Method Development of Concentration Measurements and Recoveries of Atmospheric Particle Phase Nitrophenols

CHEM 4000 Thesis

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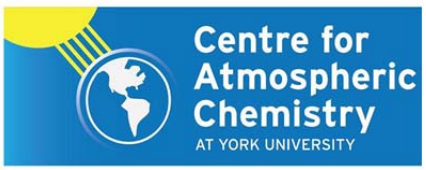

Abstract

The composition and chemistry of particulate matter (PM) found in the atmosphere has become of interest within the scientific community over recent years. The adverse effects which these atmospheric PM have on human health and climate, make understanding their origins and any possible transformations they undergo imperative. The formation of secondary organic PM, such as nitrophenols, from the photo-oxidation reaction of atmospheric volatile organic compounds (VOC's), is currently still quite poorly understood. Therefore studying nitrophenols in the atmosphere is useful, in order to gain a better understanding of the process of secondary organic PM formation. To achieve this goal, it is essential that an accurate, precise and well characterized sampling and analysis technique is created to measure atmospheric nitrophenols.

The goal of this project is to optimize the method which was developed by Dr. S Moukhtar and M. Saccon to determine the concentrations of a variety of atmospheric nitrophenols found in organic PM. Optimization of this method is important since it can offer a better understand of the origin of the loss of nitrophenols which is experienced during the process. This project specifically dealt with modifications to two of the main steps in the extraction method, the volume reduction step and the HPLC clean-up step. As well, it dealt with finding a possible solution to instrumental problems caused by XAD-4 coated filter samples.

Table of Contents

Abstract.....2

1. **Introduction**.....6

2. **Experimental Method**.....8

2.1 Sampling Procedure.....8

2.2 Extraction of Particles on Filter.....8

2.3 HPLC Sample Clean-Up.....9

2.4 Solid Phase Extraction (SPE).....11

2.5 GC-MS Analysis.....12

3. Results and Discussion.....14

3.1 Volume Reduction Tests.....14

3.2 HPLC Test 1: Determining the Optimal Injection Volume.....15

3.3 HPLC Test 2: Increasing the PostTime (PT).....17

3.4 HPLC Test 3: Testing the Reproducibility of 10 minute PT Injections.....18

3.5 HPLC Test 4: Testing the Sample Loop and Overall Injection Process.....20

3.6 HPLC Test 5: Modifying the Draw and Eject Speeds of the Injector.....21

3.7 HPLC Test 6: Determining the Amount of Sample Recovered in Each HPLC Injection.....22

3.8 HPLC Test 7: Reducing the Number of HPLC Runs Using Volume Reduction.....23

3.9 HPLC Test 8: Testing the Effect of Single and Double Injections of One Target Compound with the 200 µL Sample Loop.....24

3.10 HPLC Test 9: Testing the Effect of Single and Double Injections of Two Target Compounds with the 200 µL Sample Loop.....25

3.11 Removal of Small XAD-4 Particles Using Centrifugation.....26

4. Conclusion and Future Work.....29

References.....31

Appendix.....32

List of Figures

Figure 1: Schematic of the Entire Extraction Procedure.....8

Figure 2: Solvent Gradient Program for HPLC Separation.....11

Figure 3: Derivitization Reaction of 2-methyl-4-nitrophenol and BSTFA at Room Temperature.....12

Figure 4: Temperature Program Used in GC-MS Instrument using an Rtx-5 Capillary Column.....13

Figure 5: Calibration Curve of Injection Volumes of 2-methyl-4-nitrophenol.....15

Figure 6: Characteristic Peak Shapes of 2-methyl-4-nitrophenol from HPLC Runs.....17

Figure 7: Calibration Curve of Injection Volumes of 2-methyl-4-nitrophenol with 10 minute PT.....19

Figure 8: Comparison of Peak Broadening in 100 µL Injections.....20

Figure 9: Calibration Curve for New Sample Loop using 2-methyl-4-nitrophenol Injections.....25

Figure 10: Electron Microscope Pictures of Slurry Containing XAD-4 Suspended in ACN.....28

List of Tables

Table 1: Concentrations of Standards Used in the Experiments.....9

Table 2: HPLC Retention Times of Target Compounds and Internal Standards.....10

Table 3: GC Retention Times of Target Compounds, Internal Standards and Volumetric Standards.....13

Table 4: Recovery of Target Compounds and Internal Standards from Volume Reduction Tests.....14

Table 5: 50, 100 and 200 µL Injections of 2-methyl-4-nitrophenol at Normal Concentrations.....16

Table 6: 50 µL injections of 2-methyl-4-nitrophenol with 3 minute, 5 minute, 7 minute and 10 minute PT.....18

Table 7: 50 µL, 100 µL and 200 µL Injections of 2-methyl-4-nitrophenol with 10 minute PT.....19

Table 8: Determination of Sample Loop Size.....20

Table 9: Average Recoveries for Filter Extractions Using 4 and 8 HPLC Runs.....21

Table10: 100 µL Injections of 2-methyl-4-nitrophenol with Varying Draw and Eject Speeds.....22

Table 11: Percentage of Peak Area Recovered in Each HPLC Injection.....23

Table 12: Internal Standard Recovery for 8 HPLC Run Method and 6 HPLC Run Method.....23

Table 13: 50 µL, 100 µL and both Single and Double 200 µL Injections of 2-methyl-4-nitrophenol at Normal Concentrations.....24

Table 14: Summary of 100 µL and both Single and Double 200 µL Injections of Mixture of 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol.....26

1. Introduction

Atmospheric nitrophenols are formed primarily through the photo-oxidation of aromatic VOC's, such as toluene and benzene, that are emitted into the atmosphere by industrial sources as well as vehicle exhaust, and can undergo reactions with OH radicals and nitrogen dioxide (NO_2) in order to produce nitrophenols (Cecinato, A., et al., 2005). Approximately 10% of the toluene present in the atmosphere undergoes H abstraction to form benzaldehyde. The remaining 90% of the toluene undergoes an OH addition reaction to form a methyl hydroxycyclohexadienyl radical, where the OH group can be situated either ortho, meta or para to the methyl group. This radical species can then undergo a reaction with O_2 to form methylphenols (cresols) which can further react with NO_2 to form methyl nitrophenols (Forstner, H.J.L., et al., 1997).

The research in Dr. Jochen Rudolph's group is primarily concerned with studying the main chemical processes that involve VOC's in the atmosphere, and more specifically, determining the yield of organic PM that is formed from the oxidation of toluene that occurs under ambient conditions. Nitrophenols, which are specific to the OH initiated photo-oxidation of toluene, as well as other aromatic compounds found in the atmosphere, are very useful since they allow the comparison of isotope ratios observed in the atmosphere with existing laboratory studies of nitrophenol isotope ratios.

In recent years, both Dr. S. Moukhtar and M. Saccon have worked together to construct a method which allows for the detection and quantification of nitrophenols recovered from POM in the atmosphere. This multi-step method involves using quartz fiber filters to sample nitrophenols found in the atmosphere, extraction of these filters using acetonitrile (ACN), significant extract volume reduction, separation using High Performance Liquid Chromatography (HPLC), solid phase extraction, final sample volume reduction, and finally, analysis by Gas Chromatography - Mass Spectroscopy (GC-MS) or by Gas Chromatography – Isotope Ratio Mass Spectrometry (GC-IRMS), for isotope ratio measurements

The main objective of this research project involves optimizing the method explained above, in order to gain a better understanding of the origin of the loss of nitrophenols which occurs during this process. Recoveries of the three internal standards as well as the concentrations of the target nitrophenol compounds

found when using this experimental method, are very low and typically do not exceed 45%. Increasing the efficiency of this process will allow a higher concentration of nitrophenols to be achieved, therefore allowing more material to be available for isotope ratio measurements. Increasing the recovery of these compounds is critical since in order to make isotope ratio measurement on these nitrophenols, a few ng/μL of material are required due to the very small nitrophenol concentration found as PM in the atmosphere (sub ng/m^3). Research performed during the summer of 2010 by L. Siu Wing Wong, involved making modifications to the filter extraction step, the solid phase extraction step and the final sample volume reduction to improve the recovery of nitrophenols collected by the filters. In this research project, further modifications were completed, with special emphasis being placed on modifications to the extract volume reduction step, and the HPLC separation step.

