Development of a Sample Preparation Method for Stable Isotope Ratio Measurements of Atmospheric Particulate Organic Matter

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Abstract

Atmospheric composition and chemistry of particulate matter suspended in the atmosphere has been of great interest to the scientific community, particularly within recent years. Since atmospheric particulate matter (PM) affects health and climate, its origin and transformations in the atmosphere have been subject to numerous studies during the last decades. One of the still poorly understood sources of PM is the formation of secondary organic PM from the photo-oxidation of atmospheric volatile organic compounds (VOC). It has been proposed that combining concentration and isotope ratio measurements of secondary particulate organic matter will allow to test the applicability of the results of laboratory studies to the atmosphere.

In this project a recently developed method to determine concentrations and isotope ratios of methylnitrophenols, which are products of the atmospheric photo-oxidation of toluene, in atmospheric PM has been modified and tested.

The method is based on collection of particulate matter with high volume samplers, extraction of the filters and several sample clean-up and volume reduction steps. Analysis of the methylnitrophenols in the processed sample extract is done by gas chromatography. For concentration measurements conventional mass spectrometry is used as detection method, for isotope ratio measurements gas chromatography in combination with a combustion interface and isotope ratio mass spectrometry (GCC-IRMS) is used. Modifications to the method include the reduction of the number of target compounds, choice of internal standards, and the type of GC column used for analysis of the processed sample extract were altered. These modifications eliminated previously existing problems due to peak overlaps. Using the modified method two atmospheric

samples were analyzed. Using GC-MS in the selective ion monitoring (SIM) mode the detection limit was 4×10^{-4} pg/m^3. It is expected that this is sufficient to quantitatively determine the concentrations of 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol in most atmospheric samples. However, isotope ratio measurements by GCC-IRMS require higher concentrations in the processed extract than conventional GC-MS analysis. However, measurements of carbon isotope ratios by GCC-IRMS will only be possible for the samples collected at high levels of atmospheric pollution.

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1. Introduction

Volatile organic compounds (VOCs) are emitted into the earth's atmosphere by both natural and industrial processes in great amounts each day[1]. Consisting of thousands of different compounds, VOCs affect photochemical processes in the atmosphere which can contribute to climate changes. Toluene, a common VOC, is emitted as part of air pollution by vehicle exhaust as well as industrial sources and can undergo photo-oxidation in the atmosphere to produce secondary particulate organic matter (POM)[1]. Products of this reaction include nitrophenols, as well as numerous other compounds. Several methylnitrophenols have been detected by Satoshi Irei (Ph.D. student, Rudolph's group) in POM during a flow reactor experiment studying the reaction of toluene with OH. The formation of nitrophenols as secondary organic aerosols though the photo-oxidation of toluene is further confirmed though the smog chamber experiment of Hamilton *et al.*, 2005 [2]. To our knowledge, atmospheric methyl-nitrophenols are primarily due to the atmospheric oxidation of toluene, but may also have some primary sources such as exhaust from vehicles[1].

The target compounds, that is, the compounds to be analyzed in atmospheric PM, will be methylnitrophenols, particularly, 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol. These two compounds were the most prominent nitrophenols found in lab studies of the oxidation of toluene in the gas-phase.

Each source of pollutant has its own ^{13}C isotope ratio, and therefore is specific to the compounds it emits into the atmosphere. The $^{13}C/^{12}C$ isotope ratio is often used rather than H isotope ratios since the data available is restricted to source signatures[3]. With regards to toluene, some stable carbon isotope measurements have been performed close

to some traffic related emission sources in the greater Toronto area, as well as some suburban areas, by Rudolph *et al*., 2002 [4]. In contrast to this, no data or measurement methods for stable carbon isotope ratios of atmospheric nitrophenols have been published.

Knowledge of the isotope ratios of photo-oxidation products of toluene in POM will provide information on the chemical processing that affects toluene. This will allow for the differentiation between the influence of chemical reactions, primary emissions and mixing processes[5]. It is expected that measurements of the isotope ratios and concentrations of photo-oxidation products of toluene in POM in the atmosphere will allow testing the applicability of the results of the laboratory measurement for atmospheric conditions. Overall, a better understanding of the formation of secondary products from the atmospheric oxidation of toluene as well as of the origin of nitrophenols, which are toxic to both humans and plants, in atmospheric POM can be achieved by combining isotope ratio and concentration measurements[6].

