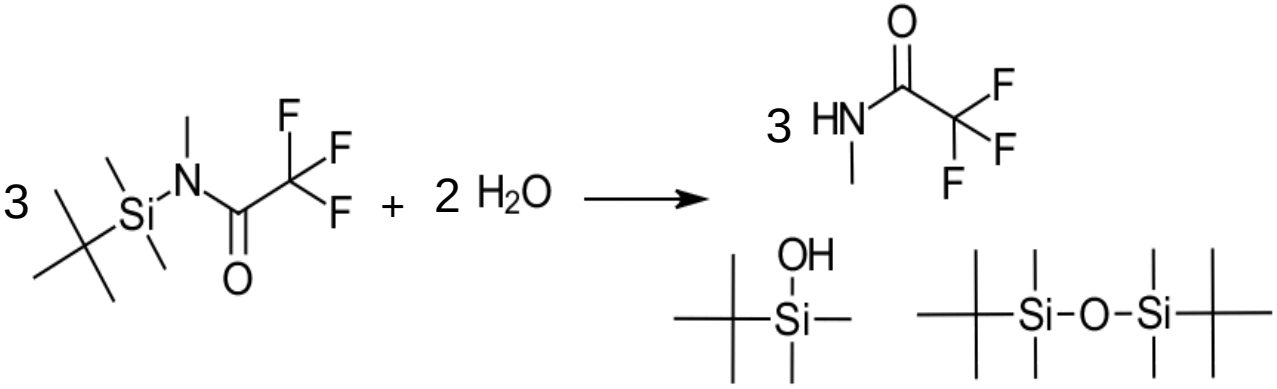
Table 5 – Effect of increasing time and deriving reagent volume on derivatization reaction efficiency.

Entry	Temp (^{o}C)	Time (min)	Amount MTBSTFA (μL)	Amount EA (μL)	Amount BMAPP (%)[a]	Amount Deriv. BMAPP (%)[a]
1	60	10	50	100	89	11
2	60	20	50	100	81	19
3	60	30	50	100	76	24
4	60	10	100	50	95	5
5	60	20	100	50	89	11
6	60	30	100	50	83	17

[a]- As determined between comparison of derived and non-derived peak height in GC/MS

Although there was an increase in derivative formation with time (Table 5, entry 1-3, 4-6) neither the larger volume (Table 5, entry 4-6) nor smaller volume (1-3) of MTBSFTA gave a considerable amount of derivative. It was noticed that in the chromatograms of each reaction is a peak that corresponds to the double reaction of MTBSTFA with water (Scheme 9) characteristic by 147 m/z.

Scheme 9 – Stoichiometry of MTBSTFA hydrolysis.



Because these reactions are moisture sensitive, a derivatization reaction was run under argon to reduce the possibility of moisture intrusion into the reaction. When analyzed, the reaction showed no greater increase in derivative formation and the hydrolysis peak was on par

with that of the other reactions. After eliminating the possibility that there is too much moisture in the reaction, the temperature was increased to examine its effect on the process.

Table 6 – Effect of elevated temperature on derivatization efficiency

Entry	Temp (°C)	Time (min)	Amount MTBSTFA (μL)	Amount EA (μL)	Amount BMAPP (%)[a]	Amount Deriv. BMAPP (%)[a]
1	90	10	50	50	71	29
2	90	20	50	50	55	45
3	90	30	50	50	50	50

[a]- As determined between comparison of derived and non-derived peak height in GC/MS chromatogram.

The elevated temperature showed an increase in derivative production for all time trials compared to lower temperature reactions (Table 6 vs Table 5). However, even after 30 minutes of derivatization only 50% of the derivative was formed (Table 6 entry 3). Next, reactions were completed using a small temperature increase and with other solvents in order to determine if a solvent switch helps the derivatization process.

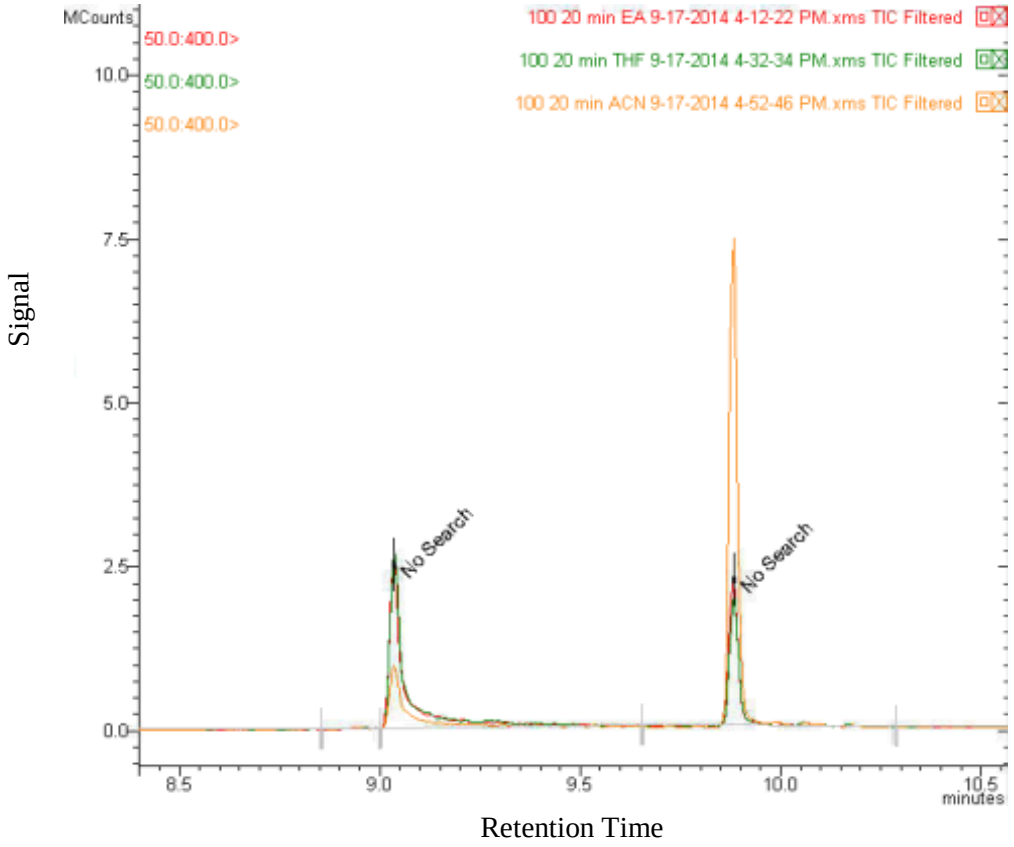
Table 7 – Efficiency of derivatization reactions performed with a variety of solvents

Entry	Solvent	Temp (°C)	Time (min)	Amount MTBSTFA (μL)	Amount Solvent (μL)	Amount BMAPP (%)[a]	Amount Deriv. BMAPP (%)[a]
1	MeCN	100	10	50	50	22	78
2	EA	100	10	50	50	71	29
3	THF	100	10	50	50	71	29
4	MeCN	100	20	50	50	12	88
5	EA	100	20	50	50	50	50
6	THF	100	20	50	50	57	43
7	MeCN	100	30	50	50	7	93
8	EA	100	30	50	50	43	57
9	THF	100	30	50	50	47	53

[a]- As determined between comparison of derived and non-derived peak height in GC/MS chromatogram.

It was seen that acetonitrile (MeCN) is the preferred solvent for this derivatization (Figure 27, Table 7, entry 1,4,7), as a marked increase in derivative formation for all times tested was seen in the MeCN chromatogram compared to other solvents.

Figure 27 – Gas chromatogram of BMAPP (left) and derived BMAPP (right) for comparison of the effect of solvent on derivatization efficiency. Orange is acetonitrile, green is tetrahydrofuran and red is ethyl acetate.



Unfortunately as a precaution in these reactions the MeCN used in testing was very dry. Even though it was already tested to see if moisture was an issue with EA, it was unknown if the moisture content found in regular lab bottle of acetonitrile would hinder derivatization. When using a flow protocol, a solvent reservoir is required to house excess MeCN while it waits to be used for derivatization and this reservoir would need to be sealed if the solvent could not be open to the atmosphere. Pumping from a sealed container would necessitate the use of inert gas to fill

the void created as the solvent is drawn out of the flask to prevent the establishment of a vacuum that would complicate pumping. Side by side reactions were run with the same ultra-dry MeCN and "wet" MeCN that had been opened and sitting in the lab prior to use here (Table 8). Fortunately there was no appreciable difference between the two different sources of acetonitrile at the 30 minute mark of reaction time. Therefore, acetonitrile was chosen as the best solvent for this derivatization.

Table 8 - Comparison of the derivatization of BMAPP using different acetonitrile sources.

Entry	Solvent	Temp (^{o}C)	Time (min)	Amount MTBSTFA (μL)	Amount MeCN (μL)	Amount Prod. (%)	Amount Deriv. (%)
1	MeCN wet	100	30	50	50	10	90
2	MeCN dry	100	30	50	50	7	93

[a]- As determined between comparison of derived and non-derived peak height in GC/MS

Moving forward, the final batch derivatization reactions were done on BMA and BMAPP (Table 9). Table 9 shows the results from the derivatization experiments completed on BMAPP and BMA and it is important to highlight the results denoted with a [b] in the table. The storage of MTBSTFA is suggested to be between 2 to 8^{o}C in a sealed ampule and to be used as soon as it is opened to prevent hydrolysis of the reagent. In the flow derivatization setup, the MTBSTFA will be sitting in a syringe for some time while waiting to be used. Even though the flow derivatization system will be a closed system and the MTBSTFA should not come in contact with air, it is not perfect. In the reactions in Table 9 (entry 10 and 11), little change in efficiency occurred when using MTBSTFA that was open to air for several days before being used when compared to MTBSTFA that was freshly opened and used (Table 9, entry 3 and 4).

Table 9 – Effect of solvent volume and reaction time on efficiency of derivatization of BMA and BMAPP.

Entry	Temp (°C)	Time (min)	Amount MTBSTFA (μL)	Amount MeCN (μL)	Amount BMAPP Deriv. (%)[a]	Amount BMA Deriv. (%)[a]	Combined Deriv.
1	100	10	50	100	63	90	153
2	100	10	50	100	67	91	157
3	100	10	50	200	50	88	138
4	100	10	50	200	56	89	144
5	100	10	50	300	50	83	133
6	100	10	50	300	39	80	119
7	100	30	50	100	86	97	183
8	100	30	50	200	82	97	179
9	100	30	50	300	71	96	167
10[b]	100	10	50	200	48	83	131
11[b]	100	10	50	200	44	86	130

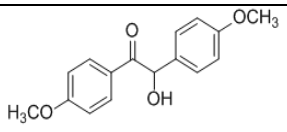
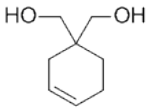
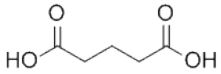
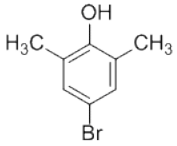
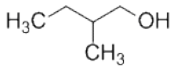
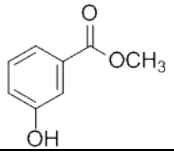
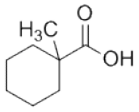
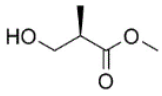
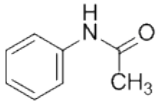
[a]- As determined between comparison of derived and non-derived peak height in GC/MS
[b] – MTBSTFA was kept out in contact with air over weekend then used on Monday in reaction

It is noted that derivatization reactions should consistently convert the target analyte to >95% derivative. The values obtained for the derivatization of BMAPP are lower than this threshold (Table 9). It was hypothesized that even though the aminoalcohol is not a large molecule, the alcohol is actually quite hindered in this reaction. Hydrogen bonding that can occur between the alcohol and the ether moiety, or the alcohol and the amine, which can stabilize the molecule making it less reactive. However, in regards to a reaction like this derivatization, when compounds are subjected to a process that was developed using a model compound that is more reluctant to undergo said reaction, the method should lead to suitable conversion for less challenging compounds. In order to show that the current developed protocol is applicable to a variety of compounds, several small molecules that have derivable functionalities were subjected to the derivatization protocol (Table 10). The developed protocol was able to affect the derivatization of compounds possessing an assortment of functionality. Alcohols, phenols,

carboxylic acids and an amide all underwent derivatization to 100% yield under the 10 or 30 minute protocol. However not all reactions proceeded efficiently, as the diol substrate in entry 2 failed to react at all, and the bromophenol derivative in entry 4 decomposed after being subjected to the 30 minute derivatization protocol. Some compounds, such as the dicarboxylic acid (Table 10, entry 3), did not elute from the column even at high temperatures; no peaks were seen in the chromatogram without any derivatization, and only one peak was observed (303 m/z) even though two reaction sites are present, 303 m/z indicates both reaction sites are derivatized.

These results demonstrated that the protocol developed can be used to derivatize a variety of different compounds. Therefore, the optimal reaction conditions in batch can be assumed valid and a flow derivatization setup was designed to mimic these conditions as best as possible. Combining the outcomes of the batch experiments led to the following set of conditions for the flow system: **Solvent:** acetonitrile **Temperature:** 100^{o}C **Volume Deriving Agent:** at least 50 μL **Volume Solvent:** Should be as small as possible **Time:** 10 minutes or 30 minutes residence time depending on how difficult all compounds in the sample are to derivatize.

Table 10 – Derivatization of various compounds using optimized batch procedure.

Entry	Compound	10 min Derivatization (% derivative)[a]	30 min Derivatization (% derivative)[b]	Notes
1		38%	100%	Similar to aminoalcohol product used to optimize derivatization method.
2		0%	0%	
3		100%	n/a	Did not come off column without derivatization.
4		88%	Decomposed	3 equal height peaks observed, no Br, use shorter reaction time
5		100%	n/a	Moves analyte peak past MTBSTFA peak[c]
6		100%	n/a	
7		100%	n/a	
8		95%	100%	Moves analyte peak past MTBSTFA peak[c]
9		51%	100%	

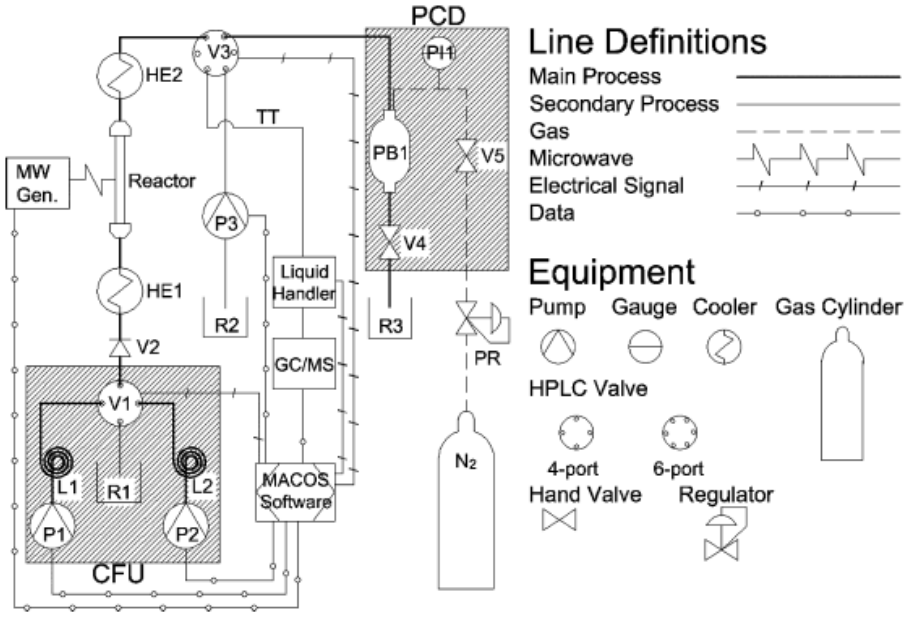
[a] – As determined between comparison of derived and non-derived peak height in GC/MS [b]- n/a as the compound went to 100% conversion after 10 minutes. [c] – Moving peak past MTBSTFA allows the detector to remain off while the large MTBSTFA peak elutes from the column.