2. Experimental Method

The entire procedure of this experimental method is illustrated below in *Figure 1*.

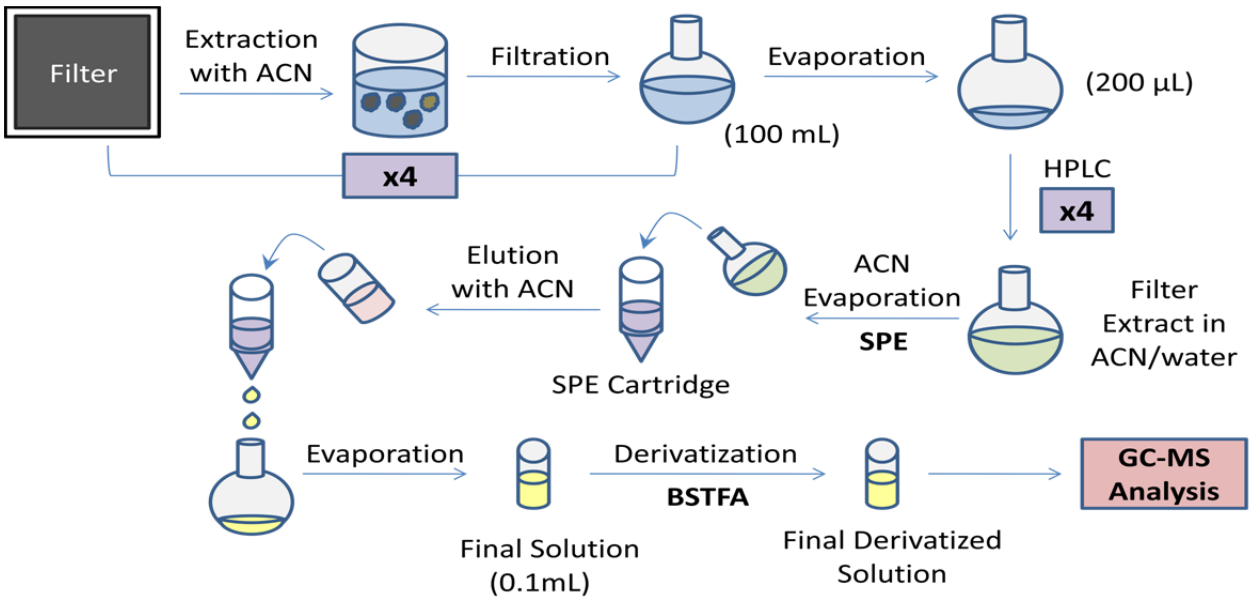

Figure 1: *Schematic of the Entire Extraction Procedure*

2.1 Sampling Procedure

Quartz fibre filters measuring 8 by 10 inches in size, were used to sample nitrophenols found in atmospheric PM. Before sampling, the filters were baked in an 850^{o}C oven for 24 hours. The filters were then used to sample organic PM with a $PM_{2.5}$ high-volume air sampler. The flow rate used in the air sampler was 1.13 m^3/min, with sampling times ranging from one to three days in duration. Once sampling was completed, the filters were placed in air-tight glass jars which were stored in a freezer until analysis.

2.2 Extraction of Particles on Filter

The sampled quartz filter was cut into eight pieces and spiked with 40 µL of each of the three internal standards used: 2-methyl-phenol (o-cresol), 2-methyl-3-nitrophenol and 2-methyl-5-nitrophenol. A list of the concentrations of all target compounds and standards used in this method is shown in *Table 1*. The filter was then transferred into an amber glass jar and extracted with approximately 15-20 mL of ACN (HPLC grade, $\geq$ 99.9%, Sigma Aldrich). The jar was sonicated in a 5510R-DTH Bransonic Ultrasonic Cleaner for 15 minutes to promote extraction. After sonication, the liquid extract was removed from the jar with a pipette and

filtered through a syringe equipped with a 0.45 µm PTFE Chromspec syringe filter, directly into a round bottom flask (RBF). This extraction procedure was repeated three additional times, with the liquid extracts being collected into the same RBF. The flask containing the combined filter extract was then evaporated, using an R110, Brinkmann rotary evaporator at a temperature of 35°C, down to a volume of approximately 1 mL. The extract was then further evaporated under nitrogen in a conical vial to a volume of approximately 200 µL.

***Table 1**: Concentrations of Standards Used in the Experiments*

		Concentration (ng/µL)
Target Compounds	4-nitrophenol	100
	4-methylphenol (p-cresol)	107
	2-methyl-4-nitrophenol	106
	4-methyl-2-nitrophenol	101
	3-methyl-4-nitrophenol	101
	2,6-dimethyl-4-nitrophenol	101
Internal Standards	2-methyl-phenol (o-cresol)	114
	2-methyl-3-nitrophenol	103
	2-methyl-5-nitrophenol	100
Volumetric Standards	heptadecane ($C_{17}H_{36}$)	223
	octadecane ($C_{18}H_{38}$)	229
	nonadecane ($C_{19}H_{40}$)	209

Note: The concentrations of target compounds are listed since these are used in tests run

2.3 HPLC Sample Clean-Up

The HPLC sample clean-up stage was employed to reduce the complexity of the extracted sample. The internal standards and atmospheric nitrophenols elute from the HPLC at specific elution times, as seen in *Table 2*. The retention times determine the window of time where the effluent from the HPLC was collected to undergo the remainder of the experiment.

The Hewlett-Packard (HP) 1050 HPLC used is equipped with a Supelco Supelcosil LC-18 column (25 cm x 4.6 mm, 5µm) and a Variable Wavelength Detector (VWD) which uses a Deuterium lamp as the light source. The wavelength selected was 320 nm, since this is the wavelength at which all target compounds and internal standards, except the cresols, absorb. Tests previously performed at wavelength 280 nm, where the cresols absorb, confirmed that the retention times of cresols fall within the same time frame. The solvent flow rate was 1.00 mL/min. A gradient elution program is performed using two solvents, ACN and Milli-Q water (Milli-Q gradient A10 Millipore, 18 MΩ) and is illustrated in *Figure 2*.

The 200 µL reduction of filter extract from *Section 2.2*, was transferred from the RBF to a 1.5 mL glass vial, and the extract was then separated using the HP 1050 HPLC. The RBF was then rinsed with approximately 5 mL of ACN, and the solution was evaporated back down to approximately 200 µL using both the rotary evaporator and evaporation under nitrogen. The remaining 200 µL was placed into another 1.5 mL glass vial and injected into the HPLC. This procedure was repeated an additional two times so that a total of four HPLC analyses are performed.

Each HPLC analysis involved a 200 µL injection and each HPLC run was 30 minutes in duration with a 1 minute PostTime (PT). The PT is a period of time between adjacent runs used to return the solvent program back to 100% water and 0% ACN in order to return the column to original conditions. Between the time of 9 and 16 minutes, as *Table 2* confirmed, the target compounds and internal standards eluted from the column. During these 7 minutes, the effluent from each of the 4 HPLC runs was collected into a clean flask.