A preparation method developed by Dr. Sophie Moukhtar (Post Doctoral Fellow in Dr. Rudolph's group) allows the GC-MS analysis of methylnitrophenols in atmospheric particulate matter. The procedure consists of a number of steps: Collection of atmospheric particulate matter from large air volumes (several 1000 m^3) on filters, extraction of the filters with acetonitrile, reduction of the extract volume, HPLC separation, a solid phase extraction step, reduction of the total volume of the processed sample to some 50 μL, and derivatization of the methylnitrophenols for GC-analysis[7].

However, analysis of ambient samples shows that two problems still exist. Firstly, the very low concentrations of methylnitrophenols in ambient particulate matter will

make accurate isotope ratio measurements, which require larger samples than concentration measurements, very difficult. Furthermore, there were problems with the recovery of 2-methyl-6-nitrophenol, which resulted in variabilities in the results.

In Dr. Sophie Moukhtar's method of determining the concentrations and isotope ratios of methylnitrophenols, two internal standards were used; 2-methyl-3-nitrophenol, and 2,6-dimethyl-4-nitrophenol. However, after several sample measurements, it was discovered that the recovery of 2,6-dimethyl-4-nitrophenol was low and there were variabilities in the results. Consequently, the choices of internal standards have to be reassessed.

In order to obtain an isotope ratio measurement, ideally, a concentration of 10 ng/μL in the processed sample extract for each analyte must be acquired to allow accurate measurements. With the existing procedure, for most ambient samples, a concentration of less than 3 ng/μL is currently obtained. Furthermore, the GC-measurements of processed PM samples still contained a number of peaks interfering with the analysis of the methylnitrophenols and some of the methylnitrophenols were not completely separated from each other.

It was found from Satoshi Irei's flow reactor experiment that were two methylnitrophenols which were found in higher abundance than others, being 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol[8]. Since there are difficulties in attaining a clean sample with concentrations that are above the detection limit, two target compounds were chosen rather than the six target compounds that were chosen previously.

The purpose of this project is to obtain higher concentrations of 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol in the processed sample extract. Furthermore, the objective will be to obtain a cleaner sample and thus reduce peak overlapping.

2. Experiment

The purpose of this research project was to improve the method that was used for the BAQS campaign by Dr. Sophie Moukhtar. This method is described below.

2.1 Sampling Procedure

Prior to sampling, 8 x 10 inch quartz filters (Pallflex Membrane Filters – 2500QAT) were baked at 900°C for a period of 24 hours in a large muffle furnace (Fisher Scientific Model 550-58)[9]. This was done as a cleaning procedure to remove organic contaminations. Particulate organic matter (POM) was collected on the cleaned Quartz filters using a high-volume air sampler, collecting particles sizes below 2.5 μm at a sample flow rate of 1.13 m^3/min. The sampling occurred for a period of three to seven days, depending on season. Once sampling finished, the filters were removed from the air sampler and stored in glass jars in a freezer until analysis.

2.2 Extraction

The concentration range of the standards used was from 5 to 50 ng/μL. An atmospheric filter was cut into eight pieces, and stored in a small glass jar because of its inertness. One of the pieces was spiked with 40 μL each of the internal standards, which were 2-methyl-3-nitrophenol and 2,6-dimethyl-4-nitrophenol. Concentrations of internal standards can be seen in *Table* 1. Each of the nitrophenols were purchased from Sigma-Aldrich. The jar was filled with approximately 15-20 mL of acetonitrile (HPLC grade – Sigma-Aldrich). This was enough to completely immerse the filter pieces. The filter was

then stirred with a glass stirring rod. The jar was then placed in a Bransonic Ultrasonic Cleaner (Model SS10R-DTH) for 15 minutes. The liquid from the jar was removed with a pipette into a syringe equipped with a PTFE Chromspec syringe filter that would remove particles larger than 0.45 μm. The solution was transferred into a round-bottom flask. This procedure was repeated three additional times.

The solution in the round-bottom flask was then evaporated by rotary evaporation at a temperature of 42°C, to a volume of approximately 1 mL. It was then transferred to a small vial and the solvent was further reduced by passing a flow of nitrogen over the solution until there were only 200 μL of solution left.

Table 1 *– Concentrations of Standards Used*

Standard	*Concentration (ng/μL)*
2-methyl-4-nitrophenol	124
4-methyl-2-nitrophenol	133
2-methyl-3-nitrophenol	100
2,6-dimethyl-4-nitrophenol	104
2-methyl-5-nitrophenol	131
heptadecane ($C_{17}H_{36}$)	182
octadecane ($C_{18}H_{38}$)	183
nonadecane ($C_{19}H_{40}$)	182
4-ethyl-resorcinol	101

2.3 HPLC Sample Clean-Up

The 200 μL volume was separated by an HP 1050 HPLC as a cleaning procedure. After transfer of the sample for HPLC separation, the round-bottom flask was filled with 5 mL of acetonitrile, and was evaporated to 200 μL. This volume then also was separated by the HPLC. This was repeated two more times, for a total of four HPLC analyses.