2.2.5 Flow Derivatization

2.2.5.1 Microwave assisted continuous organic synthesis (MACOS) system

The reactor and analysis system for which the flow derivatization protocol is being developed can be seen in Figure 28**.** In this microwave assisted continuous organic synthesis (MACOS) system, automated technologies are used to run a chemical reaction in flow and to analyze reactions in-line, combining a GC/MS instrument with the reactor to monitor the transformation.[1] For the sake of simplicity, briefly described are the parts of the reactor and analysis system that are directly related to the flow derivatization setup only.

Figure 28 – Complete flow reactor system with in-line analytics for which the flow derivatization protocol is being developed.[1] P1 and P2: syringe pump, P3: carrier solvent pump L: holding loop, R1: reactant reservoir, CFU: Continuous flow units, R2: carrier solvent reservoir, R3: product collection reservoir, V: valve, V3: sampling valve, HE: heat exchanger, PB: pressure ballast, PI: pressure indicator, PR: pressure regulator, TT: transfer tube.



All components of the MACOS system are run by one software interface, and this software will also be used during flow derivatization experiments. V3, a 6-port 2-position valve,

is used to isolate a sample from the flow reaction being run (see Figure 29). The reaction samples isolated in V3 are delivered to N2 (an in-house created upgrade), a side-arm needle connected to the liquid handler (autosampler) (Figure 30). The samples are delivered using P3, a positive displacement pump. Here solvent is flowed through V3 into plastic tubing then through N2 into a clean vial on the autosampler deck. The autosampler's automated sequences (macros) are used to complete automated sample preparation steps and a GC injection sequence. This reactor-analysis system was used to run a model reaction (see scheme 1) using completely automated reaction and analysis technologies.[1]

Figure 29 – Close up view of V3 as used to isolate a sample from a MACOS reaction A) Valve position when a reaction stream is being pushed directly to collection (red arrows). B) Valve position to divert the reaction stream into the isolation loop to take an aliquot before being pushed to collection (N2 – see Figure 30).

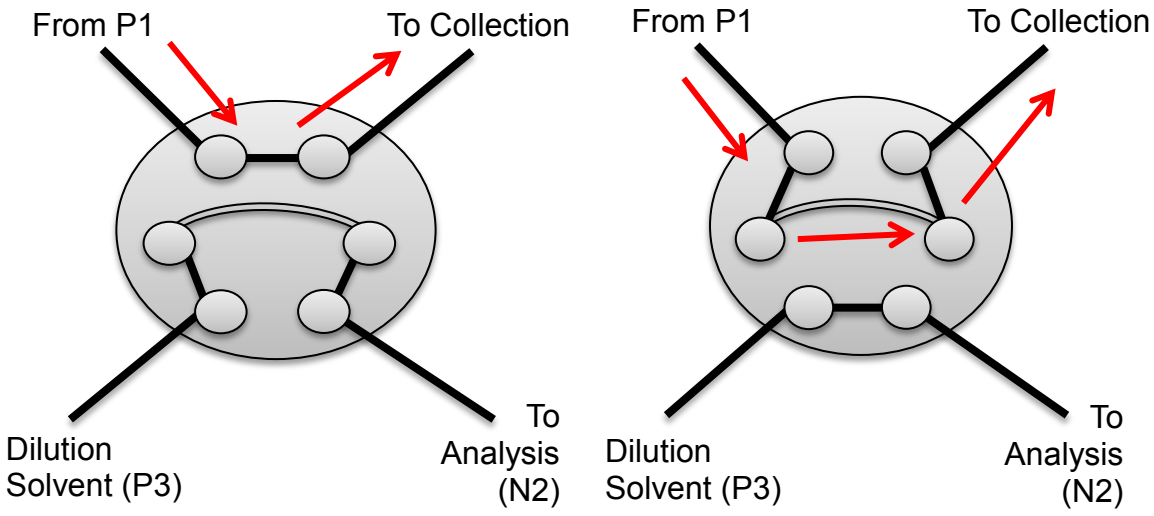
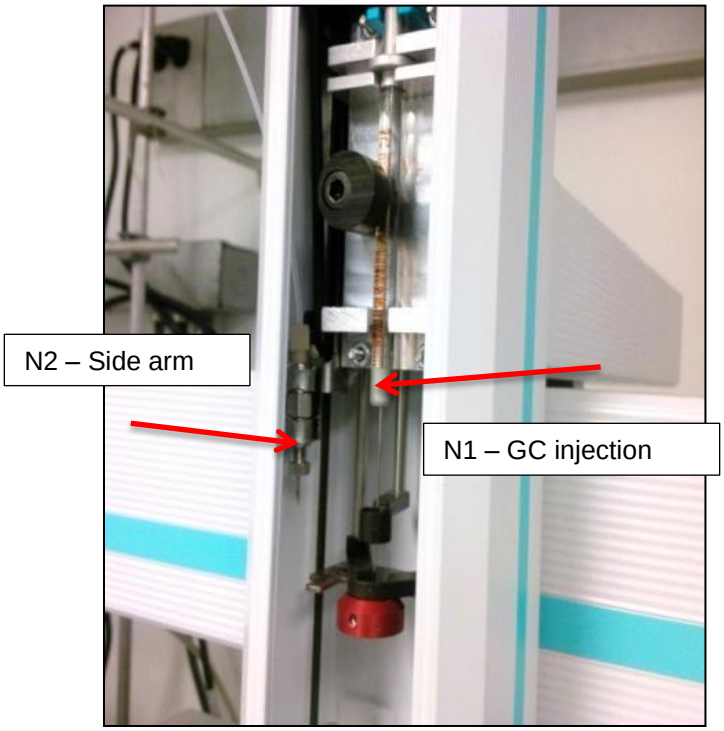


Figure 30 - Side-arm needle (N2) that facilitates delivery of reaction sample from sampling valve. GC syringe (N1) is used for GC injections.



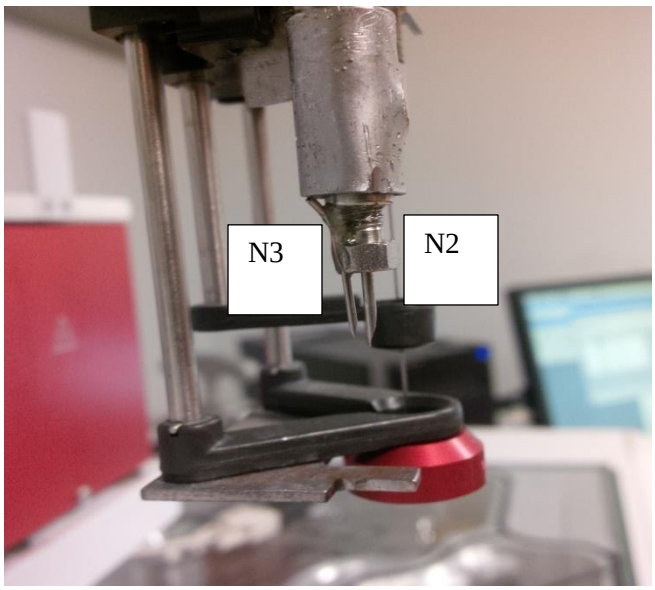
2.2.5.2 Upgrades to the Sample Delivery System to Incorporate Derivatization

2.2.5.2.1 Autosampler

The addition of a second needle, N3 (Figure 31), onto the side-arm of the autosampler that houses N2 was the first required upgrade to the automated sampling system used with MACOS previously.[1] Initially used with MACOS, N2 facilitated sample transfer from the reactor into a GC vial. However, the lid on the collection vial was left loose to help prevent pressure build up during filling. If the vial lid was left slightly ajar during derivatization, which requires heating, analytes would assuredly be lost and the sampling would not be quantitative. N3 is responsible for piercing the septum of each collection vial in order to eliminate pressure build up while the vial is being filled. As proof of concept, a common 18 gauge laboratory needle was affixed onto the back of the bolt that holds N2. The tip of N3 was situated higher than N2 as to

not be contaminated by solvent entering the vial. Once N3 was affixed to the side-arm, the previously prepared autosampler macros used for automated sample preparation and GC injection were employed (Figure 12); this time with the side needle piercing the collection vial to alleviate pressure instead of having a loose vial lid. Initial tests were successful in that N3 allowed for complete filling of the vial without the indicative sound of escaping gas when N2 is withdrawn from a vial that has built up pressure.

Figure 31 – Close up of N3 attached to the back of N2 used to prevent pressure build up in sample collection vials.



Having successfully demonstrated that N3 can facilitate the filling of capped vials while using N2, the GC side arm attachment syringe plate was permanently modified with a needle for pressure relief.. The initial design was unsuccessful as N3 was placed too close in front of N2. Instead of relieving the pressure by letting gas escape as the fill volume increased, solvent flowed out of N2, directly into N3 and out of the vial; this flow of solvent was due to the increased pressure in the collection vial and close proximity of N3 to N2. Moving N3 to the

back of the needle and a little farther away eliminated this issue and created a reliable method for filling a vial with a completely closed lid without dangerous pressure build up (Figure 31).

The initial macro sequence that was used to collect a sample from the reactor (Figure 12) also required an upgrade to include the derivatization steps in the preparation sequence.

Figure 32 – Macro sequence of autosampler to include derivatization step in automated reaction monitoring.

01 Set Temp	12 Home
02 Wait Temp	13 Wait
03Wait Signal	14 Aspirate
04 Aspirate	15 Wait signal
05 Wait Signal	16 Transport vial
06 Transport Vial	17 Home
07 Home	18 Start Agitator
08 Start Agitator	19 Wait Agitator
09 Clean Syringe	20 Transport Vial
10 Wait Agitator	21 GC Inject
11 Transport Vial	22 Clean Syringe

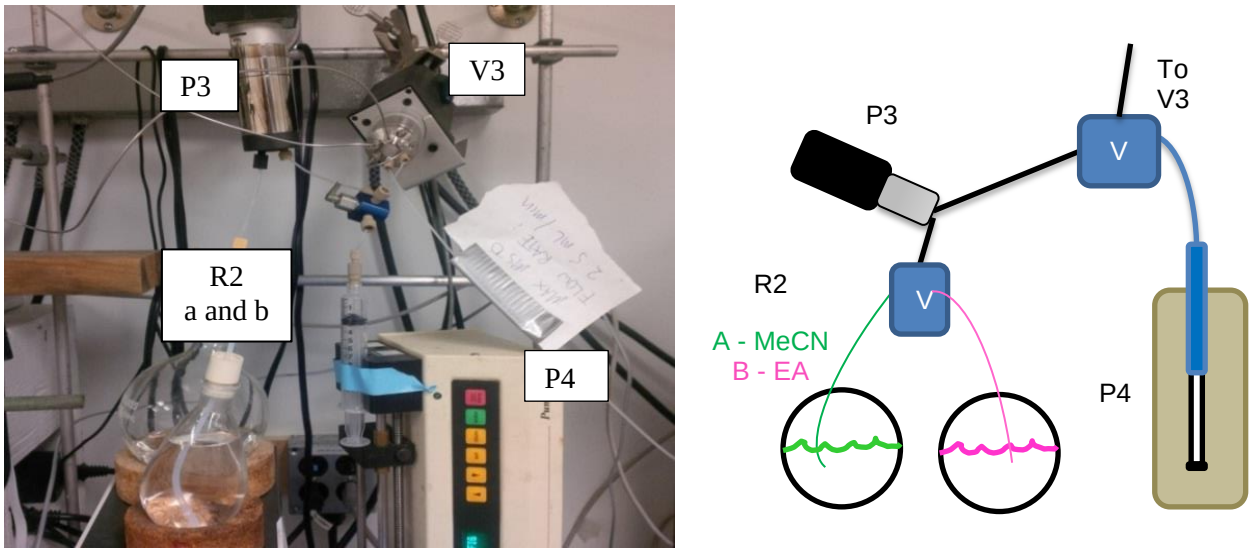
The derivatization sequence in Figure 32 begins with set temperature (01) and wait temperature (02) commands. These commands set the agitator temperature to 100^{o}C and wait for it to get to that temperature before the next command begins. This prevents any sample from being derivatized at the wrong temperature. Wait signal (03) puts the autosampler into a state of waiting to receive a signal from MACOS software to continue with the rest of the sequence. Macro 04 is used to move the autosampler over to the collection vial where P3 then pumps the isolated sample into the vial. Wait Signal 05 is used to keep the autosampler on the vial until the entire 350 μL pumped with P3 is collected. Transport vial macro 06 moves the collection vial into the agitator to start derivatization. The autosampler arm returns to the home position (07) before the agitator starts (08, 5 minutes at 700 rpm, 5 minutes motionless, 100^{o}C). The autosampler cleans the GC injection syringe (09) and then waits for the agitator (10) to finish

heating. Then, the autosampler transports the vial to cool it (11) placing it in a position on a vial tray away from all other vials. After moving back home (12) the autosamplers waits (13) for 30 seconds for the vial to cool down. Then, the autosampler moves so that P3 can fill the vial (14) with the final 8 mL MeCN dilution. A wait signal (15) is used to prevent the autosampler from moving before the entire dilution volume is delivered. The autosampler transports the vial to the agitator (16) then returns home (17) before the agitator stirs the vial for 30 seconds at 450 rpm (18/19). Then the autosampler transports the vial (20) back to the original vial position. A GC injection sequence (21) is initiated and the autosampler syringe undergoes cleaning (22) before the sequence starts back at 01. The entire isolation, transfer, preparation, derivatization and injection sequence takes approximately 17 minutes (with a subsequent GC method run time of 18 minutes, but this will vary depending on what is being analyzed). Because the GC/MS and autosampler function individually, the autosampler can prepare a new sample while the GC/MS is still analyzing the previous sample.

2.2.5.2.2 Sample Delivery

The original sample delivery mechanism on the automated MACOS system combined one pump, P3, and one valve, V3, to deliver solvent with GC internal standard from a reservoir, R2, through transfer side-arm needle N2 (Figure 28). The derivatization procedure requires a slightly more complicated arrangement. Originally set up for the flow derivatization (Figure 33) were two large solvent containers (RBF's, R2 a and b), one RBF (a) MeCN used for derivatization and (b) one for GC grade EA/internal standard used for a final 8 mL dilution.

Figure 33 – First pump and valve set up used to deliver isolated V3 sample for flow derivatization reactions. P3 –Pump for solvents, V3 – Valve with isolation loop, R2 a and b – solvent reservoirs for MeCN and EA, P4 – MTBSTFA delivery pump

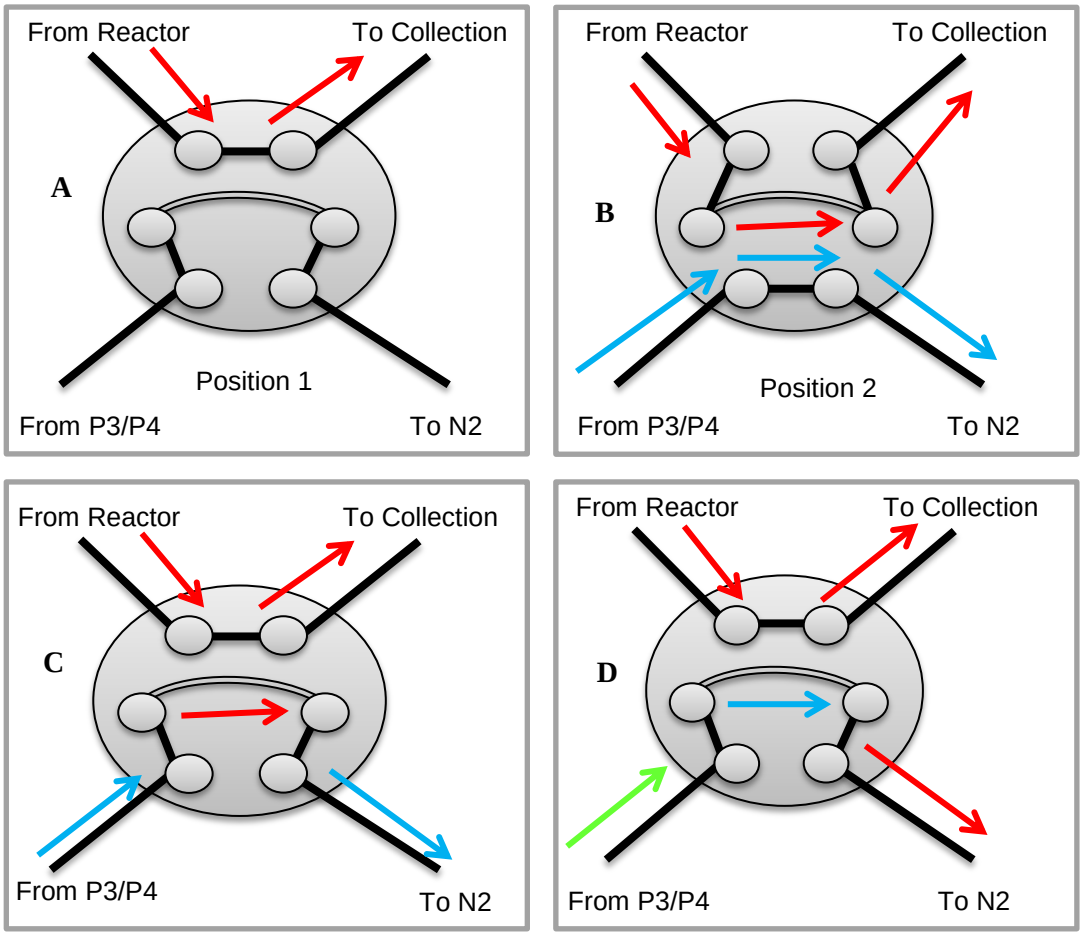


The RBF's a and b are connected via small valve that is installed upstream of the M50 and downstream of the solvent reservoirs; this valve splits the tubing into two separate lines, allowing for flow of EA when the valve is in one position, and flow of MeCN when in the other. This valve is manually operated, as there were no available automated valves when the system was being compiled; although the derivatization should be completely automated, it was not possible at the time. However, these valves are very easily exchanged with automated valves like V3 and the derivatization procedure can become completely automated with little effort.