Table 2: *HPLC Retention Times of Target Compounds and Internal Standards*

	HPLC Retention Time (min)
4-nitrophenol	9.9
3-methyl-4-nitrophenol	12.2
4-methylphenol (p-cresol)	12.4
2-methylphenol (o-cresol)	**12.7**
2-methyl-4-nitrophenol	13.0
2-methyl-3-nitrophenol	**13.2**
2-methyl-5-nitrophenol	**13.7**
2,6-dimethyl-4-nitrophenol	14.0
4-methyl-2-nitrophenol	14.4

Note: compounds in **bold** are internal standards

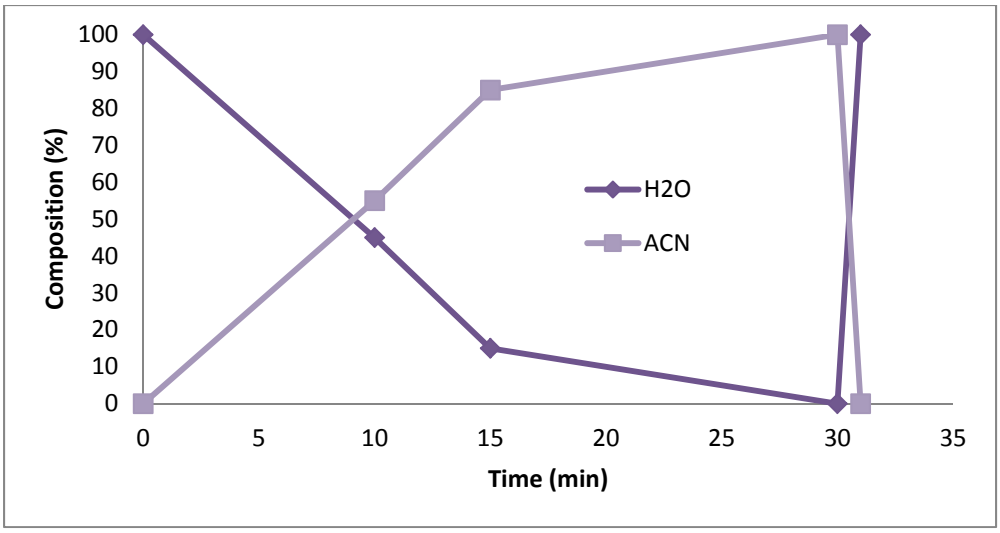

***Figure 2**: Solvent Gradient Program for HPLC Separation*

2.4 Solid Phase Extraction (SPE)

The eluent fraction collected from the HPLC step contained nitrophenols, internal standards, ACN, water and some unknown compounds. The ACN contained in the eluent solution was evaporated using the rotary evaporator at room temperature until the volume of the solution had been reduced about 50%. The residual solution was then acidified with 50 µL of phosphoric acid (H_3PO_4) and subjected to a solid phase extraction (SPE) step, in order to remove any water contained in the solution.

The SPE step employed a Waters Oasis Hydrophilic-Lipophilic Balance (HLB) Extraction Cartridge. The cartridge itself contains polymeric sorbents (N-vinylpyrrolidone and divenylbenzene) which act as a stationary phase to trap the nitrophenols from the aqueous solution (Waters Corporation, 2008). First, the cartridge was conditioned with 1 mL of ACN followed by 1 mL of Milli-Q water. The acidified eluent solution was then passed through the cartridge. The empty flask which contained the sample was then rinsed with 3 mL of Milli-Q water and was acidified with 30 µL of H_3PO_4. This acidified solution was also passed through the cartridge. Once all the sample solution had eluted through the cartridge, approximately 10 mL of ACN was passed through the cartridge and collected into a clean flask, recovering the nitrophenols and the internal standards. The solution was then placed on the rotary evaporator at 35^{o}C and evaporated down to a volume of approximately 200 µL. This final solution was then transferred to a 4 mL conical glass vial to be further

evaporated down under a soft stream of nitrogen. The flask was then rinsed, two separate times, with approximately 3 mL of ACN, and evaporated on the rotary evaporator down to approximately 200 µL. The two rinsing solutions were transferred to the same vial and then combined solution was evaporated to a final volume of approximately 50 µL under nitrogen.

2.5 GC-MS Analysis

Just before GC-MS analysis, 20 µL of a volumetric standard mixture containing heptadecane ($C_{17}H_{36}$), octadecane ($C_{18}H_{38}$) and nonadecane ($C_{19}H_{40}$), was added to the 50 µL sample solution in the same conical vial. Volumetric standards are used in this method to allow the total volume of the sample volume prior to GC-MS analysis, to be exactly determined. The solution was mixed with a magnetic stirring bar and half of the solution was transferred to a labelled glass vial which was stored in the freezer, allowing for future IR-MS analysis. The remaining half of the solution was derivatized with 20 µL of bis(trimethylsilyl)trifluoroacetamide (BSTFA). The solution was capped and left to mix with a magnetic stirring bar for 5 minutes. The BSTFA replaced labile hydrogens on the target compounds with a $–Si(CH_3)_3$ (TMS) group, producing volatile and more thermally stable derivatives of the parent compounds, which ensured more efficient analysis by GC-MS (Schummer, C., et al., 2009). A sample derivatization reaction of one of the target compounds (2-methyl-4-nitrophenol) with BSTFA is shown below in *Figure 3*.

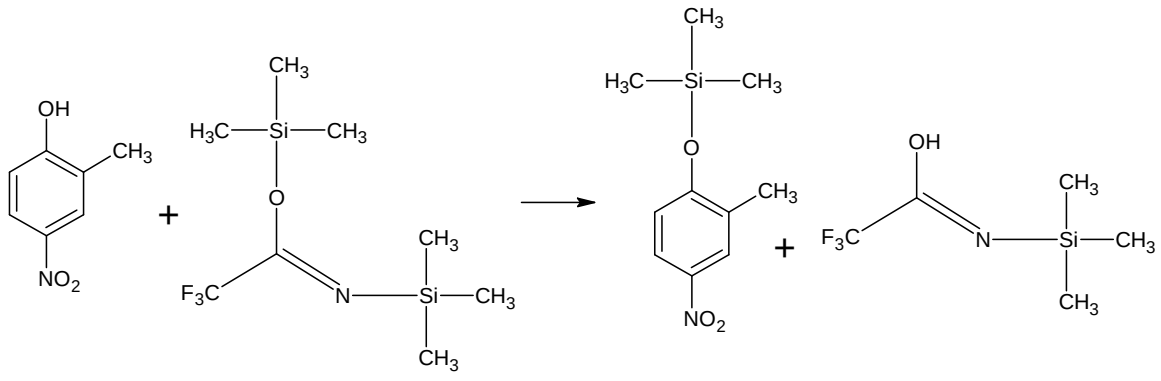

***Figure 3**: Derivitization Reaction of 2-methyl-4-nitrophenol and BSTFA at Room Temperature*

The derivatized solution is then transferred into a glass vial and analyzed using a Hewlett-Packard (HP) 5890 Series II GC, which was equipped with a HP 5972 Series MS Detector. The column employed in

the GC was an Rtx-5 (Restex, 5%-diphenol-95%-dimethylpolysiloxane) capillary column, 60 m in length with an inner diameter of 0.25 mm and a 1.0 µm film thickness. The analysis involved a 125 minute temperature program, which is illustrated in *Figure 4*. The retention times (*Table 3*) and peak abundances from the chromatograms were analyzed in order to determine the recoveries of the internal standards and the concentrations of the atmospheric nitrophenols.