The separation was done using an (name type of HPLC) instrument equipped with a Supelco Supelcosil LC-18 column, as well as a Variable Wave Detector (VWD). The wavelength selected was 320 nm and the solvent flow rate used was 1.00 mL/min. A

gradient system was set up using two solvents – acetonitrile and milli-Q water (18Ω), which was deionized using a Milli Q Gradien A10 Millipore system. The gradient can be seen in *Figure 1*.

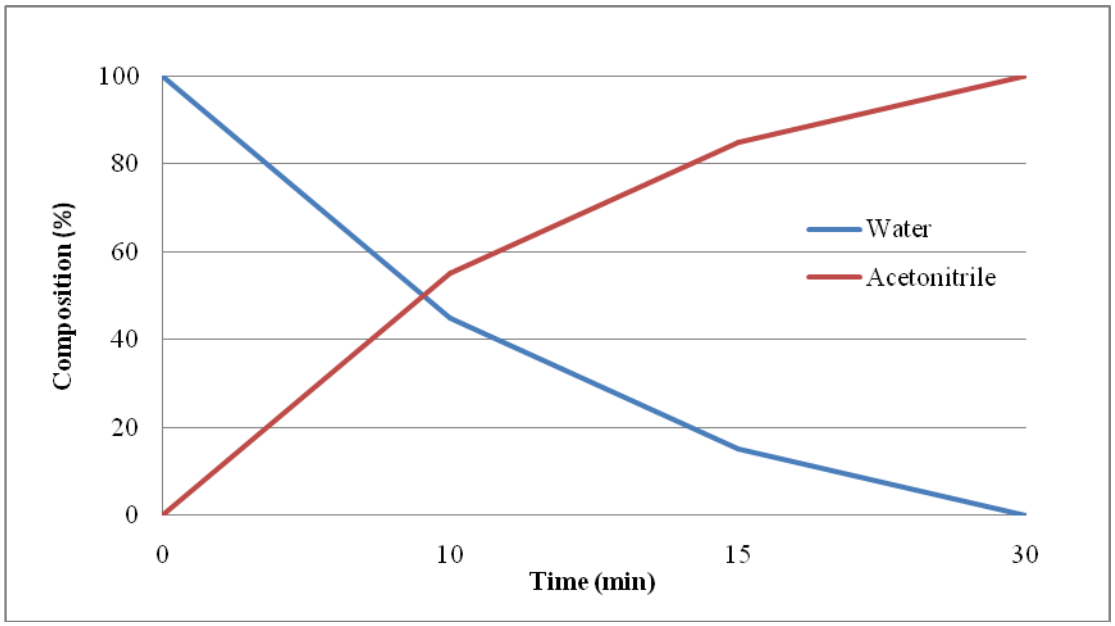

Figure 1 *– Gradient program used for HPLC separation*

The eluent was collected between 11 and 19.5 for each of the four runs into the same flask.

2.4 Solid Phase Extraction

The HPLC eluent fraction contains water, acetonitrile, nitrophenols, and other unidentified compounds. The acetonitrile is evaporated using a temperature-controlled rotator evaporator. The temperature is kept between 5 and 10°C and the solution is evaporated until it is reduced to half of its volume which takes approximately 2 hours. This step removes most of the acetonitrile from the solution.

The evaporated solution is acidified with 50 μL of H_3PO_4 so that the final pH is approximately 2. This solution is then subjected to solid phase extraction, so to remove water.

In order to condition the cartridge, 1 mL of acetonitrile is pipetted into a Waters Oasis HLB Extraction Cartridge[10]. Once the acetonitrile was eluted from the cartridge into a waste flask, 1 mL of milli-Q water was eluted through the cartridge into waste. The acidified solution is then added to the cartridge, and eluted through. 3 mL of milli-Q water is dispensed into the flask, acidified with 30 μL of H_3PO_4 and added to the cartridge. The pH of the solution is approximately 2. As the solution elutes through the cartridge, water passes through, while the methylnitrophenols, as well as other compounds remain in the stationary phase of the cartridge. Once the solution has eluted, but before the cartridge is completely dry, approximately 10 mL of acetonitrile is eluted through, so to extract the methyl nitrophenols from the stationary phase of the cartridge The eluent is collected in a flask. The solution is then evaporated until the final solution is in the region of 60 μL. The evaporation is repeated twice by adding approximately 3 mL of acetonitrile to the flask, evaporating it, and adding it to the same vial as the first evaporation.