The derivatization reagent is introduced upstream of P3 and just before V3 via P4 (Figure 33). This introduction is facilitated with one of the manually manipulated valves as well. This valve switches between the derivatization reagent and the main line allowing MTBSTFA to be cut off from all other solutions used during normal operation, helping MTBSTFA to remain dry. When V3 is in position to collect a sample from the reaction (position two, B, in Figure 34), the

derivatization reagent is flowed through V3 into the analytical transfer tubing (position two, B, in Figure 36). This ensures that the reaction sample is sandwiched between deriving reagent on each side.

Figure 34 – V3 positions as used to isolate sample, allow P3 to deliver MTBSTFA, and allow P4 to deliver MeCN. A) Position 1 - Normal position of valve B) Position 2 – Isolation position. Sample (red) is collected in the loop while P4 pumps MTBSTFA (blue) through tubing connected to N2 C) V3 back to position 1 to sandwich the sample (red) in between plugs of MTBSTFA (blue) D) P3 pushes MeCN (green), MTBSTFA and sample to N2.



2.2.5.3 Determining Solvent Flush Volume Required for Reproducible Sampling from V3

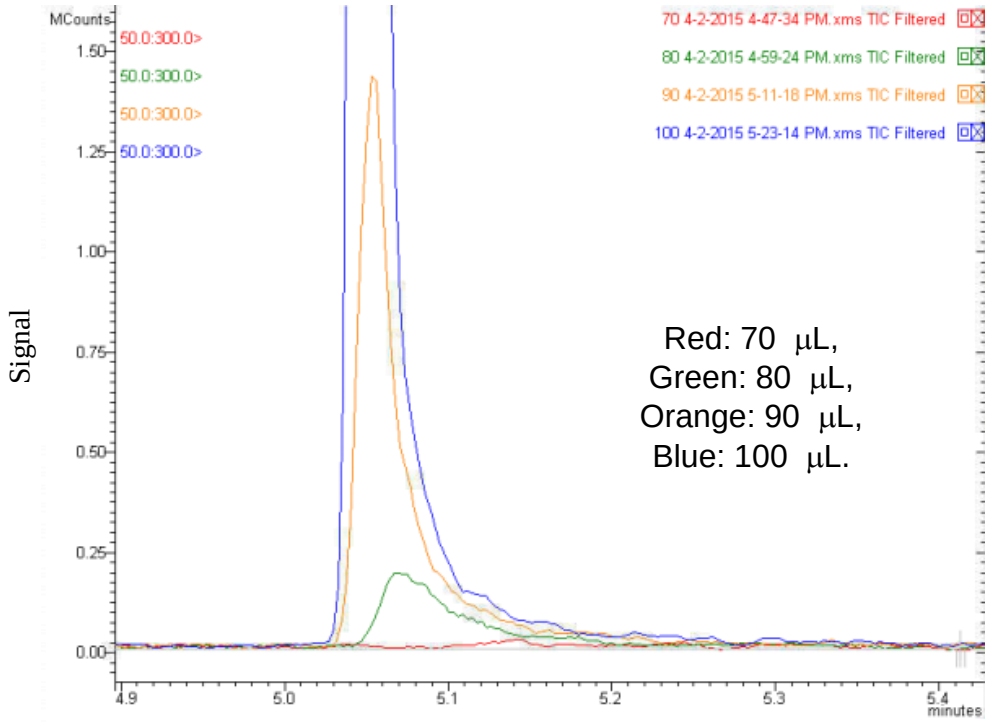
To recreate the batch derivatization reaction conditions with the flow derivatization setup in Figure 34, each sample isolated in V3 from the synthetic reaction must be reproducibly delivered for analysis with minimal solvent from P3, and the required amount of deriving reagent (50 μL) must also be delivered into the collection vial via P4. Diffusion of the sample and/or MTBSTFA into MeCN can occur as they flow through the tubing towards N2 and it is important to know the extent of this diffusion and how much solvent is required to capture the entire sample reproducibly. Unfortunately, there is a large area in which the actions of the autosampler arm are performed that requires the transfer tubing between V3 and N2 to include a bit of slack in order for the arm to reach the farthest point away from V3. This increases the total required tubing length and which then requires the use of smaller inner diameter (ID) tubing to keep volumes minimal. Unfortunately P3, which is responsible for the final push of sample through the tubing to N2, has a pressure limit of 125 psi; a long tube with a small ID creates a higher back pressure than P3 can handle if it were to be used at a relatively quick sample delivery speed. Although it is not ideal to use a slower flow rate as it will significantly increase sample preparation time, it is required here. The maximum sampling flow rate is now limited to 2 mL/min to prevent damage to P3.

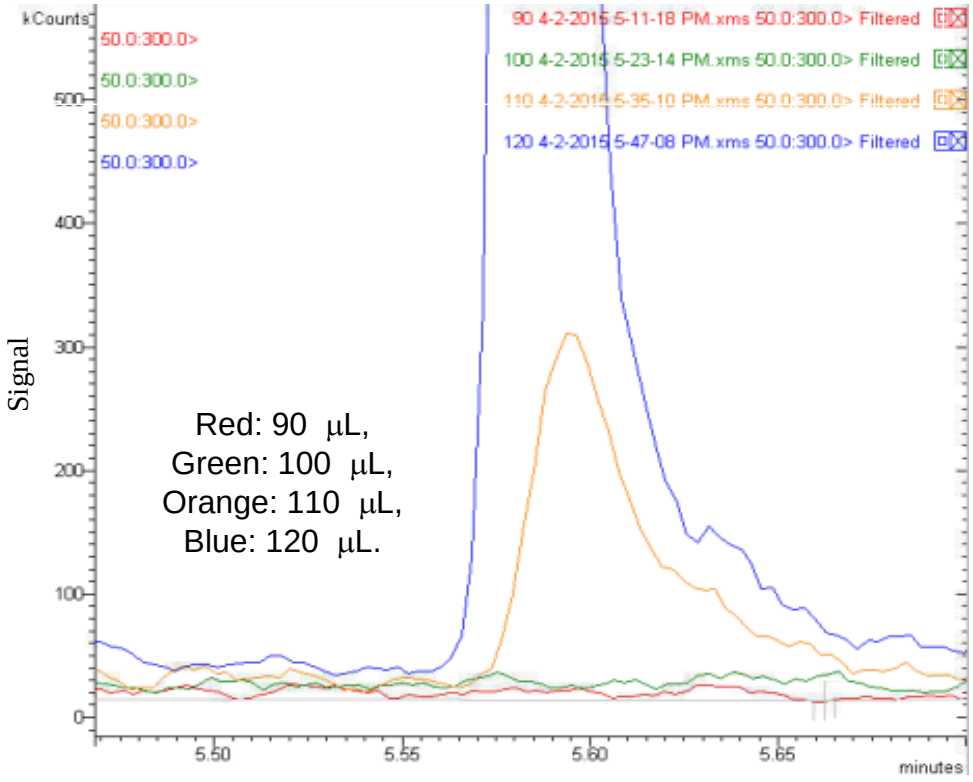
The volume in the tubing between V3 and N2 is much larger than the volume of sample and deriving reagent being collected. This means that there is a large dead volume in front of the deriving reagent/sample that can be discarded to keep volume entering the collection vial low as it does not house any compounds of interest. An experiment to determine how much MeCN can be flowed before analytes reach the derivatization vial was set up using a compound readily analyzed by GC. This was done so that no derivatization is required before the samples are

analyzed and the samples can be injected as soon as they are collected. For these experiments, a solution of EPP was made up in EA and was flowed through the reactor system into V3 using pump P1/P2 (see Figure 28). Acting as a simulated derivatization reagent, 2-allyl phenol (2AP) was dissolved in dimethylsulfoxide (DMSO) and was flowed using P4 through V3 (Figure 33).

Once all lines in the system were primed, P1/P2 delivered the solution of EPP through its lines, into V3 and out into collection until such time V3 switches positions and the EPP solution enters the valve loop for isolation. As the solution is isolated, 60 μL of 2AP in DMSO is pumped by P4 through V3 into the transfer tubing to N2. Since all lines in the derivatization setup are primed before the experiments starts, 60 μL is pushed out of N2 due to this delivery of 2AP. This 60 μL is collected in a waste container that sits on top of the GC/MS. Once P1 pumps enough solution through V3 to collect a sample, V3 switches back to its original position. P3 pushes MeCN (and 2AP/DMSO, EPP) through V3 into the transfer tubing and through N2 into a collection vial. To minimize how much volume enters the vial, it is important to determine how much solvent can be pushed to waste after a sample is isolated. This is determined by monitoring the chromatogram of samples that are collected from N2 in 10 μL increments. Here, P3 pumps 10 μL of MeCN at a time into a vial after a sample is isolated in V3, changing vials between each 10 μL pumped. Each sample is analyzed using the same GC method; if no peaks occur in the chromatogram, it indicates that that amount of solvent can be pushed to waste without losing any sample (Figure 35).

Figure 35 – Appearance of analyte peaks after sample isolation in V3 and various amounts of MeCN to flush uing flow derivatization setup. (top) - Appearance of 2AP (bottom) - Appearance of EPP





It was determined after multiple 10 μL increment experiments that 70 μL of solution can be flowed after a sample is taken without losing any of the analyte or derivatization reagent. The 2AP peak consistently appeared in the 80 μL chromatogram while the EPP appears first in the 110 μL chromatogram (Figure 35). From these experiments, a protocol was developed for the derivatization setup where, after a sample is isolated in the loop and MTBSTFA has been pumped into the lines, V3 switches back to its original configuration, 70 μL is pushed out N2 into waste, before the rest of the sample is collected in a vial. Here, to aid in sample collection, after V3 switches to stop sample isolation, P3 is programmed to flow 70 μL then stop. P3 then waits until N2 moves over the collection vial so that the sample can then be collected.

In order to determine the minimum volume required to reproducibly deliver the entire isolated sample and required 50 μL of MTBSTFA, experiments were set up with the same flow setup as the isolation experiments above, however after a sample was taken and 70 μL was pushed to waste, ten 50 μL samples of MeCN were pumped into individual vials and these samples were analyzed against a calibration curve. A plateau in the isolated sample volume after successive injections would indicate that no more sample is left over in V3 or the transfer tubing and that increasing the flush volume would be unnecessary. Noted is that the first increment of MeCN was only 30 μL since 70 μL was already pushed to waste. In these calibrated experiments, two compounds instead of just one, EPP and tetrabutylbenzene (TBB), at 0.1 mol/L were flowed using P1 though V3 for isolation. This was done to collect extra data that can be compared to help validate the sampling technique. The lowest volume required to push the entire isolated sample into a vial will be the volume used to deliver samples when using the derivatization protocol to monitor a reaction. In the 50 μL increment isolation experiments, there was good agreement between TBB and EPP with respect to the amount of sample that was

isolated and at what point they plateaued (Table 11). An unpaired, 2 tail T test was done to compare the isolated averages of both TBB and EPP at 350 μL. Here the two-tailed P value equals 0.3130 and by conventional criteria, this difference is considered to be not statistically significant at the 95% confidence interval. As well, an unpaired 2 tail t-test comparing the isolated volume combined average for TBB and EPP from the 300 μL and 350 μL flushes gives a number of 0.0013, which is considered to be statistically significantly different at the 95% confidence interval. Whereas the t-test used comparing the 350 μL isolation with the 400 μL isolation shows a value of 0.183 which is not statistically different at 95% confidence interval. The 350 μL isolation volume has a similar average compared to the 400, 450 and 500 μL samples where the average isolation volume of EPP and TBB were 7.400, 7.411, 7.341, 7.341 μL for 350, 400, 450 and 500 respectively (average between them being 7.37 μL and a 0.03 standard deviation). At the 350 μL isolation volume, a sufficient amount of MTBSTFA (~56 μL) is also collected, indicating that the flow set up is capable of matching the volumes determined with the batch optimization studies.

Table 11 – Isolated TBB, EPP and 2AP volumes after isolation in V3 and flushing with different P3 solvent volumes.

Solvent Volume Flush (μL)	TBB Volume (μL) [a]	EPP Volume (μL) [a]	2AP Volume (μL) [a]
250	7.107	6.958	53.35
250	7.020	7.015	53.46
250	6.929	6.922	53.58
250	7.043	6.863	52.46
300	7.373	7.343	56.42
300	7.161	7.142	54.75
300	7.089	7.074	57.18
300	7.218	7.145	56
350	7.287	7.301	54.15
350	7.402	7.439	56.04
350	7.364	7.422	58.02
350	7.342	7.406	57.79
400	7.477	7.441	57.74
400	7.454	7.459	59.83
400	7.432	7.550	59.64
400	7.178	7.460	57.09
450	7.266	7.269	56.18
450	7.345	7.431	57.42
450	7.333	7.344	57.44
450	7.345	7.392	59.09
500	7.164	7.189	54.73
500	7.392	7.331	57.01
500	7.503	7.394	55.77
500	7.335	7.422	56.25

[a] –isolated volumes determined through GC/MS calibration with undecane internal standard

The results obtained through the solvent flush experiments helped create the protocol for delivering a sample while using the derivatization setup combined with the flow reactor system. When a reaction is being completed with the MACOs reactor, V3 is used to isolate a reaction sample while 60 μL MTBSTFA is flowed through the transfer tubing while sample isolation occurs. Once the sample is collected, 70 μL of solution is pushed to waste, then 280 μL MeCN

(350 μL [the plateau volume from Table 11] minus 70 μL [already pushed to waste]) is pushed into a vial to reproducibly collect the isolated sample with as little volume as possible.

Moving forward, a new reaction was selected (due to the aminoalcohol derivatization problem) to validate that the derivatization flow setup created can produce accurate and precise information on isolated samples.