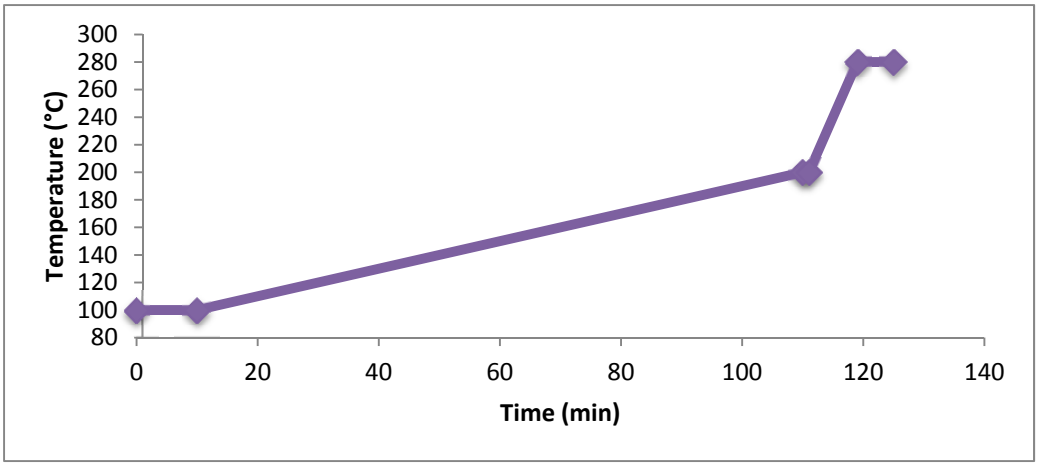

***Figure 4**: Temperature Program Used in GC-MS Instrument using an Rtx-5 Capillary Column*

***Table 3**: GC Retention Times of Target Compounds, Internal Standards and Volumetric Standards*

	GC Retention Time (min) Rtx-5 – 1.0 µm Film Thickness
2-methylphenol (o-cresol)	**28.0**
4-methylphenol	30.2
4-methyl-2-nitrophenol	72.6
4-nitrophenol (p-cresol)	73.7
2-methyl-3-nitrophenol	**74.0**
2-methyl-5-nitrophenol	**75.7**
3-methyl-4-nitrophenol	79.7
2-methyl-4-nitrophenol	84.5
2,6-dimethyl-4-nitrophenol	98.7
heptadecane ($C_{17}H_{36}$)	95.7
octadecane ($C_{18}H_{38}$)	106.4
nonadecane ($C_{19}H_{40}$)	114.6

3. Results and Discussion

3.1 Volume Reduction Test

The first test was conducted to see if loss of nitrophenols is a result of the volume reduction step. Testing was specifically done on the volume reduction occurring when evaporating the solution under nitrogen (see Section 2.2). To do this, 400 µL of each of the six target compounds and each of the three internal standards were mixed together in a 4mL glass vial. From this solution, 500 µL was placed into a conical glass vial and evaporated under a soft stream of nitrogen down to a volume of approximately 50 µL. Addition of 20 µL of the volumetric standard solution (3 alkane solution) as well as 20 µL of BSTFA was done, following the procedure stated in Section 2.5, before GC-MS analysis.

***Table 4**: Recovery of Target Compounds and Internal Standards from Volume Reduction Tests*

	Recovery (%)		
2-methylphenol (o-cresol)	62	67	62
2-methyl-3-nitrophenol	94	97	92
2-methyl-5-nitrophenol	101	107	103
4-methylphenol (p-cresol)	68	76	72
4-methyl-2-nitrophenol	95	102	98
4-nitrophenol	91	97	92
3-methyl-4-nitrophenol	103	107	102
2-methyl-4-nitrophenol	104	110	106
2,6-dimethyl-4-nitrophenol	137	143	134

The results from three separate tests are shown in *Table 4*. The results show that there was not much loss seen by the evaporation done under nitrogen. The recoveries for each of the target compounds and the internal standards were very reproducible and averaged close to 100% for most of the species. The low recoveries seen by the cresols (2-methylphenol and 4-methylphenol) are typical of the cresols in almost all experiments due to their high volatility. Results for the repeat experiments were similar, and therefore it was concluded that there was not significant loss of nitrophenols due to the volume reduction step.

3.2 HPLC Test 1: Determining the Optimal Injection Volume

Efforts were focused next on testing losses taking place during the HPLC separation, which was where most of the loss of nitrophenols was believed to be occurring. The first test done was the determination of the optimal injection volume. The method that had been developed involved a 200 µL injection, since it was believed that the 1050 HPLC contained a 200 µL sample loop. A calibration curve was created using injections of one of the target compounds, 2-methyl-4-nitrophenol, which in the atmosphere is the most abundant of the target compounds. Injections of different sample volumes of 2-methyl-4-nitrophenol were done in the range of 25 µL to 250 µL, and the data obtained from area of the eluting peak are shown in the calibration curve illustrated in *Figure 5*.

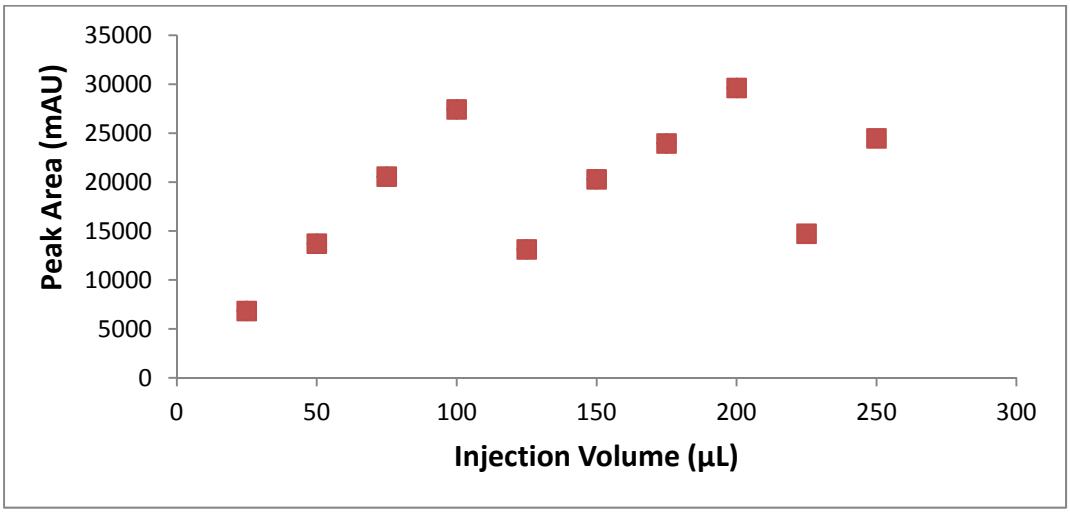

***Figure 5**: Calibration Curve of Injection Volumes of 2-methyl-4-nitrophenol*

***Table 5**: 50, 100 and 200 µL Injections of 2-methyl-4-nitrophenol at Normal Concentrations (106 ng/µL)*

Injection Volume (µL)	Retention Time (min)	Peak Area (mAU)	Average Peak Area (mAU)
50	13.2	12930	13990 ± 780
	13.2	13970	
	12.9	14300	
	12.9	14750	
100	12.5	21780	25150 ± 4320
	13.1	21780	
	13.2	30830	
	12.7	26200	
200	12.9	23170	29070 ± 4440
	12.9	28320	
	13.2	33320	
	12.1	31450	

Note: Data points in red represent peak broadening or multiple peaks for the same signal and data points in purple represent retention time shifts

The calibration curve obtained, as seen in *Figure 5*, was very different from what was expected. Instead of a continuously linear function, the calibration curve depicted three distinct regions of linearity. A significant loss in area was seen after the 100 µL injection, as well as after the 200 µL injection. Also, the area of the 200 µL injection was almost equivalent to the area of the 100 µL injection, which meant about half of the injection at 200 µL was being lost.

By specifically looking at the 50 µL, 100 µL and 200 µL injections done in this test, the results, as seen in *Table 5*, illustrated that some sort of loss was indeed occurring between the 100 µL and 200 µL injections. The peak area obtained by a 50 µL injection was indeed half the value of the peak area obtained by the 100 µL injection, but the peak area of the 200 µL injection was certainly not double the peak area obtained by the 100 µL injection.

The results in *Table 5* also depict data points where there were shifts in retention times and broadening, or even doubling, of peaks occurring. Illustrations of these peak characteristics are found below in *Figure 6*. This was a significant problem in most of the large volume injections done on the HPLC. One reason for both the peak broadening and possible shifts in retention times may be that too much ACN remained in the column, influencing solvent strength and therefore the retention time. A good possibility is that these effects

could be removed if the column was flushed out for a longer period of time between runs. Therefore, the next step was to look at the effect of increasing the time between runs where the solvent program is returned to 100% Milli-Q water, and the column was flushed out.