2.5 GC-MS Analysis

20 μL of 4-ethyl-resorcinol (101 ng/μL), purchased from Sigma Aldrich, was added to the 60 μL solution. It was stirred, and half of the solution was saved in a glass vial and stored in a freezer if a second analysis is required. The solution was then derivatized with bis(trimethylsilyl) trifluoroacetamide (BSTFA), purchased from Regis Technologies Inc. 10 μL of the BSTFA was added to the remaining solution, and the solution was capped and mixed with a magnetic stirring bar for a period of 5 minutes. An example of a typical derivatization reaction using BSTFA and 2-methyl-4-nitrophenol can be seen in *Figure 2*. An advantage to using BSTFA as a derivatizing agent is that the

byproducts produced are high in volatility and elute from the GC column before analytes, and often with the solvent[12].

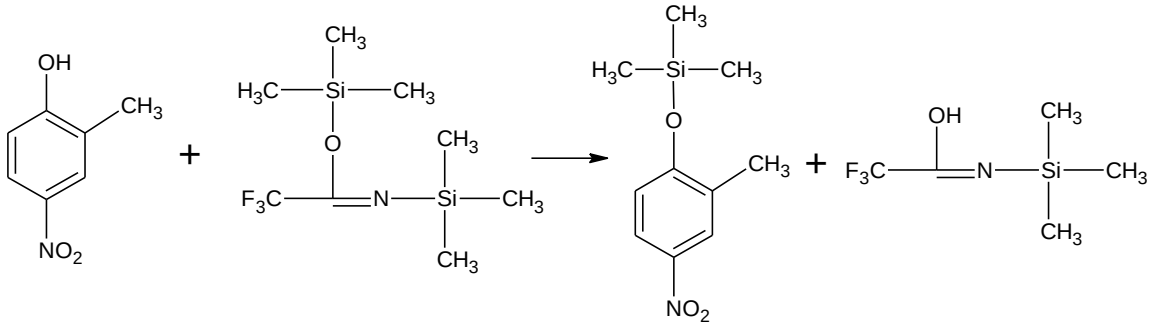

Figure 2 *– Derivatization reaction of 2-methyl-4-nitrophenol and BSTFA at room temperature*

The solution is then deposited in a glass vial, and analyzed using a HP 5890 Series II GC, equipped with an HP 5972 Series MS detector. The gas chromatographic portion of the experiment was based on an 82 minute temperature program (*Figure 3*). The column used was a DB-1 (J & W Scientific – 100% dimethyl polysiloxane) 100 m x 0.252 mm i.d. (0.5 μm film thickness) capillary column.