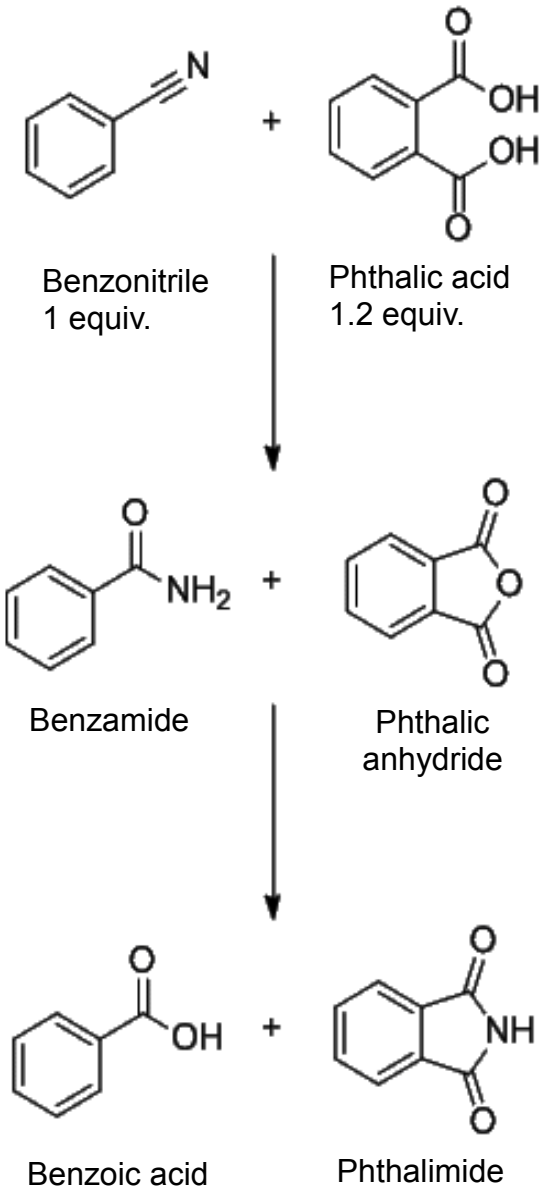
2.2.6 Reaction Selection - Ideal Derivatization Targets

It is important to study and calibrate the derivatization of all compounds in a synthetic reaction and not just mixtures of random derivable compounds because components of a synthetic reaction may react during the derivatization procedure. Since the flow setup is ultimately being developed to monitor reactions, it needs to be shown that this is not occurring. The entirety of a reaction needs to be studied all at once using the derivatization protocol as it is the only way to determine if the derivatization will be quantitative when monitoring a reaction from the automated reactor system. A reaction was found that has all compounds involved commercially available for ease of validation and calibration.

2.2.6.1 Hydrolysis of Nitriles

The preparation of carboxylic acids without solvent from their corresponding nitriles was studied as a model reaction in 1994 when microwave irradiation was first being introduced into the laboratory.[45] Several reactions were examined with a variety of compounds and aimed to study the effect that microwave irradiation had compared to normal heating methods on yield and selectivity of these reactions.

Scheme 10 – Hydrolysis of benzonitrile with phthalic acid.



This reaction uses benzonitrile and phthalic acid to form benzamide and phthalic anhydride, which subsequently react together to form phthalimide and benzoic acid (Scheme 10). The original paper uses both HPLC/MS and GC/MS to monitor the reaction with significant separation and extraction steps required in order to get two separate samples for analysis. The derivatization procedure aims to quantify the reaction with only one sample preparation step (with subsequent dilution) and only one analytical instrument.

The original reference completes reactions in batch and not flow; no solvent was used and the reaction relied on the dissolution of the phthalic acid in benzonitrile as heat was applied. To adopt this reaction to a flow format, a solvent was required in order to solubilize the solid reaction components. The ratio of reactants in the reaction remains the same as the reference but the addition of solvent decreased the reaction concentration. It was determined that solutions of 1 mol/L for all compounds except for 1.2 mol/L for phthalic acid (as per the reaction stoichiometry) were to be created and used for quantification of the derivatization procedure.

2.2.7 Validation of the Derivatization Process and its Use

The hydrolysis reaction chosen to be studied with derivatization has several advantageous qualities to it that can help validate the derivatization protocol and analytical method. The reaction has one starting material that is not affected by derivatization which acts as an internal standard to the process. As the reaction creates two products, assuming no side products or decomposition occurs, they should always be created in equal amounts (which the calibration should be able to verify). If the calibration indicates that the compounds are not created in equal amounts, it indicates that something is wrong with the process. Also, because the first two compounds that are created together are also the same compounds that react to form the final products, the calibration can be set up such that when the conversion percent of each step of the reaction are added together it should equal 100%. If values of isolated reaction samples deviate from 100%, it is an indicator that the process is not quantitative. There are also several different types of functional groups present on compounds in this reaction that will undergo derivatization, helping again to demonstrate the wide applicability of the developed protocol.

The first requirement for validating the derivatization process involved choosing a solvent to be used to adapt the synthetic reaction to flow. The application of solvent to the compounds lead to the first issue that needed to be addressed: the limited solubility of the phthalimide and phthalic acid. After several solvents were tested (e.g., EA, acetone, 1,3-Dimethyl-2-imidazolidinone (DMI), dimethylacetamide (DMA)), DMSO, often referred to as the universal solvent, was used. However, after several derivatizations of the compounds in DMSO, it was noted that DMSO as well as the target analytes were derivatized by MTBSTFA, eliminating DMSO for the use as an ideal solvent.

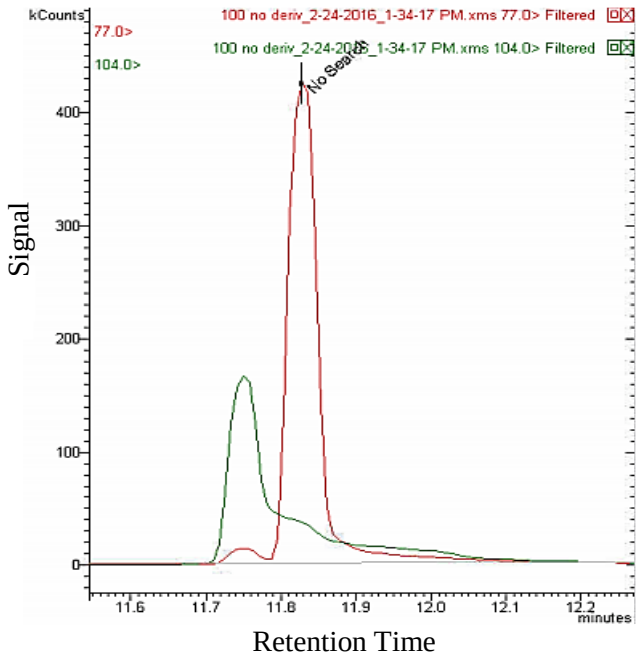
After several other solubility tests it was determined that DMF can be used to dissolve 5 of the 6 compounds with no problem. Phthalimide required a small amount of heat be applied to dissolve completely but once in solution it was stable long enough to handle. DMF is also sometimes used in MTBSTFA derivatizations and is not affected by the process.

Initially, each individual compound in the hydrolysis reaction was injected without derivatization into the GC/MS, and then was also injected after derivatization with MTBSTFA. Phthalimide, benzoic acid, benzamide reacted and benzonitrile didn't react as expected with 204, 179, 178 and 103 m/z peaks appearing in their respective spectra. However, the phthalic acid and phthalic anhydride did not inject as expected. The phthalic acid, when injected without derivatization, undergoes a dehydration reaction and turns into the phthalic anhydride in the injector. Alternatively, when the anhydride is injected into the GC without derivatization it shows up as expected, but after derivatization the anhydride is turned into the acid. This is obviously unfavourable but seems to be unavoidable in this case. However, because the acid is used in 20% excess in the hydrolysis reaction, it would not be used to monitor the progression of the reaction; this is because even at 100% yield of final product there is still 20% of acid left over. Therefore the anhydride turning into the acid during derivatization is tolerable due to the fact that the acid would not be used to monitor the % conversion of a reaction anyway. Benzonitrile, the other starting material, is used at 1 equivalent in the reaction and is more appropriate to use for monitoring purposes; furthermore, benzonitrile can be used to track the progression of the first step of the reaction. The second step of the reaction can still be monitored even though the anhydride turns into the acid through the formation of the benzamide derivative because when the anhydride is produced during a reaction, the benzamide is produced in equal quantities and derivatizes as expected. It is noted that the derivatization reaction is also beneficial

to complete in this case due to the creation of water in the injector through the acid to anhydride reaction. If this water were to be continually injected into the GC/MS it can cause irreversible damage when it enters the column.

Another clear advantage of this derivatization is that the peaks of the nonderived amide and anhydride significantly overlap, even after some GC method development to try and separate the overlapping peaks (Figure 36). If no derivatization occurs, the anhydride is unable to be quantified due to the acid changing into it during injection, and since the amide nonderived peak has significant peak tailing (Figure 36), the second step of this reaction would not be able to be accurately quantified without derivatization.

Figure 36 – Overlapping peaks in GC/MS chromatogram of benzamide (green) and phthalic anhydride (red).



Finally, one further advantage of derivatization is highlighted through a quick comparison of the chromatograms created when samples of the same concentration of non-derived and derived samples are injected. A stark increase in signal-to-noise (S/N) ratio is seen in the derived chromatogram (example: ~200,000 S/N for derived benzoic acid ~5600 for non-

derived) and peak shape is improved for all compounds as well. In the most extreme example, the peak asymmetry factor (A_s), which is a measure of how symmetrical a peak is, of the derived and non-derived amide was determined according to the equation A_s= b/a, where a is the width of the front half of the peak, and b is the width of the back half of the peak measured at 10% of the peak height from the leading (b) or trailing (a) edge of the peak to a line dropped perpendicularly from the peak apex. Most column manufacturers consider asymmetry factors of 0.9–1.2 to be acceptable. The non-derived amide had an asymmetry factor of 2.5 (b=0.125, a = 0.05) and the derived amide peak had a A_s of 1 (0.08 for both a and b). There are no unknown peaks that show up in the chromatogram when all compounds are derivatized together, which signifies no side reactions occur during the derivatization process. Calibrated injections are used to determine if the chemical reactions between the compounds of interest (the synthetic reaction being simulated) occurs during the derivatization process.

Figure 37 – Derivatized products of the hydrolysis reaction compounds

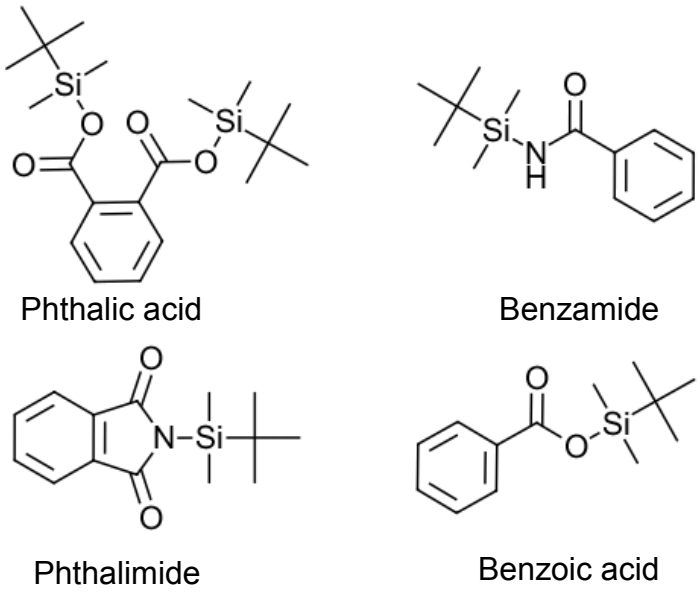
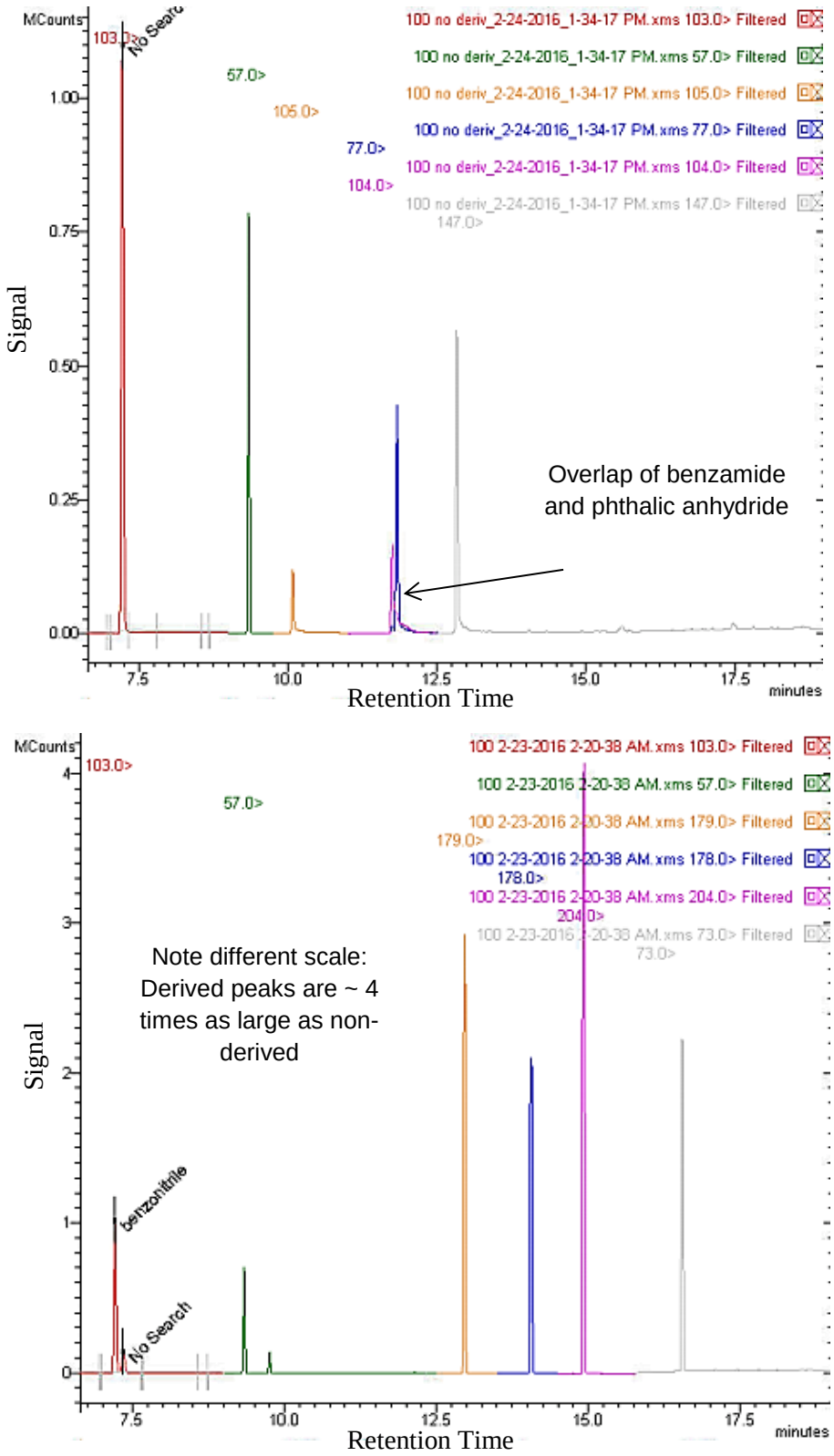


Figure 38 – Chromatogram for comparison of non- derivatized (top) and derivatized (bottom) samples of all compounds in the hydrolysis reaction.



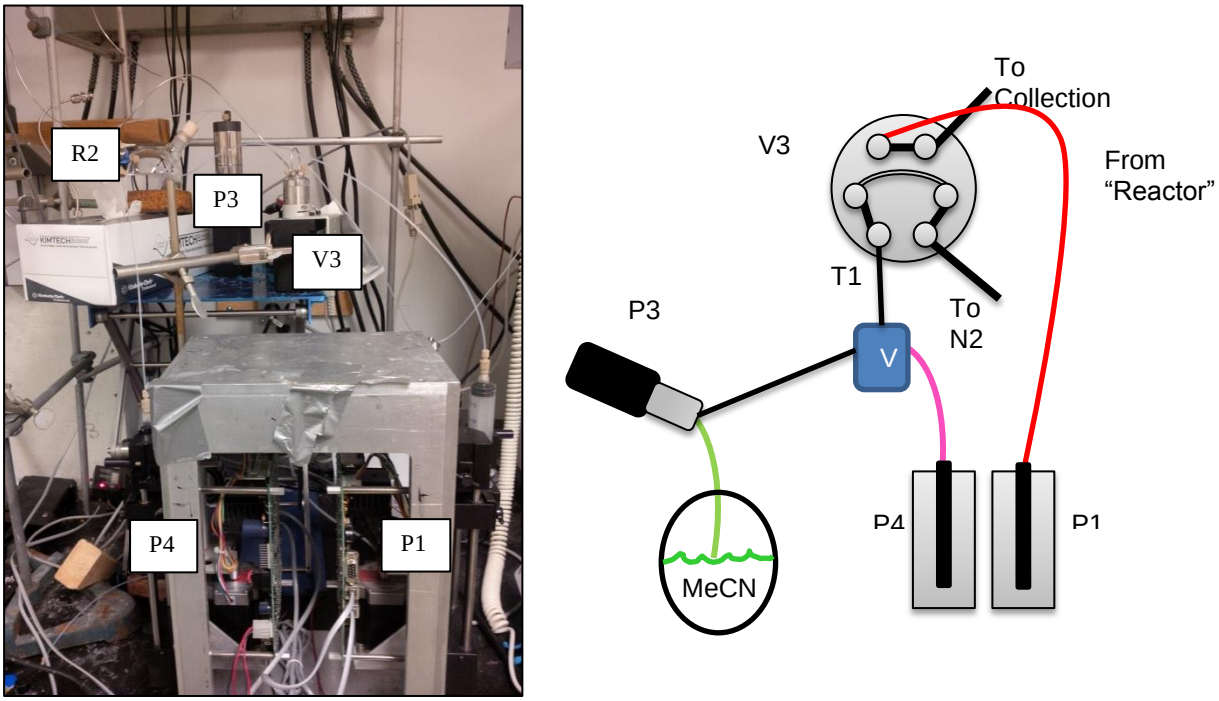
2.2.8 Calibrated Derivatization

Stock solutions of each compound involved in the hydrolysis reaction were created in DMF and combinations of these stock solutions were used to simulate samples from a real reaction.