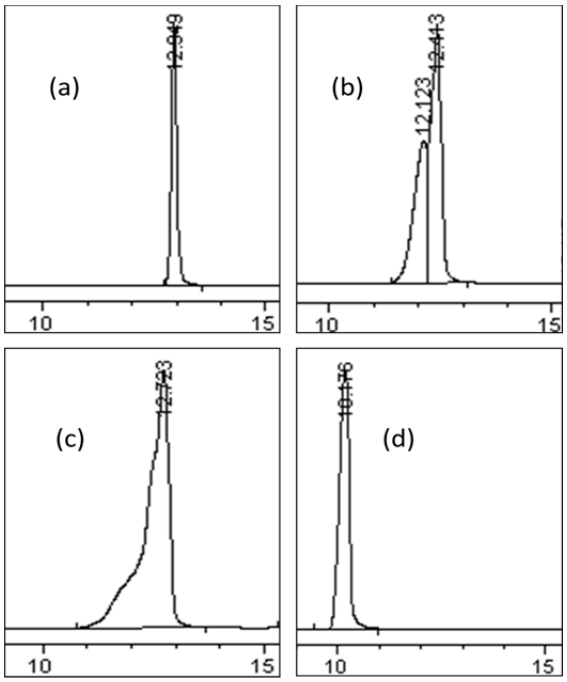

Figure 6: *Characteristic Peak Shapes of 2-methyl-4-nitrophenol from HPLC Runs: (a) ideal peak shape – narrow and at correct retention time; (b) multiple peaks; (c) peak broadening; (d) retention time shifting*

3.3 HPLC Test 2: Increasing the PostTime (PT)

As stated in Section 3.2, the PostTime (PT) is the time after the 30 minute HPLC run when the solvent program is returned to 100% Milli-Q water, allowing the column to be returned to initial conditions. As seen in *Figure 2*, the method currently used only employs a 1 minute PT. To conduct this test, four new HPLC methods were created that involved 50 µL injections of 2-methyl-4-nitrophenol, which had 3 minute, 5 minute, 7 minute and 10 minute PT's respectively. Testing involved use of 50 µL injections, since small injections are more favourable in HPLC. Some of the results are shown in *Table 6*.

***Table 6**: 50 µL injections of 2-methyl-4-nitrophenol with 3 minute, 5 minute, 7 minute and 10 minute PT*

	Retention Time (min)	Peak Area (mAU)	Average Peak Area (mAU)
3 minute PT	10.8	8940	7030 ± 3300
	8.6	3210	
	10.8	8940	
5 minute PT	11.3	5220	9490 ± 3880
	12.7	10440	
	12.9	12800	
7 minute PT	11.6	9100	12750 ± 3180
	12.6	14230	
	12.7	14920	
10 minute PT	12.7	16310	16370 ± 60
	12.8	16380	
	12.8	16420	

Note: Data points in red represent peak broadening or multiple peaks for the same signal and data points in purple represent retention time shifts.

With reference to *Table 6*, it can be seen that only the HPLC runs with 10 minute PT resulted in no shifting of retention time and no changes in peak shape (no peak doubling or broadening). As well the runs with the 10 minute PT were the only ones that were reproducible in both peak area and retention time. Before concluding that the 10 minute PT was the optimal PT, reproducibility tests were conducted with 100 µL and 200 µL injections.

3.4 HPLC Test 3: Testing the Reproducibility of 10 minute PT Injections

To conclude that the 10 minute PT was indeed optimal for the method, tests to determine the reproducibility of 10 minute PT runs were conducted with 50 µL, 100 µL and 200 µL injections of 2-methyl-4-nitrophenol. Some results from these tests are illustrated in *Table 7 and Figure 7*.

***Table 7**: 50 µL, 100 µL and 200 µL Injections of 2-methyl-4-nitrophenol with 10 minute PT*

Injection Volume (µL)	Retention Time (min)	Peak Area (mAU)	Average Peak Area (mAU)
50	12.8	16110	16380 ± 250
	12.8	16310	
	12.8	16380	
	12.7	16710	
100	12.9	32430	32520 ± 360
	12.9	32800	
	12.9	32050	
	13.0	32790	
200	12.7	34670	36330 ± 1630
	13.0	35760	
	12.9	36320	
	12.9	38550	

Note: Data points in red represent peak doubling occurring for the same signal.

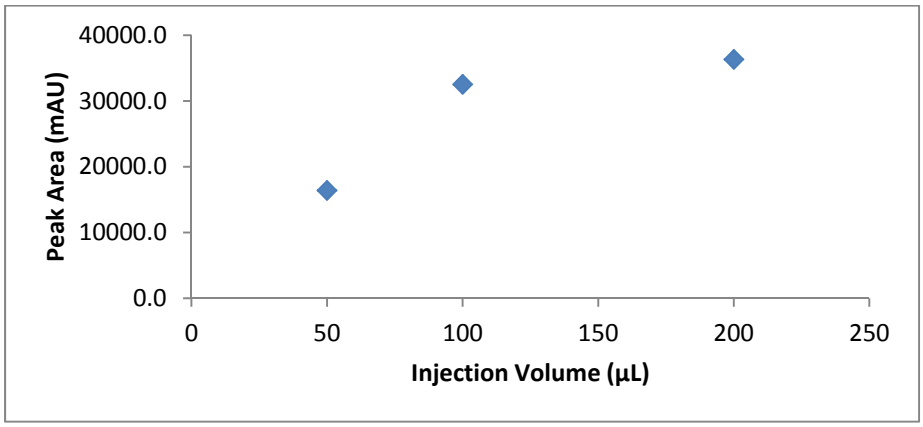

***Figure 7:** Calibration Curve of Injection Volumes of 2-methyl-4-nitrophenol with 10 minute PT*

The results shown in *Table 7* illustrate good reproducibility in both peak area and retention time for all three injection volumes. Therefore, a 10 minute PT is seemingly the best choice for use in this method. The 10 minute time interval was significantly long enough that all the ACN remaining in the column was flushed out by the Milli-Q water, resulting in no retention time shifting and practically no peak broadening, compared to runs with lower less than 10 minute PT as seen in the chromatograms shown in *Figure 8*. Increasing the PT from 1 minute to 10 minutes resulted in a 40 minute increase in the overall duration of the HPLC clean-up step, since there are 4 HPLC runs in the method.

The same non-linearity is seen between 100 µL and 200 µL injections as illustrated in *Figure 7*, suggesting that 200 µL injections are simply not reliable for this method, since there is some significant loss in sample occurring. As well, significant peak doubling was seen with almost all the 200 µL injections, which may be a result of too large an injection volume being injected or an inadequate injection procedure being used. To fully understand why this loss was occurring with the 200 µL injections, further study had to be done on the HPLC sample injection process.

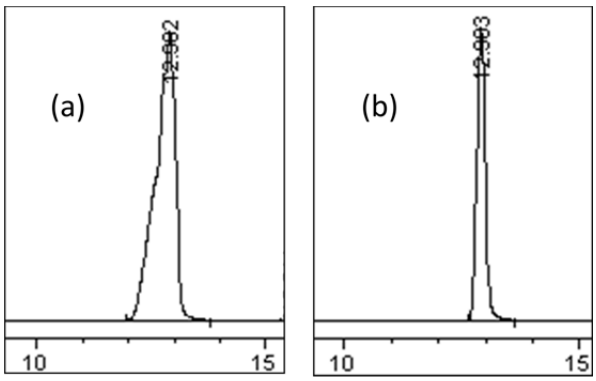

***Figure 8**: Comparison of Peak Broadening in 100 µL Injections with (a) 1 minute PT and (b) 10 minute PT*

3.5 HPLC Test 4: Testing the Sample Loop and Overall Injection Process

In order to gain a better understanding of why a loss was seen in the 200 µL injections, further study on the sample loop contained in the HPLC as well as on the overall injection process was conducted. Firstly, the sample loop, which was thought to be 200 µL in size, was removed from the HPLC and completely emptied by passing a soft stream of nitrogen through one end. The emptied sample loop was weighed. One end of the loop was then plugged using a rubber disc and the loop was then completely filled with Milli-Q water. The sample loop filled with water was then weighed. The difference in the masses allowed the sample loop size to be determined.