2.2.8.1 Simplified Flow Setup

To minimize the amount of stock solution that is required to prime the lines between P1 and V3, a simplified sample delivery set up was devised that by-passes the microwave reactor since it is not being used (Figure 39). As well, only MeCN with undecane internal standard is used for both the derivatization and final dilution. This simplifies the system by eliminating ethyl acetate as the GC solvent and no longer using one hand manipulated valve.

Figure 39 - Final flow derivatization configuration used for isolating and delivering a reaction sample from V3.



The proposed design has no effect on the volume of solvent required to push a sample into a vial for analysis as the only volume/tubing that matters for this is located after V3, which

remains exactly the same, and T1, which was reused from the initial setup in Figure 33. For all experiments reported from this point on, each isolated sample is pushed into a vial with 350 μL MeCN. Since the compounds involved in the hydrolysis reaction have shown to be much easier to derivatize that the aminoalcohol first used, the 70 μL push-to-waste that was determined to be required earlier does not need to be used here. However, if the derivatization protocol is used for compounds that seem to be resistant to derivatization when a larger volume of solvent is present, then the volume of those reactions can still be reduced via the push-to-waste method.

2.2.8.2 Flow Experiments

2.2.8.2.1 Analysis of Stock Solutions

Individual stock solutions of each compound in the hydrolysis of nitrile reaction were created. The reaction concentration that is used is 1 mol/L for all compounds except for phthalic acid which is 1.2 mol/L (as per the reaction stoichiometry). The solutions were diluted 10 fold and 5 solutions for each compound, equal to 100, 80, 60, 40 and 20 % reaction conversion of each compound, were created. Calibration curves were created for benzonitrile, benzamide, phthalimide, and benzoic acid in the absence of the anhydride or acid. This prevents any MACOS reaction from propagating during derivatization due to the absence of required starting material and intermediate.

Next, the 1 and 1.2 mol/L stock solutions of all reaction compounds were combined in mock reaction ratios (e.g. 40 20 40 = 40% benzonitrile and phthalic acid, 20% benzamide and anhydride, 40% phthalimide and benzoic acid). If these solutions, when measured against the calibration curves created in absence of some of the compounds, do not measure as expected compared to the known concentration of stock solution used, it could indicate that there is some synthetic reaction occurring during the derivatization procedure (i.e. samples would analyze with

lower than expected starting material concentration as well as higher final product concentration). To measure this, the stock solutions were placed into P4 and each solution was flowed through the setup in Figure 40. Each isolated stock solution sample was subjected to the flow derivatization protocol and analyzed against a calibration curves in order to determine if the procedure is quantitative. For comparison, manual samples were also derivatized where an Eppendorf pipette instead of V3 isolation was used to take an aliquot of stock solution.

Table 12 - Comparison of calibrated flow and hand derivatization methods of mock nitrile hydrolysis reaction solutions.

Flow Derivatization					
Entry	**Solution (% conversion each step)**			**% Add Up**	**Statistic**
1	100	0	0	97.66	**Average**
2	80	20	0	98.76	99.07
3	60	40	0	101.37	**Std Dev**
4	60	20	20	99.52	1.81
5	40	20	40	101.00	
6	0	40	60	96.15	

Manual Derivatization					
Entry	**Solution (% conversion each step)**			**% Add Up**	**Statistic**
1	100	0	0	99.92	**Average 6**
2	80	20	0	102.10	101.21
3	60	40	0	103.25	**Std Dev 6**
4	60	20	20	102.93	2.44
5	40	20	40	102.73	
6	0	40	60	96.32	

An unpaired, 2 tail t-test comparing the flow and manual derivatization samples indicates a value of 0.1485 at the 95% confidence interval. This is not statistically different and therefore it can be assumed that the theoretical value of 100 is accurate (supporting the null hypothesis). Therefore, it can be argued that the smaller standard deviation and closer average value to the theoretical indicates that the flow method has matched and exceeded the reproducibility of the manual samples.

Table 13 – Calibrated results of flow derivatization protocol used to analyze mock reaction solutions.

Compound	Isolation 1 (2 injections)		Isolation 2 (2 injections)		Difference Isolations (%)
	Average (%)[a]	Std Dev	Average (%)[a]	Std Dev	
Mock Solution - 100 0 0					
Benzonitrile	97.65	0.11			
All others read 0%, omitted for space					
Mock Solution - 80 20 0					
Benzonitrile	81.39	0.31	78.97	0.3	2.42
Benzoic acid	0	0	0	0	
Benzamide	18.30	0.17	18.86	0.06	0.56
Phthalimide	0	0	0	0	
Mock Solution - 60 40 0					
Benzonitrile	62.71	0.44	62.61	0.38	0.1
Benzoic acid	0	0	0	0	
Benzamide	37.86	0.47	39.56	0.06	1.69
Phthalimide	0	0	0	0	
Mock Solution - 60 20 20					
Benzonitrile	59.57	0.58	61.22	0.16	1.65
Benzoic acid	18.40	0.17	19.85	0.01	1.44
Benzamide	20.01	0.10	19.98	0.05	0.03
Phthalimide	17.56	0.22	18.97	0.11	1.41
Mock Solution - 20 40 40					
Benzonitrile	25.90	0.16	24.04	0.03	1.85
Benzoic acid	36.27	0.17	37.35	0.00	1.08
Benzamide	38.90	0.06	39.51	0.18	0.61
Phthalimide	37.40	0.19	38.12	0.16	0.72
Mock Solution - 0 40 60					
Benzonitrile	0	0	0	0	
Benzoic acid	56.23	0.15	56.91	0.45	0.67
Benzamide	39.57	0.51	0	0	
Phthalimide	56.06	0.24	56.63	0.17	0.57

[a] –averages determined through GC/MS calibration with undecane internal standard

Referring to Table 13, one can see that there were no discrepancies between the nominal reading and what isolated samples analyzed at. This indicated that only derivatization takes place and no synthetic reaction occurs. The standard deviation between injections of the same samples is an indicator of how well the analytical method and calibration work. All standard deviations were below 1% with most being below 0.5%. This indicates that the analytical method used is reproducible and that there is no carry over in the GC/MS between subsequent injections of the

same solution. The average data for all injections shows that the solutions are analyzed very close to the theoretical value, indicating that the calibration is accurate, precise and that the results of samples analyzed against it are indeed representative of the actual sample isolated in V3. With a 1% overall average difference between subsequent V3 isolations of the same mock solution (Table 13, average of final column), the flow derivatization protocol in which each sample isolated in V3 is pushed only by 350 μL MeCN into a vial is verified to be reproducible. These results are in accordance with the findings earlier with EPP and TBB that showed 350 μL is capable of pushing the entire sample. In further verification of the derivatization process, to ensure that there is no sample loss during heating, a sample of the 100% stock solution of benzonitrile (the compound not affected by derivatization) was analyzed against the derivatized calibration curve without undergoing the derivatization (heating) procedure. If sample is lost during the heating procedure, a 100% solution that has not been heated would read much higher than 100% on the calibration. After several injections, the 100% benzonitrile unheated sample injections all read 100% (+/- 2%) on the derived benzonitrile calibration. This indicates that there is no difference between the "derived" and nonderived sample of benzonitrile and that there is no sample loss during the sample heating process.

2.2.8.2.2 Analysis of Reaction Solutions

After it had been decided that the flow setup created is in fact capable of producing quantitative sampling and monitoring of a mock reaction solution, it was decided that real reaction solutions should be analysed with the system. Therefore, a solution of pthalic acid and benzonitrile were made up at 1.2 and 1 mol/L in DMF respectively. This solution was split up into several different microwave vials to undergo batch microwave reactions. The idea is to run reactions at various temperatures and times that would simulate what the automated reaction

system would do to try and optimize a reaction's conditions. I.e. start a reaction at low temperature with short reaction time and increase temperature and time as necessary. These batch solutions were then to be flowed through the simplified flow set up used for the stock solution analysis. Unfortunately, there was one unexpected set back: the reaction does not run with solvent. A large peak at 250 m/z (among other smaller unknown peaks) starts to appear once the solution is heated above 200^{o}C. This peak is of unknown exact structure, only shows up in the chromatogram after derivatization, and was determined to be formed when the phthalic acid alone is heated in DMF. Unfortunately this means that studying this reaction run in DMF is not possible. However, because this reaction runs well without solvent, the reaction was run neat and then the entire reaction was diluted in a volumetric flask to create solutions of real reactions at known concentrations. These reaction solutions were then flowed through the system as if they were synthetic reaction samples that had been run in DMF. Two samples of each reaction were isolated and derivatized.

Table 14 – Comparison of benzoic acid and phthalic anhydride percent conversion results from analysis of real reaction samples using flow derivatization.

Flow Reaction Monitoring						
Reaction / Compound	Trial 1 Average Reading (%)[a]					
	160°C 20 min[b]	200°C 20 min	240°C 20 min	250°C 20 min	250°C 40 min	250°C 60 min
Benzoic acid	n/a	4.96	17.19	23.34	37.20	59.22
Phthalimide		4.47	17.23	23.93	37.70	59.06
Difference		0.49	-0.04	-0.59	-0.50	0.16
% Difference		10.36	0.22	2.48	1.34	0.28

Reaction / Compound	Trial 2 Average Reading (%)[a]					
	160°C 20 min[b]	200°C 20 min	240°C 20 min	250°C 20 min	250°C 40 min	250°C 60 min
Benzoic acid	n/a	5.14	17.00	23.31	37.02	55.99
Phthalimide		4.80	17.01	23.45	36.69	55.85
Difference		0.34	0.01	-0.15	0.33	0.14
% Difference		6.76	0.01	0.62	0.90	0.25

Reaction / Trial	Average of Benzoic Acid and Phthalimide in Both Trials (%)[a]					
	160°C 20 min[b]	200°C 20 min	240°C 20 min	250°C 20 min	250°C 40 min	250°C 60 min
Trial 1	n/a	4.71	17.21	23.63	37.45	59.14
Trial 2		4.97	17.00	23.38	36.86	55.92
Difference		-0.26	0.21	0.25	0.59	3.22
% Difference		5.37	1.21	1.08	1.58	5.59

[a] –averages of 4 injections determined through GC/MS calibration with undecane internal standard

[b] – No phthalimide or benzoic acid present in reaction

To monitor how well the derivatization protocol works for real reaction solutions, phthalimide and benzoic acid were analyzed and compared as they should be produced in identical quantities in the reaction and therefore analysis of them should also be identical. Table 14 shows the values for both benzoic acid and phthalimide as an average of 2 different isolated samples of the same reaction solution with each sample injected twice into the GC (4 calibrated injections per benzoic acid and phthalimide average in Table 14, 8 values for average of both in

each trial). Within the same injection, the difference between the phthalimide and benzoic acid was never larger than 0.34% in any reaction. Unpaired, 2 tail t-tests were completed on all samples comparing benzoic acid and phthalimide. The 200^{o}C at 20 minute t-test does show a statistical anomaly (most likely due to error in analyzing low percentages using the GC/MS calibration that starts at 20%), but all unpaired t-tests performed on the other reactions showed no statistical difference between the benzoic acid and phthalimide analyzed. As one example, the comparison of benzoic acid and phthalimide in trial 2, 220^{o}C reaction for 20 minutes (Table 14) gives a unpaired, 2 tail t-test value of 0.998 at the 95% confidence interval, which is considered to be not statistically significant.

Table 15 – Combined conversion percentages of all components in the real hydrolysis reaction solutions analyzed using flow derivatization

	Trial 1		**Trial 2**	
Reaction	**% All[a]**		**% All[a]**	
160^{o}C, 20 minutes	103.72		95.47	
200^{o}C, 20 minutes	105.92		102.44	
240^{o}C, 20 minutes	97.62		94.27	
250^{o}C, 20 minutes	101.70		96.42	
250^{o}C, 40 minutes	102.46		101.92	
250^{o}C, 60 minutes	102.06		103.57	
	Average	**Std Dev**	**Average**	**Std Dev**
	102.25	2.50	99.01	3.71
		Average		
		100.63		

[a] – Average sum from every injection of each reaction analyzed on GC/MS calibration with undecane internal standard – Theoretical is 100%

As one final comparison, the average percentage conversion of all the compounds in the real reaction solutions (only counting the average of phthalimide and benzoic acid) for all reactions were added up (with a theoretical value being 100%). The overall average of all reactions equalled 100.63%, a remarkably accurate presentation of the derivatization process. An unpaired, 2 tail t-test was performed in ordered to determine if the two trials gave statistically

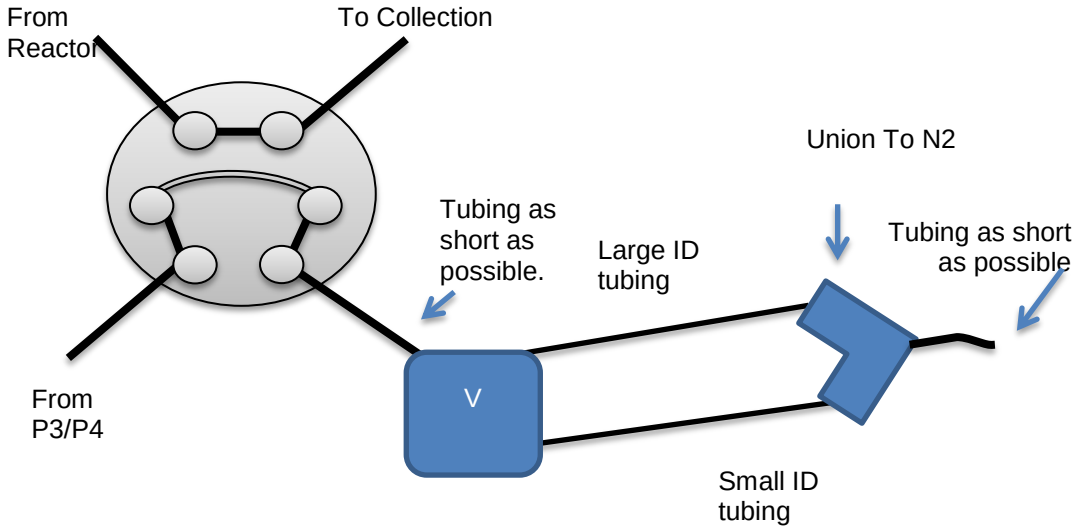
different analyses. Here, comparing trial 1 and trial 2, the t-test results were 0.137 at the 95% confidence interval. This value is not statistically significant and shows that the method is highly reproducible.

These results highlight the ability of the flow derivatization procedure to produce accurate and precise monitoring of a reaction. Theoretically, this process can be used with the automated reaction system to monitor the progression of a reaction that has many different functional groups. Alternatively, there are several other applications in which the flow derivatization protocol can be utilized.

2.3 Possible Applications

In the future to speed up the overall sampling time and eliminate the stress placed on P3 when pumping at top pressure, two sample delivery lines can be used. Smaller ID tubing can be used to deliver the derivatization reagent at a slower flow rate as only a small volume needs to be pushed, whereas larger ID tubing can be used for the final dilution volume to accommodate the large 8 mL dilution that needs to be pushed quickly without large pressure build up (Figure 40).

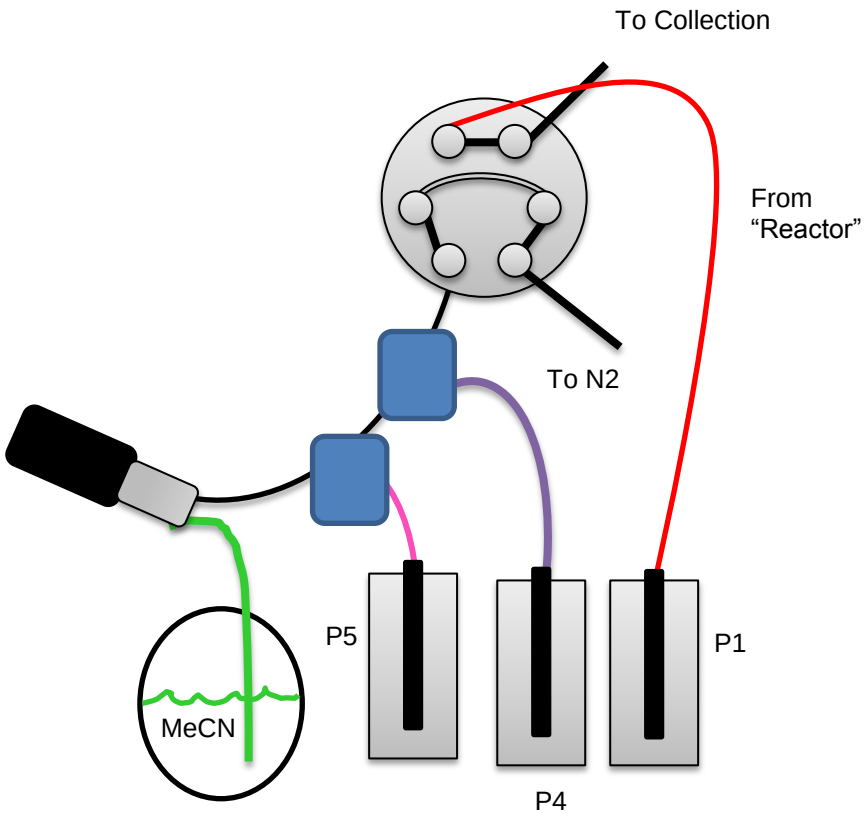
Figure 40 - Theoretical design for a derivatization setup where sample delivery rates can be increased to decrease sample preparation time.



Even though the 3-port 2-position valve that is used in the derivatization setup is turned manually making the current set up not completely automated, that valve can very easily be exchanged with an automated valve. Using the set up in which the microwave was bypassed, the automated derivatization procedure can be used to monitor compounds in other matrices besides from a reaction. Environmental samples, for example, that require testing for small molecules, illegal drugs, various organic compounds and other derivable analytes can use this method of sample preparation to eliminate human interaction with the samples. Since it was shown the procedure is highly reproducible, the technique can be used to sample, derivatize, inject and analyze as many samples as required. This can also be applied to biological samples that required derivatization before analysis. Essentially, any sample that requires qualitative or quantitative derivatization and is capable of being flowed can be reproducibly derivatized through this methodology. Furthermore, it is quite possible that other derivatization reagents can be used instead of MTBSTFA in case a set of mixtures is extremely resistant to MTBSTFA derivatization.

To further the application of this procedure to include the ability to study chemical reactions that have catalysts or additives that need to be removed before derivatization can occur, extractions can be completed using different solvents and acid/base washes can be implemented in order to help eliminate compounds that would interfere with the derivatization. This can be completed through low means using an identical pump as P4 and another valve. This would allow for flow of different solutions in the same stream (Figure 41).

Figure 41 – Theoretical apparatus used to help quench and/or extract reaction samples.



2.4 Conclusions

Flow derivatization has been demonstrated to be a viable option to add as a sample preparation step for quantitative monitoring of synthetic reactions conducted in flow. The technique has been used to quantitatively monitor a 2 step microwave reaction with remarkable accuracy and precision. The flow technique matched or exceeded the hand derivatization technique when compared side by side and showed that the flow sampling technique developed is highly reproducible and robust. Whereas literature references indicate that the synthetic reaction should be analyzed using both HPLC and GC/MS techniques to monitor the reaction progress, in this approach the introduction of a single derivatization technique allows for only one analytical instrument to be used with minimal sample preparation for full quantification of the reaction.

The replacement of the hand operated small valves with automated analogues would allow this protocol to be used for various other matrices requiring derivatization. This type of system can produce highly accurate samples and can eliminate the human error present in sampling methods completed by hand.

2.5 Experimental

All chemicals were purchased from Sigma-Aldrich Chemical Company unless otherwise stated and were used without further purifications. All chromatographic separations were performed using a Bruker 450 GC outfitted with a VF-5ms 30 x 0.25 mm x 0.2 μm column. Detection was performed on a Bruker 300 triple quadrupole electron ionization mass spectrometer (EI) with single ion monitoring (SIM) and full scan methodologies employed. All NMR experiments were completed with a Bruker Topspin instrument of either 300 or 400 MHz strength.

2.5.1 Epoxide Ring Opening Optimization

Each reaction was completed in a biotage batch microwave in a 2-5 mL microwave vial with magnetic stirring and crimp top metal lid with septa.

1,2-epoxyphenoxypropane (EPP) (134 μL, 1 mmol) and N-benzylmethylamine (BMA) (192 μL, 1.5mmol) were combined in 2.5 mL solvent to form 1-[Benzyl(methyl)amino]-3-phenoxypropan-2-ol (BMAPP). Ethyl acetate, tetrahydrofuran, dimethylethoxide or methanol were used as solvents and initial reactions were carried out for 4 minutes at 140^{o}C.

In the second experiments, BMA (479 μL, 3.75 mmol) and EPP (338 μL, 2.5 mmol) were dissolved in 2.5 mL ethyl acetate. These reactions were run at 200^{o}C for 4 minutes.

2.5.2 Epoxide Ring Opening Analysis

Each epoxide ring opening reaction was analyzed in a 400 MHz NMR. Deuterated chloroform was used as a solvent and quantification was completed by integrating peaks representing the shift of the proton signal of the CH_2 epoxide ring protons at 2.8 ppm which shift downfield after the epoxide is broken to 2.6 ppm in the final product.

2.5.3 Isolation of 1-[Benzyl(methyl)amino]-3-phenoxypropan-2-ol (BMAPP)

1-[Benzyl(methyl)amino]-3-phenoxypropan-2-ol (BMAPP) is easily isolated from the other reaction compounds through column chromatography. After rotoevaporation of reaction solvent, the yellow transparent oil that remains is dissolved in a minimal amount of a mixture of 2:1 ethyl acetate:hexane. This mixture is loaded onto a column prepared with the same EA:hexane mixture. The epoxide (EPP) comes off the column quickly with this solvent system and then after all EPP is eluted, the solvent is switched to 3:1 EA:hexane to help elute BMAPP. The amine sticks to the silica gel and does not elute.

After collection of all fractions containing the product, the samples are evaporated and analyzed via ^{1}H NMR in deuterated chloroform as solvent. Comparison of the NMR of the final product obtained to the literature values shows that the product formed is indeed what is expected**.**

2.5.4 Batch Derivatization Optimization

All reactions heated were done with the on-board agitator/heater on the autosampler. Each reaction was completed with 5 minutes of agitation then the vial remained still for the rest of the reaction time.

2.5.4.1 BMAPP Derivatization

A 6.85 μL sample of a 1 mol/L (2.72 g, 10 mmol, in 10 mL solvent) of 1-[Benzyl(methyl)amino]-3-phenoxypropan-2-ol (BMAPP) was subjected to various derivatization conditions in a 10 mL glass GC vial with metal screw lid with septa. Experiments were undertaken at RT, 50, 60, 70, 90, and 100 ^{o}C with ethyl acetate, tetrahydrofuran, and acetonitrile at various volumes. Derivatization reagent MTBSTFA was used in either 50 or 100 μL volumes and reactions were run for 5, 10, 20 or 30 minutes.

2.5.4.1.1 GC/MS Method

Pre-dried helium gas was used as the carrier gas at 36 cm/s of linear velocity to produce a chromatographic flow-rate 1.0 mL/min. GC injections were made using a 1:50 split protocol, changing to 1:10 after 2 minutes, at an injector temperature of 265 °C. The GC oven temperature was initially set at 50 °C and held for 1 minute then the temperature was increased by 30°C/min until 280 °C was reached. 280^{o}C was held for 3 minutes until method end. The transfer line and the EI source temperatures were set at 260 °C and 280 °C, respectively. The MS was

programmed to monitor a full scan channel between 50-400 m/z, using a ionization potential of 70 eV.

Retention time of the final product is 9 minutes with a base peak of 91 m/z. The derived product elutes at 9.8 minutes and has a base peak of 134 m/z. For rough quantification purposes, the heights of the derived and underived peaks were compared to determine the % conversion of the reaction.

2.5.4.2 BMAPP and BMA Derivatization

In these experiments, solutions of BMAPP (2.72 g, 10 mmol) and BMA (768 μL, 15 mmol) in 10 mL ethyl acetate were created. 6.85 μL samples of each solution were added to the same vial and derivatization procedures were undertaken. Samples were subjected to a reaction temperature of 100^{o}C at 10, 20 and 30 minute reaction time. Various acetonitrile volumes were used to determine the ideal volume for derivatization.

2.5.4.2.1 GC/MS Method

The GC/MS method that was used for the derivatization done only on BMAPP was continued to be used with this set of experiments. As well as the peaks for BMAPP and it's derivative, the starting material amine peak shows up at 4.7 minutes with base peak 147 m/z and the derived amine peak shows up at 6.5 minutes with base peak of 179 m/z.

2.5.5 Batch Derivatization Protocol Applicability Tests

2.5.5.1 Derivatization Compound Screen

Several compounds were subjected to the optimized batch derivatization conditions to determine the applicability of the protocol to other various functionalities. All reactions were completed with an approximately 5 mg sample of each compound, 200 μL acetonitrile and 50 μL

of MTBSTFA in a 10 mL glass GC vial with metal screw top lid and septa. Reactions conditions were 100^{o}C for 10 or 30 minutes.

2.5.5.1.1 GC/MS Method

Pre-dried helium gas was used as the carrier gas at 36 cm/s of linear velocity to produce a chromatographic flow-rate 1.0 mL/min. GC injections were made using a 1:10 split protocol at an injector temperature of 265 °C. The GC oven temperature was initially set at 50 °C and was increased by 30°C/min until 280 °C was reached. The transfer line and the EI source temperatures were set at 260 °C and 280 °C, respectively. The MS was programmed to monitor only one channel between 50-400 m/z, using a ionization potential of 70 eV.

4-Bromo-2,6-xylenol – compound elutes at 8 minutes, base peak 200/202 m/z (due to presence of bromine), derived compound at 10.2 minutes, base peak at 257/259 m/z.

Cyclohex-3-ene-1,1-diyldimethanol – compound elutes at 11.25 minutes with base peak 66 m/z. No derived peak detected.

Glutaric Acid – compound does not elute or elutes too soon from column without derivatization. Derived compound elutes at 10.1 minutes with base peak 73 m/z.

1-methyl-1cyclohexanecarboxylic acid – compound elutes at 6.5 minutes with base peak 97 m/z. Derived compound elutes at 7.9 minutes with base peak 199 m/z.

(+/-)-2-methyl-1butanol – compound elutes at 2.5 minutes with base peak 57 m/z. derived compound elutes at 5.1 minutes with base peak 75 m/z.

Methyl-3-hydroxybenzoate – compound elutes at 8 minutes with base peak 121 m/z. Derived compound elutes at 9.75 minutes with base peak 209 m/z.

(+)Methyl L-β-hydroxyisobutyrate – compound elutes at 3.75 minutes with base peak 88 m/z. Derived compound elutes at 6.6 minutes with base peak 175 m/z.

Acetanilide – compound elutes at 7.5 minutes with base peak 135 m/z. Derived compound elutes at 8.3 minutes with 192 m/z base peak.

4,4′-Dimethoxybenzoin – compound elutes at 8.3 minutes with base peak 136 m/z. Derived compound elutes at 9.2 minutes with base peak 193 m/z.

2.5.6 Design of Flow Derivatization Setup

2.5.6.1 Determining Flush Volumes for Reproducible Sampling

The experiments to determine the flush amount of solvent required for producing reproducible sample volumes were completed using the flow setup seen in Figure 33. Experiments involved solutions of 2-allyl phenol (0.1 mol/L, 130 μL in 10 mL DMSO) acting as MTBSTFA with tertbutylbenzene (TBB, 0.1 mol/L, 157 μL in 10 mL) and 1,2-epoxyphenoxypropane (EPP, 0.1 mol/L, 136 μL in 10 mL) in ethyl acetate being used as analytes isolated in V3.

All experiments were completed after the following priming sequence was done:

1) Using P3, the transfer tubing was primed with the GC injection/dilution solvent EA, also ensuring full prime of the V3 loop.
2) Using P3, the derivatization solvent lines were primed with MeCN
3) Using P4, the derivatization reagent lines were primed with 2AP solution in DMSO.
4) Using P1/P2, the process lines were primed with the TBB/EPP solution (happens simultaneously during steps 1-3)

Once priming was complete, P1/P2 were used to continuously deliver the TBB/EPP solution through the process lines. A TBB/EPP sample was isolated in V3 while P4 pumped 60 μL of MTBSTFA through the derivatization reagent lines and V3 and into the transfer tubing. Once the TBB/EPP solution was finished being isolated, P3 pushed MeCN through the lines into V3 loop and through to the transfer tubing, bringing the sample into a clean vial. Various volumes of MeCN were flushed through the lines and the eluent from the end of the transfer tubing was collected in 10 μL increments from 10 to 150 μL. Samples were then diluted to 8 mL with EA. Qualitative information was obtained by the appearance of either 2AP or TBB/EPP in the chromatogram.

For quantitative analysis, calibration curves were used. In these experiments, the same priming sequence was used and how much solvent is required to reproducibly push the entire TBB/EPP V3 isolated volume into the vial was determined. Various volumes of MeCN were flushed through the lines after a sample was isolated in V3. Then, the eluent from the end of the transfer tubing was collected in 50 μL increments from 100 to 500 μL. Samples were then diluted to 8 mL with EA.