***Table 8**: Determination of Sample Loop Size*

	Mass (g)
Mass of Empty Sample Loop	17.1129
Mass of Sample Loop Filled with Milli-Q Water	17.2124
Mass Difference	0.0995

Since the mass difference was determined to be approximately 100 mg, as seen in *Table 8*, the sample loop was concluded to be 100 µL in size, not 200 µL as thought, as is shown in a calculation found in the *Appendix*. This explained why 200 µL injections were not efficient, since the sample loop was being overfilled and therefore sending most of the sample to waste. This was confirmed by examining the injection process and collecting the waste, which exited the instrument during the injection of the sample into the sample loop.

As it was realized that 200 µL injections were not functioning well within the method, the number of HPLC runs required doubled, increasing from four HPLC runs to eight. This increase occurred because now each of the four 200 µL injections previously done, need to be injected into the HPLC in two separate 100 µL injections. The increase in HPLC runs from four to eight resulted in the more than doubling of recovery, as seen with the majority of internal standards illustrated in *Table 9*. The recovery of o-cresol may not have experienced the same doubling of recovery for two possible reasons. One may be the fact that the recovery of cresols was variable due to their high volatility, and the other may be that there were significantly more filter extractions averaged to obtain the data for the four HPLC runs than for the eight HPLC runs. Since the recoveries did significantly increase for the majority of the species, eight HPLC runs was concluded to be more effective than the four HPLC runs done previously. This significant increase in recoveries does come at a price though. The HPLC clean-up step with eight, 40 minute HPLC runs, now took 5 hours and 20 minutes, compared to the 2 hours it took with the four, 30 minute HPLC runs.

***Table 9**: Average Recoveries for Filter Extractions Using 4 and 8 HPLC Runs*

	Recovery (%)	
	4 HPLC Runs	**8 HPLC Runs**
2-methylphenol (o-cresol)	13 ± 10	10 ± 12
2-methyl-3-nitrophenol	29 ± 9	66 ± 18
2-methyl-5-nitrophenol	33 ± 11	72 ± 20

3.6 HPLC Test 5: Modifying the Draw and Eject Speeds of the Injector

A test was performed to see if modifications to the draw and eject speeds of the injector had any effect on the efficiency of the HPLC clean-up step. The draw speed is the speed at which the syringe draws up the sample from the vial, and the eject speed is the speed at which the sample is ejected from the syringe into the

sample loop. New methods involving 100 µL injections with draw and eject speeds of 100 µL/min, 500 µL/min and the normal 1000 µL/min were created, and injections of 2-methyl-4-nitrophenol occurred.

***Table10**: 100 µL Injections of 2-methyl-4-nitrophenol with Varying Draw and Eject Speeds*

	Retention Time (min)	Peak Area (mAU)	Average Peak Area (mAU)
Draw and Eject Speed = 1000 µL/min	13.0	30600	31730 ± 1100
	13.0	31810	
	13.0	32790	
Draw and Eject Speed = 500 µL/min	12.9	30630	27330 ± 2930
	13.0	25030	
	13.0	26340	
Draw and Eject Speed = 100 µL/min	12.4	14700	19130 ± 4190
	12.7	19650	
	12.8	23040	

The results of changing both draw and eject speeds at the same time are shown in *Table 10*. The most reproducible and efficient results occurred when the draw and eject speeds were set to the maximum speed of the instrument, 1000 µL/min, which was what was automatically set by the instrument. Losses were seen for injections done at the lower draw and eject speeds that could possibly be attributed to issues with timing. If the delay that occurs between injection of the syringe and the rotation of the injection valve is insufficient for complete transfer of sample, some of the sample volume will be lost and not be injected onto the column. The tests established that decreasing the draw and eject speeds did not have a positive effect on efficiency of the HPLC clean-up step, so the maximum set speed of 1000 µL/min was used in this method. Possible future tests where the draw and eject speeds are changed individually may gain more insight into the true effect that altering these speeds has on injection efficiency.

3.7 HPLC Test 6: Determining the Amount of Sample Recovered In Each HPLC Injection

To see if there was any possibility in reducing the number of HPLC runs in order to reduce the overall experimental time, the amount recovered in each HPLC injection needed to be determined. A number of filter extractions where eight HPLC runs were conducted, were analyzed. The eight HPLC injections involved two injections of the original extract and then two injections for each of the three rinses performed, as explained in

Section 3.4. Since two injections were performed for each of the four original injections, the results for each pair of injections were added together, and a percentage of the overall signal was calculated, as illustrated in *Table 11*.

***Table 11**: Percentage of Peak Area Recovered in Each HPLC Injection*

	Percentage of HPLC Signal (%) During Collection Period (9-16 min)
Original Sample	86 ± 5
Rinse 1	10 ± 4
Rinse 2	2 ± 2
Rinse 3	1 ± 1

The results in *Table 11*, showed that the percentage of overall signal found in Rinse 3 (or HPLC runs 7 and 8) and even Rinse 2 (HPLC runs 5 and 6) was very minimal. This suggested that maybe a reduction of the number of HPLC runs could be attempted. One option available was to further reduce the volume of the final two rinses from 200 µL to 100 µL, by further evaporating under nitrogen, as will be discussed in *Section 3.8*.

3.8 HPLC Test 7: Reducing the Number of HPLC Runs Using Volume Reduction

This test was conducted to assess the possibility of reducing the number of HPLC runs in a filter extraction by further evaporating the final two rinses down to 100 µL from 200 µL. A filter was extracted using the experimental procedure discussed in *Section 2*. The only difference was that the filter was cut in half and both halves were extracted separately, as if they were separate filters. The first half of the filter was subjected to eight HPLC runs, while the last two rinses of the second half of the filter were further reduced to 100 µL, resulting in only 6 HPLC runs. The filters underwent the rest of the experimental procedure and were analyzed using GC-MS to determine their internal standard recoveries which are seen in *Table 12*.

***Table 12**: Internal Standard Recovery for 8 HPLC Run Method and 6 HPLC Run Method*

	Recovery for 8 HPLC Runs (%)	**Recovery for 6 HPLC Runs (%)**
2-methylphenol (o-cresol)	24	54
2-methyl-3-nitrophenol	61	84
2-methyl-5-nitrophenol	53	83

The results seen from the filter extraction showed that the volume reduction from 200 µL to 100 µL performed during the last two rinses in the six HPLC runs method, did not experience a decrease in recovery. This confirms the results found in *Section 3.7* which express that there is not significant amount of nitrophenol solution contained in the last two rinses. Therefore an extraction method using six HPLC runs instead of eight should be sufficient for substantial recovery of nitrophenols, but further testing on more sampled filters should be conducted to confirm these results.

3.9 HPLC Test 8: Testing the Efficiency of Single and Double Injections of One Target Compound with the 200 µL Sample Loop

In order to reduce the length of time the HPLC clean-up step required, a larger (200 µL) sample loop was purchased and installed. With the new sample loop, 200 µL injections could be done as a multiple injection process since the HPLC was equipped with a 100 µL syringe. Therefore, a 200 µL injection could be achieved by telling the instrument to perform either a 200 µL injection using a single method injection program, or a 200 µL injection using two consecutive injection programs. The 200 µL injection using two consecutive injection programs consisted of a quick 100 µL injection with a 0.3 minute run time, followed half a second later, by a normal 100 µL injection with a 40 minute run time. A series of tests were run with the new sample loop to see which method of 200 µL injections was most efficient. Testing was conducted using a variety of 50 µL, 100 µL and 200 µL injections of 2-methyl-4-nitrophenol, and results are presented for some of the injections performed.