2.5.6.2 GC/MS Method

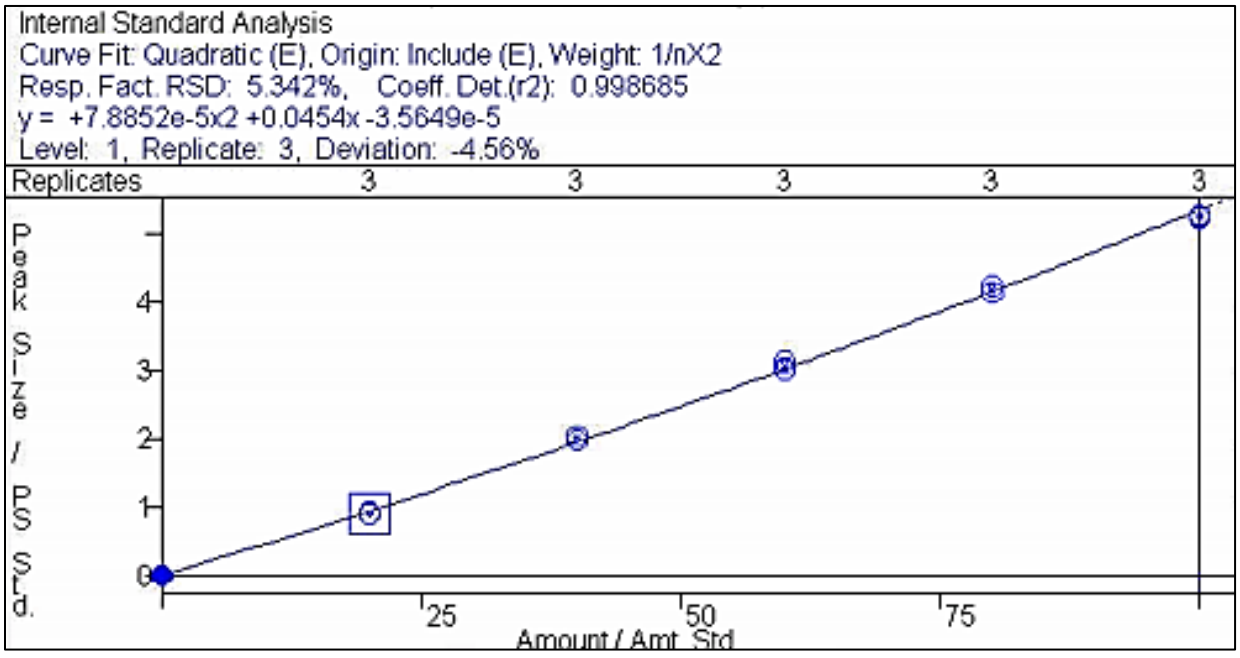
Pre-dried helium gas was used as the carrier gas at 36 cm/s of linear velocity to produce a chromatographic flow-rate 1.0 mL/min. GC injections were made using a 1:50 split protocol, changing to 1:10 after 2 minutes, at an injector temperature of 265 °C. The GC oven temperature was initially set at 50 °C and held for 1 minute then the temperature was increased by 30°C/min until 220 °C was reached. The transfer line and the EI source temperatures were set at 260 °C and 280 °C, respectively. The MS was programmed to monitor between 50-300 m/z and SIM

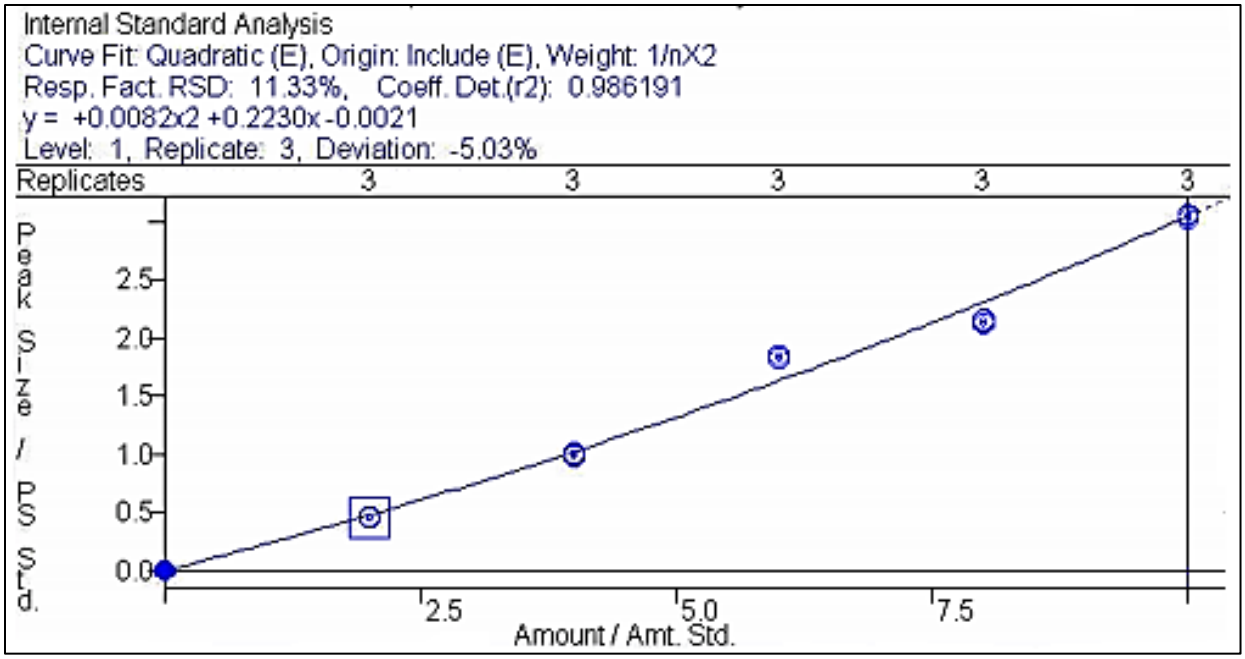
monitoring of 119 m/z for TBB (3.95 minutes), 134 m/z for 2AP (5 minutes), 57 m/z for undecane (4.5 minutes) and 94 m/z for EPP (5.6 minutes), using a ionization potential of 70 eV.

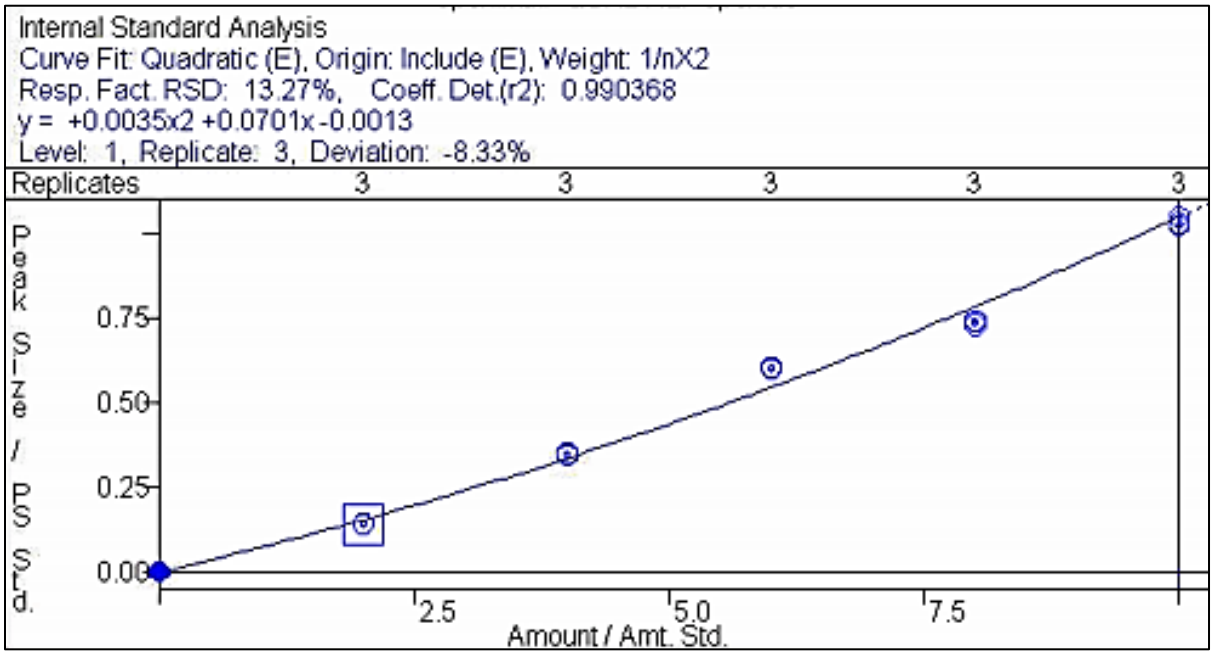
2.5.6.3 Calibration

The solution that was flowed through the system with TBB and EPP was also used to create the calibration solutions. 2,4,6,8 and 10 μL aliquots were taken of the 0.1 mol/L solution (see *Determining Flush Volumes for Reproducible Sampling*) of both TBB and EPP. The 0.1 mol/L solution of 2AP in DMSO had aliquots of 20, 40, 60, 80 and 100 μL added to the vials with the 2,4,6,8 and 10 μL aliquots respectively. A volume of 1.81 μL (0.008 mmol) of undecane internal standard was added via Eppendorf pipette to 200 mL ethyl acetate to form a solution of 0.04 mmol/L. Each vial with 2AP, TBB and EPP was then diluted with this solution to make up 8 mL.

Figure 42 - Calibration curve used for quantification of 2AP in DMSO (top), TBB (middle) and EPP (bottom) in flush volume determination experiments.







2.5.7 Process Validation

2.5.7.1 Calibrated Monitoring – Flow vs Hand Derivatizations

Plastic syringes were used to house the mock reaction solutions and then these syringes were connected to the flow set up and placed in P1. P1 was programmed to flow 500 μL of each mock solution to prime the lines before a sample is taken. Then, V3 was instructed to switch

positions to start isolating a mock reaction sample. Simultaneously, MTBSTFA was pumped via P4 through V3 into the transfer tubing. Once a mock reaction sample was isolated, V3 switches back to its original position, and P3 is used to pump 350 μL of MeCN through the V3 loop and into a clean vial. Samples were then derivatized for 10 minutes at 100^{o}C.

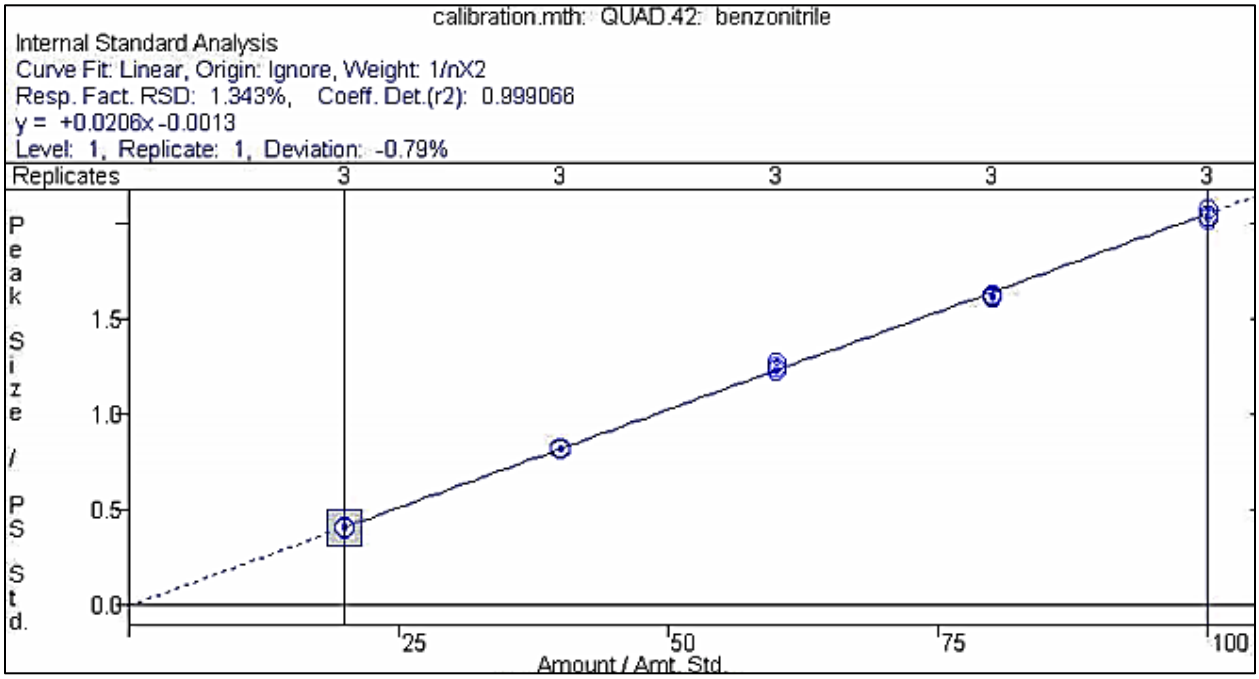
An Eppendorf pipette was used to take a hand sample of these mock solutions and these samples were diluted in the same manner as the samples above to create hand derivatization samples for comparison.

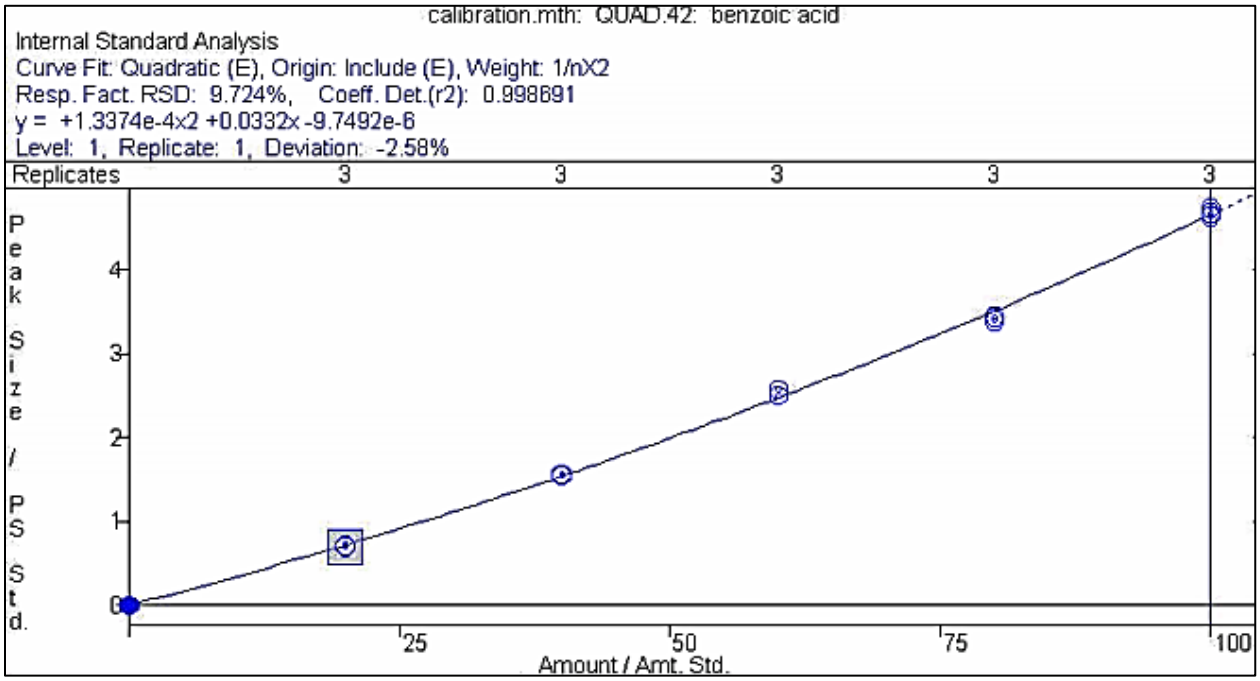
2.5.7.2 Calibration and Mock Reaction Solutions

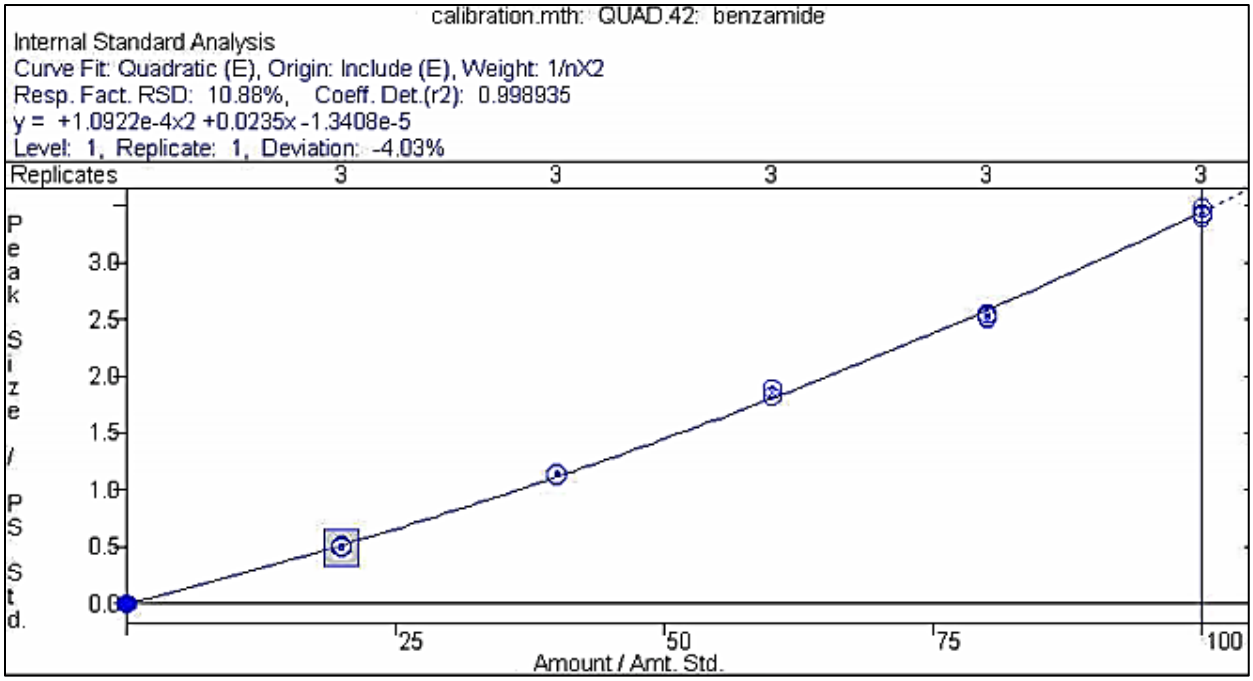
25 mL stock solutions in DMF of 1.0 mol/L of benzoic acid (0.03 mol, 3.66 g), phthalimide (0.03 mol, 4.41 g), benzamide (0.03 mol, 3.63 g) benzonitrile (0.03 mol, 3.09 g) and phthalic anhydride (0.03 mol, 4.44g) were made and a solution of 1.2 mol/L phthalic acid (0.036 mol, 5.9 g) was also created. To make the highest calibration solution (representative of the highest possible concentration of all compounds in the reaction), 100 μL of each solution was added into a vial. Then, the vial was diluted to 1 mL with DMF. To make the 80% calibration solution, 80 μL of each stock solution was added to a vial which was then diluted with DMF to 1 mL. The 60, 40 and 20 % calibration solutions had 60, 40 and 20 μL respectively of each solution added and then each was diluted to 1 mL. 6.85 μL samples of each calibration solution was added to a 10 mL GC glass vial with metal screw top with septum. Then the flow set up was used to pump 60 μL MTBSTFA and 350 μL MeCN into each vial. MeCN was added with undecane internal standard (10 mL of stock solution in 100 mL MeCN, stock solution: 1.51 μL undecane in 150 mL MeCN) already in it. Each vial then underwent a 10 minute reaction at