***Table 13**: 50 µL, 100 µL and both Single and Double 200 µL Injections of 2-methyl-4-nitrophenol at Normal Concentrations*

	Retention Time (min)	Peak Area (mAU)	Average Peak Area(mAU)
50 µL Injection	12.8	12870	13440 ± 550
	12.9	13490	
	13.0	13970	
100 µL Injection	13.0	28780	28960 ± 160
	13.0	29000	
	12.5	29100	
200 µL Injection (single injection)	12.6	34200	34200 ± 380
	12.6	34570	
	12.7	33820	
200 µL Injection (sum of two 100 µL injections)	12.7	57730	56750 ± 2080
	12.8	58150	
	12.8	54360	

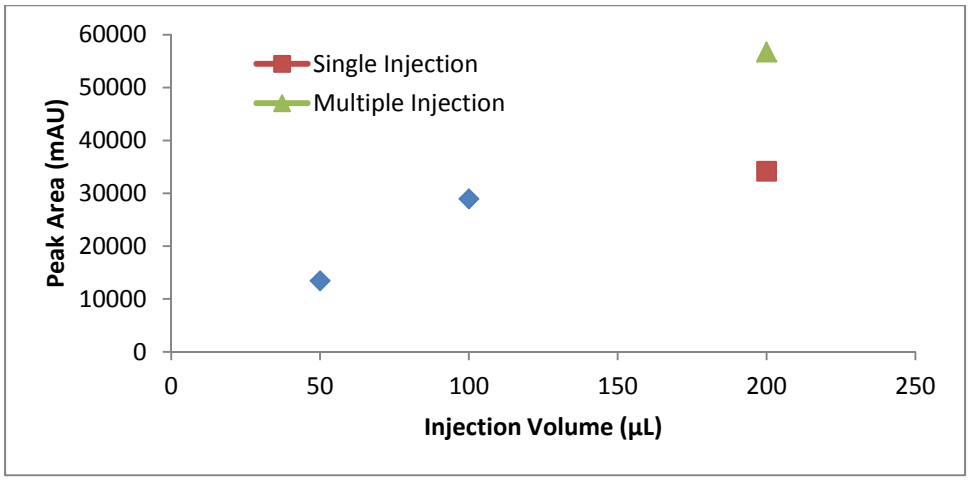

***Figure 9**: Calibration Curve for New Sample Loop using 2-methyl-4-nitrophenol Injections*

The results seen above demonstrated that the 200 µL injection using a single injection program experienced the same non-linearity as in tests done with the old sample loop. The results for the 200 µL injections done using two back to back 100 µL injections were much more linear. It was determined that the single 200 µL injection was not working accurately, most likely due to some miscommunication between the software and the hardware, since the sample loop was now 200 µL and the syringe was only 100 µL. Since the peak area of the 200 µL injection using the double injection program was almost exactly double the peak area of the 100 µL injection, as seen in *Table 13*, double injection was determined to be a possible way to inject the large sample volume of 200 µL using the current instrument.

3.10 HPLC Test 9: Testing the Efficiency of Single and Double Injections of Two Target Compounds with the 200 µL Sample Loop

In order to confirm the conclusion made in the previous section, more testing needed to be done that involved the injection of more than one compound at a time. A mixture was created that contained 2 mL of 2-methyl-4-nitrophenol and 2 mL of 4-methyl-2-nitrophenol, both at normal concentrations, which were listed previously in *Table 1*. The compounds contained in the mixture, 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol, were chosen since they had significantly different retention times (*Table 2*). Injections of 100 µL as well as 200 µL injections using both the single and double injection program methods discussed in *Section*

3.9, were conducted. The average retention times and average peak areas for the two compounds for each of the injection volumes used, are presented in *Table 14*.

***Table 14**: Summary of 100 µL and both Single and Double 200 µL Injections of Mixture of 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol*

	2-methyl-4-nitrophenol		4-methyl-2-nitrophenol	
	Average Retention Time (min)	Average Peak Area (mAU)	Average Retention Time (min)	Average Peak Area (mAU)
100 µL Injection	12.2 ± 0.9	12340 ± 1220	14.4 ± 0.5	2070 ± 10
200 µL Injection (Single Injection)	12.8 ± 0.1	13740 ± 1420	14.8 ± 0.3	2440 ± 60
200 µL Injection (sum of two 100 µL injections)	12.8 ± 0.1	26500 ± 190	14.6 ± 0.1	4290 ± 10

The results illustrated in *Table 14*, determined that even with multiple compounds in a mixture, linearity is seen with the 200 µL injection that was performed with the double injection method program and not with the 200 µL injection performed as a single injection. The peak areas of the single 200 µL injections are nowhere near close to double the peak area values seen for the single 100 µL injections, while the 200 µL double injections clearly are double the peak area values of the 100 µL injection for both 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol. This confirmed the conclusion made at the end of *Section 3.9*, which stated that the only reliable and efficient way to perform a 200 µL injection using the new sample loop was to use a quick 100 µL injection program with a 0.3 minute run time, followed shortly after by a normal 100 µL injection with a 40 minute run time.

3.11 Removal of Small XAD-4 Particles Using Centrifugation

Amberlite XAD-4 is a polystyrene-divinylbenzene resin that is coated on filters being studied by M. Saccon to measure total amounts of gas and particle phase methyl-nitrophenols. Extraction of these XAD-4 coated filters, using the same experimental procedure described in *Section 2*, begun in early 2011, and since then, a significant increase in pressure occurs when performing HPLC runs with the XAD-4 coated filters. This was thought to be occurring due to small XAD-4 particles entering the HPLC and accumulating at the guard column. Once a significant amount of these small XAD-4 particles accumulated, the guard column became

blocked and the pressure increased rapidly until it reached the instrument maximum, where the instrument automatically shut off.

Small modifications were made to prevent the pressure from building to the instrument maximum. Firstly, the 0.45 μm PTFE Chromspec syringe filter used, as stated in *Section 2.2*, was replaced with the smallest syringe filter available, a 0.20 μm PTFE Chromspec syringe filter, getting rid of the majority of the larger XAD-4 particles. Filter extractions using the new syringe filters resulted in the same outcome, which implied that the problem involved the XAD-4 particles that were less than 0.20 μm in size. Another solution that was proposed was to centrifuge the extract solution, as stated in *Section 2.2*, for a longer duration. If centrifuged for a longer duration, it was possible that the small XAD-4 particles would sediment to the bottom of the centrifuge tube, which would prevent them from entering the HPLC instrument.

To see if centrifugation successfully removed many of the small particles, pictures were taken using a scanning electron microscope (SEM). A 1 mL portion of a slurry containing ACN and XAD-4 particles that were ground for 34 hours, as was done when coating the filters, was placed in a centrifuge tube. The XAD-4 slurry was centrifuged for 60 minutes, with 5 μL being removed from the syringe every 15 minutes during the 60 minute centrifugation period. The 5 μL drop of the slurry removed after 15, 30, 45 and 60 minutes, respectively, were placed and allowed to dry on a specimen stub. Once the stubs had completely dried, they were coated with a thin layer of gold-palladium using a Hummer VI Sputtering System. The SEM samples were then placed in the Hitachi S-520 Scanning Electron Microscope, and pictures were obtained for each of the four samples.

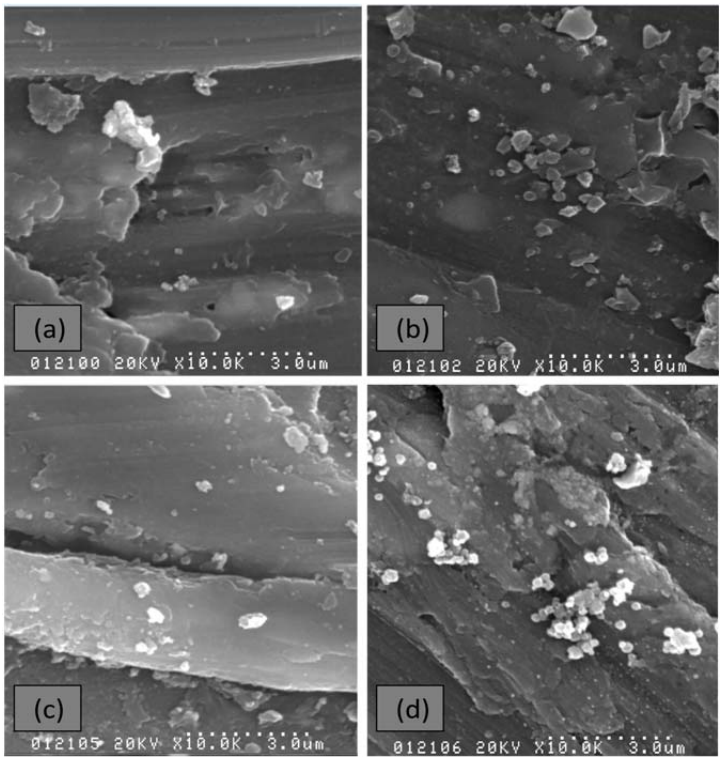

***Figure 10**: Electron Microscope Pictures of Slurry Containing XAD-4 Suspended in ACN – (a) centrifuged for 15 minutes; (b) centrifuged for 30 minutes; (c) centrifuged for 45 minutes; (d) centrifuged for 60 minutes*

The pictures in *Figure 10* illustrated that many small particles remained in the solutions after 15, 30, 45 and 60 minutes of centrifugation, respectively. All four pictures contained XAD-4 particles that were smaller than 0.3 μm in size, with many of the particles being even smaller than this. The calculation for the sedimentation of XAD-4 suspended in ACN from centrifugation, as seen in the *Appendix*, confirms that very small particles (of radius 0.05 μm) will not sediment after 1 hour. This confirmed that XAD-4 particle accumulation in the HPLC will be an ongoing problem for future extractions of XAD-4 coated filters, so further study of other methods to try to remove these small particles must be conducted.

4. Conclusion and Future Work

The goal of this research project was to optimize the experimental method used to sample and analyze atmospheric nitrophenols in order to improve the recoveries of these nitrophenols and to increase the overall efficiency of this method. A number of tests were performed in which specific modifications were made to both the volume reduction step and the HPLC sample clean-up step.

The results found in *Section 3.1* showed that the volume reduction step, where filter extract was evaporated under nitrogen, did not have a significant effect on nitrophenol recovery for this method. The results found in *Sections 3.2 – 3.10* showed that improvements to the HPLC clean-up step procedure allowed for significant gains in nitrophenol recovery. The results from *Section 3.2* determined that a 100 µL injection was more optimal than a 200 µL injection, suggesting that smaller injections be used, effectively doubling the number of HPLC runs for each filter extraction, from four to eight. *Sections 3.3 and 3.4* confirmed that an adjustment to the solvent gradient program, adding 10 minutes to the each HPLC run time, allowed the column to return to 100% ACN, which resulted in better reproducibility of the HPLC results. The results found in *Section 3.5* determined that sample analysis which used the eight 100 µL injections, each with a 10 minute longer run time, effectively doubled the recoveries of two of the internal standards, from around 30% to about 70%. The results from Section 3.6 indicated that the optimal injection draw and eject speeds should be set at the maximum speed allowed for the instrument, which in this case was 1000 µL/min. Results shown in *Section 3.7* suggested that it was possible to reduce the number of HPLC injections done on the final two rinsing steps in order to decrease the duration of the overall HPLC clean-up process, since a minimal percentage of original filter extract was recovered in these runs. The results from *Section 3.8* supported the results in *Section 3.7*, since it was determined that internal standard recoveries on filters extracted were not affected by decreasing the number of HPLC runs performed on the final two rinsing steps. *Sections 3.9 and 3.10* showed that 200 µL injections of both single and multiple compound mixtures could only efficiently be performed on the new 200 µL sample loop, using two consecutive 100 µL injections instead of as a single 200 µL injection. The testing performed in *Section 3.11* looked at fixing a pressure build-up problem occurring with the HPLC instrument when extracting filters coated with XAD-4. These results showed that centrifugation was not a solution for

preventing very small (less than 0.2 μm) XAD-4 particles from entering the HPLC and clogging the guard column.

Overall, this research project was able to identify an area in the extraction procedure where significant nitrophenol loss was occurring. The resulting method modifications were able to more than double the nitrophenol recovery, from 30% to 70%, making the overall procedure more efficient. The additional nitrophenol material that is recovered in filter extractions will aid successful future GC-IRMS measurements.

For future work, further testing using a series of sampled filters should be conducted in order to compare possible ways of reducing the number of HPLC runs required. Since *Sections 3.9 and 3.10* confirmed the successful use of a 200 μL injection with the new sample loop using a multiple injection program, tests should be conducted to see if the number of HPLC steps can effectively be reduced back to four 200 μL injections. If successful, this would reduce the duration of the HPLC clean-up step in half. As well, effort needs to be placed on finding a way to prevent the very small XAD-4 particles from entering the HPLC instrument. Since sampling with XAD-4 coated filters is something that will continue within this research group, frequent replacement of the guard column to remove the blockage is a time consuming, and quite expensive task. Further filtration methods for the extract entering the HPLC instrument should be explored in order to eliminate this problem.

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Appendix

Calculation of Sample Loop Volume

- Volume of the sample loop is calculated using the density of water (ρ_{H2O} = 1.00 g/mL) and a conversion factor to get the volume in µL:

$$0.0995\ g \times \left(\frac{mL}{1.00\ g}\right) \times \left(\frac{1000\ \mu L}{1\ mL}\right) = 99.5\ \mu L$$

- The sample loop was determined to be approximately 100 µL in volume

Calculation of XAD-4 Sedimentation in ACN from Centrifugation

- Stokes Law depicts the force required (F) to move a sphere with radius (r) at velocity (u) through a fluid which has viscosity (η):

$$F = 6 * \pi * r * \eta * u$$

Note: Force can be either gravitation ($F = F_g = m * g$, where g is the gravitational acceleration of the earth = 9.81 m s^{-2}) or centrifugal acceleration ($F = F_s = m * \omega^2 * r_c$, where r is radius of centrifuge)

- Effective mass is determined using the particle radius (r) and the particle density (ρ_{XAD}) minus the solvent density (ρ_{ACN})in order to correct for buoyancy:

$$m_{eff} = 4/3 * \pi * r^3 * (\rho_{XAD} - \rho_{ACN})$$
$$m_{eff} = 4.189 * r^3 * (1.02\ g\ cm^{-3} - 0.785\ g\ cm^{-3})$$
$$m_{eff} = 0.984\ g\ cm^{-3} * r^3$$

- Therefore:

$$F_s = m * \omega^2 * r$$

$$6 * \pi * r * \eta * u = 4/3 * \pi * r^3 * (\rho_{XAD} - \rho_{ACN}) * \omega^2 * r$$

- The angular velocity, ω, is determined by $\omega = 2 * \pi *$ number of rotations per second:

$$\omega = 2 * \pi * (3300\ rpm/60\ s\ min^{-1}) = 345.6\ s^{-1}$$

- Isolating for u:

$$u = \frac{2 * r^2 * (\rho_{XAD} - \rho_{ACN}) * \omega^2 * r_c}{9 * \eta}$$

- Solving for u, assuming a particle with radius of 1 µm ($1*10^{-6}$ m), and knowing r_c = 0.12m and viscosity of ACN (η) is 0.34 mPa s = 0.34 m^{-1} g s^{-1}:

$$u = \frac{2 * (1*10^{-6}\ m)^2 * 0.235*10^6\ g\ m^{-3} * (345.6\ s^{-1})^2 * (0.12m)}{9 * 0.34\ m^{-1}\ g\ s^{-1}} = 2.2*10^{-3}\ m\ s^{-1} = 7.9\ m/h$$

Note: This determines that an XAD-4 particle with a 1 µm radius will sediment at a rate of 7.9 m/h and an XAD-4 particle with a 0.1 µm radius will only sediment at 7.9 cm/h. Assuming the centrifuge tube is filled to about 2.5 cm, it will take about 20 minutes to remove a 0.1 µm radius particle. However, with particles smaller than this (0.05 µm radius), the particle will not be removed within 1 hour.