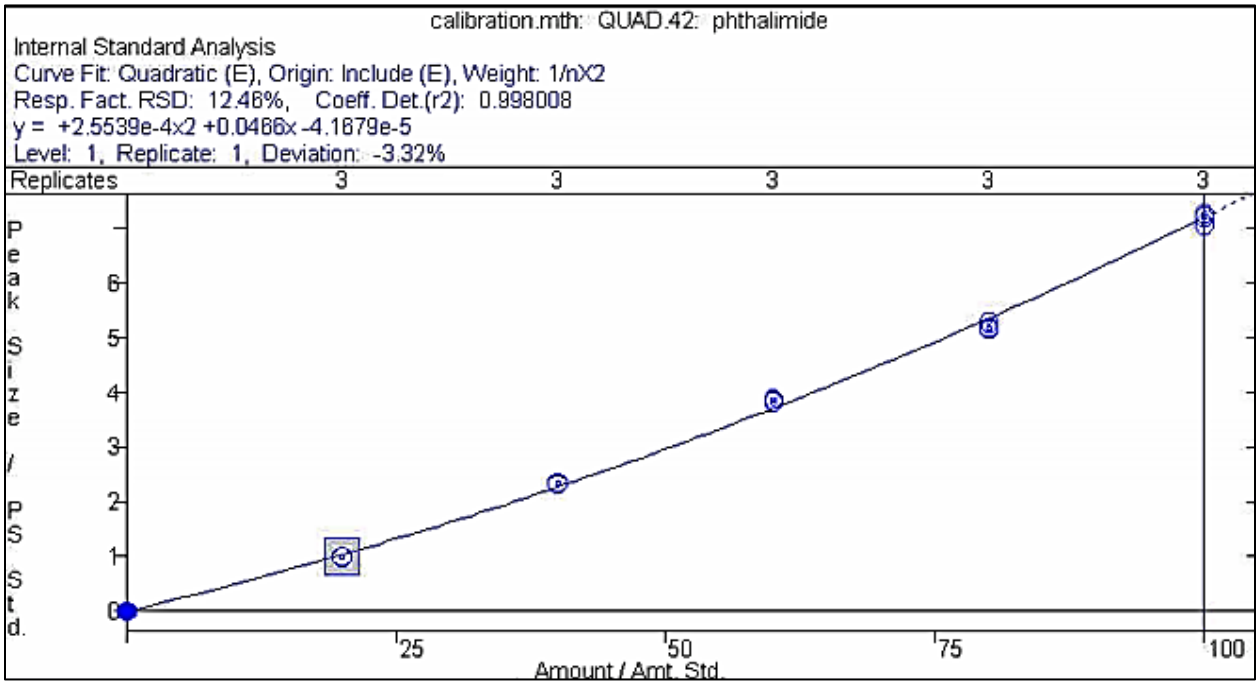
100°C. Afterwards, the flow setup was then used to dilute each sample to 8 mL with the MeCN/internal standard solution.

Figure 43 – Calibration curves used for monitoring hydrolysis of nitrile reactions









Mock reaction solutions were created using the 25 mL stock solution of each compound. For the reaction “40 20 40” 40 μL of benzonitrile and phthalic acid, 20 μL of phthalic anhydride and benzamide and then 40 μL of phthalimide and benzoic acid were all added into one vial. Then

that vial was diluted to 1 mL (or multiply all volumes added by 3 if the solution was being diluted to 3 mL instead of 1).

2.5.7.3 GC/MS Method

Pre-dried helium gas was used as the carrier gas at 38 cm/s of linear velocity to produce a chromatographic flow-rate 0.9 mL/min. GC injections were made using a 1:50 split protocol, changing to 1:10 after 2 minutes, at an injector temperature of 265 °C. The GC oven temperature was initially set at 50 °C and held for 1 minute then the temperature was increased by 20°C/min until 80°C was reached. 80°C is held for 3 minutes then 20^{o}C increase until 280^{o}C is reached. 280oC is held for 2 minutes until method end. The transfer line and the EI source temperatures were set at 260°C and 280 °C, respectively. The MS was programmed to monitor between 50-300 m/z and SIM monitoring .

Benzonitrile appears at 7.15 minutes with base peak 103 m/z/.

Derived Benzoic acid appears at 13.1 minutes with base peak 179 m/z.

Derived Benzamide appears at 14.2 minutes with a base peak of 178 m/z.

Derived Phthalimide appears at 14.9 minutes with a base peak of 204 m/z.

Derived Phthalic Acid/Phthalic anhydride appears at 16.6 minutes with a base peak of 73 m/z.

2.5.8 Calibrated Analysis of Real Reaction Solutions

Added to each of 6 clean 0.5-2 mL pointed tip microwave vials was 1.03 g of benzonitrile (1 mmol) and 1.97 g phthalic acid (1.2 mmol) . 4 vials were reacted for 20 minutes, one at each of 160, 200, 240 and 250^{o}C. 1 vial was reacted at 250^{o}C for 40 minutes and another at 250^{o}C for 60 minutes. Once complete, all reaction materials were transferred with DMF into a 5 mL volumetric flask. Then, 300 μL of solution was taken and diluted into 3 mL DMF. These solutions were then each individually flowed through the flow derivatization set up in order to

undergo flow derivatization. Each sample was isolated twice in V3 and injected twice in succession into the GC/MS.

2.5.8.1 Calibration

The same methodology for creating calibration solutions that was employed for the mock reactions was also used to make the solutions for this calibration.

2.5.8.2 GC/MS Method

The same method file that was used for the analysis of the mock reaction solutions was also used to monitor the real reaction solutions.

3 References

[1] Kristina Somerville, Michael Tilley, GuanLong Li, Debasis Mallik, Michael G. Organ. *Org. Process Res. Dev.* **2014**, 18, 1315−1320.

[2] Baber Razwan, Luca Mazzei, T. K. Thanh Nguyen, Gavriilidis Asterios. *RSC Adv.*, **2015**, 5, 95585–95591.

[3]Francesca Sapuppo, Andreu Llobera, Florinda Schembri1, Marcos Intaglietta, Victor J. Cadarso, Maide Bucolo1. *Biomicrofluidics***. 2010,** 4, 024108.

[4] N. S,Wilson, C. R. Sarko, G. P. Roth. *Org. Process Res. Dev*. **2004**, 8, 535-538.

[5] Jens Wegner, Sascha Ceylan, Andreas Kirschninga. *Adv. Synth. Catal*. **2012**, 354, 17 – 57.

[6] Cara E. Brocklehurst, Hansjorg Lehmann, Luigi La Vecchia. *Org. Process Res. Dev*. **2011**, *15*, 1447–1453

[7]Farman Ullah, Thiwanka Samarakoon, Alan Rolfe, , Ryan D. Kurtz, Paul R. Hanson,; Michael G.Organ. *Chemistry – A European journal,* **2010,** 16, 36, 10959-10962.

[8] A) Michael G. Organ, Paul R. Hanson, Alan Rolfe, Thiwanka B. Samarakoon, Farman Ullah. *J. Flow Chem.* **2011**, 1, 32–39. B) Teresa Cablewski, Alan F. Faux, and Christopher R. Strauss. J. Org. Chem. **1994**, 59, 3408-3412.

[9] For a thorough review of microwave reactions: Pelle Lidstrom, Jason Tierny, Bernard Wathey, Jacob Westman. *Tetrahedron,* 57, **2001,** 9225-9283.

[10] Mark Zell, Brian Marquez, Dave am Ende, Pascal Dube, Eric Gorman, Robert Krull Don Piroli, Kimberly Colson, Michael Fey. *Pharmaceutical Technology. Analytical Technology and Testing.* **2011,** s19-s21.

[11] Lucio Frydman. *C. R. Chimie.,* **2006,** 336–345.

[12] M. S Tswett. *Proceedings of the Warsaw Society of Naturalists, Biology Section*. **1905,** 14, 6, 20–39. (original in Polish)

[13] Danijela R. Kocic, Andrew Shalliker. *Jour, Mass. Spec.* **2015**, 50, 1396-1399.

[14]David A. Foley, Jian Wang, Brent Maranzano, Mark T. Zell, Brian L. Marquez, Yanqiao Xiang, and George L. Reid. *Anal. Chem.* **2013**, 85, 8928−8932

[15] William W Christie. *Lipids* **1998,** 33, 4, 343-353.

[16] DA Armbruster, MD Tillman, L Hubbs. *M.Clin Chem.* **1994,** 1233-8.

[17] Mo Yang, Tae-Young Kim, Hyun-Chul Hwang, Seok-Kyung Yi, Do-Hoon Kim. *Journal of the American Society for Mass Spectrometry*. **2008**, 19, 1442–1448.

[18] A. N. Verenchikov , A. P. Kolosov. *J. Anal. Chem.* **2015**, 70, *13*, 1527-1532.

[19] John W. Dolan, Lloyd R. Snyder. "Troubleshooting LC Systems: A Comprehensive Approach to Troubleshooting LC Equipment and Separations". *Springer Science and Business Media*, Walnut Creek, CA. **1989**, page 243.

[20]Syed Salman Lateef. Vinayak. `Injecting variable volumes using the partial loop-fill method with the Agilent 1260 Infinity Analytical SFC System` Technical Overview. Agilent Technologies, Inc., 2013 Published in the USA, January 1, 2013 5991-0514EN

[21] A) Ralph Zwagerman, Pierre Overman. *Eur. J. Lipid. Sci. Technol.* **2015,** 117, 0000-0000. B) Jingyi Lee and Hian Kee Lee *Anal. Chem*. **2011**, 83, 6856–6861

[22] H.Kobayashi, B. Driessen, D.J. van Osch, A. Talla, S. Ookawara, T. Noel, V. Hessel. *Tetrahedron* **2013**, 69, 2885−2890.

[23] Considerations for Selecting GC/MS or LC/MS for metabolomics. Agilent Technologies, Inc. **2007.** 5989-6328EN

[24] United Nations Office on Drugs and Crime. *Guidelines for the Forensic analysis of drugs facilitating sexual assault and other criminal acts.* English, Publishing and Library Section, United Nations Office at Vienna (accessed online) 2011.

[25] Y, Liu, S, Wang, C.A. McDonagh, M, Khairy, DC Muir, P.A.Helm , Lohmann R. *Environmental Science and Technology*. Article ASAP. DOI:10.1021/acs.est.5b04586 Publication Date (Web): December 07, 2015

[26] Thomas J. Wenzel, James D. Wilcox. *Chirality*. **2003** *15*, 3, 256–270.

[27] Mireia Oromi-Farrus, Merce Torres, Ramon Canela. *J .Anal Meth Chem.* **2012,** *2012*, 1-10.

[28] Neil D. Danielson. Patricia A. Gallagher, James J. Bao. *Encyclopedia of Anal Chem.* John Wiley & Sons Ltd, Chichester, **2000**. R.A. Meyers (Ed.) pp-7042-7076.

[29] D.R. Knapp. *Handbook of Analytical Derivatization Reactions.* Wiley, New York, **1979**.

[30] M. Sittig. *Handbook of Toxic and Hazardous Chemicals and Carcinogens*. 2nd ed. Noyes Publications, Park Ridge, NJ. **1985.**

[31] N. C. Jain, 1 D. M. Chinn, R. D. Budd, T. S. Sneath,W. J. Leung. *J Forensic Sci*, **1977**, 22, 1, 7-16.

[32] E. Barceló-Barrachina, F.J. Santos, L. Puignou, M.T. Galceran. *Analytica Chimica Acta.* **2005,** 545, 2, 209–217.

[33] Tamer Awad, Jack DeRuiter, Tarek Belal, C. Randall Clark. *J. Chroma. Sci.* **2009,** 47, 279-286.

[34] R.C. Baselt. *Disposition of Toxic Drugs and Chemicals in Man*, 10th edition, Biomedical Publications, Seal Beach, CA, **2014**, ISBN 978-0-9626523-9-4. pp 531.

[35] United Nations Office of Drugs and Crime. *Recommended methods for the identification and analysis of amphetamine, methamphetamine and their ring-substituted analogues in seized materials.* Vienna, Austria: United Nations Office of Drugs and Crime; **2006.**

[36] Calum Morrison, Frank J Smith, Tomasz Tomaszewski, Karolina Stawiarska. *Chirality.* **2011,** 23, 519-522.

[37] John A. Bowden, Dominic M. Colosi, Whitney L. Stutts, Diana C. Mora-Montero, Timothy J. Garrett, Richard A. Yost. *Anal. Chem.* **2009**, *81*, 6725–6734.

[38] Vladimir Zaikin, John Halket. A Handbook of Derivatives for Mass Spectrometry. IM Publications LLP. West Sussex, **2009**. pp 3.

[39] Serban C Moldoneanu, Victor David. Modern Sample Preparation for Chromatography.Elsevier Amsterdam. **2015**, pp 362.

[40] A) S. M. Hörst, R. V. Yelle, A. Buch, N. Carrasco, G. Cernogora, O. Dutuit, V. Vuitton. *Astrobiology*, **2012,** *12*(9), 809–817. B) F. Stalport, D.P.Glavin, J.L.Eigenbrode, D.Bish, D.Blake, P.Coll, C.Szopa, A. Buch, A.McAdam, J.P.Dworkinc, P.R.Mahaffy. *Planetary and Space Science* **2012,** 67, 1–13.

[41] C. Freissinet1, D. P. Glavin1, A. Buch, C. Szopa, S. Kashya, H. B. Franz1, J. L. Eigenbrode, W. B. Brinckerhoff1, R. Navarro-González6, S. Teinturier, C. A. Malespin , B. D. Prats, P. R. Mahaffy1 and the SAM and MSL science teams. *First in situ wet chemistry experiment on Mars using the SAM instrument: MTBSTFA derivatization on a martian mudstone.* 46th Lunar and Planetary Science Conference, **2015.**

[42] A, Buch, R. Sternberg, C. Szopa, C. Freissinet, C. Garnier, El J. Bekri, C. Rodier, R. Navarro-González, F. Raulin M. Cabane, M. Stambouli, D.P. Glavin, P.R. Mahaffy. *J. Chrom. A*, **2009**, *43*, 143-151.

[43] Aelig Robin, Fraser Brown, Noemi Bahamontes-Rosa, Binghua Wu, Eric Beitz, Jurgen F. J. Kun and Sabine L. Flitsch. *J. Med. Chem.* **2007**, *50*, 4243-4249.

[44] A. Shareef. *J. Chromatogr. A.* **2006**, *1108*, 121-128.

[45]Farid Chemat, Martine Poux, Jacques Berlan *J. Chem. Soc. Perkin Trans. 2*. **1994,** 2597-2602.