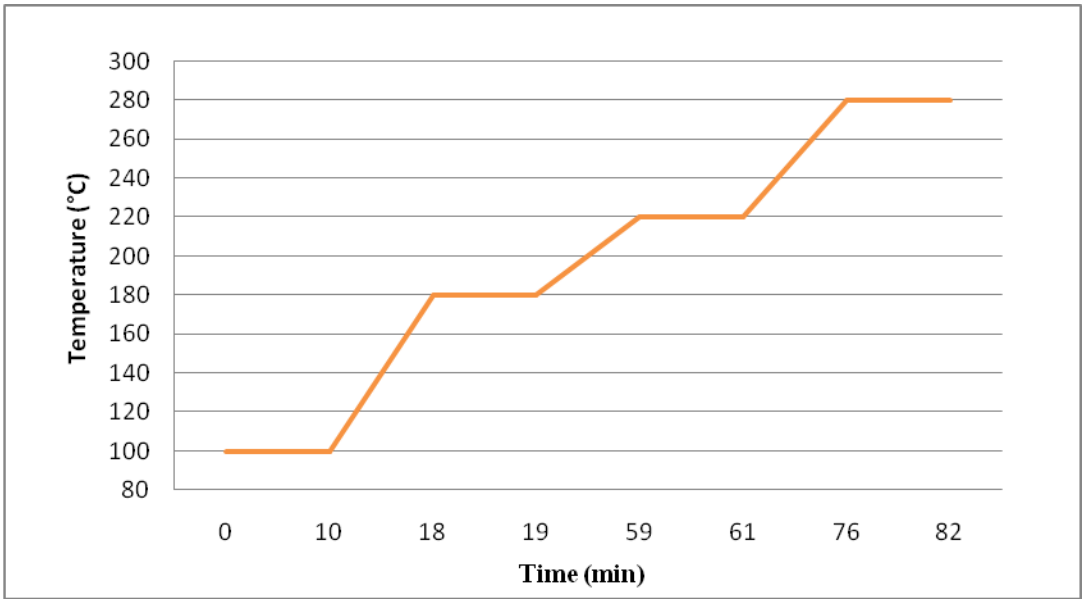

Figure 3 *– Temperature program of GC instrument using a DB-1 column*

2.6 Method Modifications

These method modifications were designed and conducted by Marina Saccon for the purpose of this project. The *filter extraction* was altered such that the internal standards spiked on the filter were 2-methyl-3-nitrophenol and 2-methyl-5- nitrophenol. The target compounds were 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol. Since only two target compounds were used, the collection time window for the *HPLC sample clean-up procedure* was decreased to 3.5 minutes, and was collected from 12 minutes to 15.5 minutes. The column was changed for the *GC-MS Analysis*. The column was changed to an Rtx-5 (Restex, 5% diphenol & 95% dimethyl polysiloxane) 60m x 0.25mm i.d. (0.5 µm film thickness). Due to the change in column, the analysis time was 62.5 minutes rather than 82 minutes. The temperature program used can be seen in *Figure 4*.

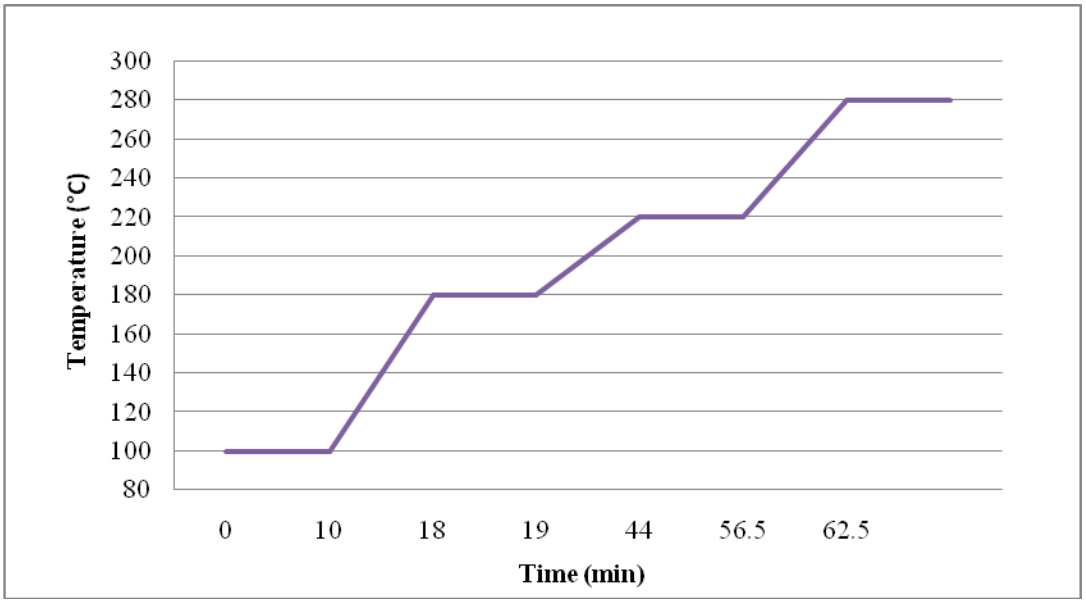

Figure 4 *– Temperature program of GC instrument using an Rtx-5 column*

2.7 Experiments Conducted

- Extracted atmospheric filter **Q110907A** prior to method modifications
- GC instrument was calibrated each time a modification was made to the GC instrument, including change of threshold, change of standards used, change of column and use of SIM or TIC
- Standards were run on HPLC to verify retention times and test for candidate internal standards
- Recoveries of target compounds and internal standards during new collection time window were tested
- Blank filter spiked with internal standards was extracted and tested for contaminant
- Standard solution (acidified 2-methyl-4-nitrophenol, 4-methyl-2-nitrophenol and 2-methyl-3-nitrophenol) ran through SPE cartridge and eluted with acetonitrile to test for overlap on Rtx-5 column
- Standards were ran through GC-MS to test for retention times
- Applicability of change of ISRC from 4-ethyl resorcinol to 3 alkanes was tested by the GC-MS
- Stability of 2-methyl-3-nitrophenol was tested with atmospheric filters **Q1180108A** and **Q061208A**
- Atmospheric filters **Q261007B** and **Q011107A** were spiked with target compounds and internal standards to test recoveries
- Atmospheric filters **Q100807A** and **Q280907B** were tested to show method improvements with all method modifications

3. Results and Discussion

3.1 Selection of Target Compounds

The target compounds that were chosen and will be the focus of this work are methylnitrophenols. In Dr. Moukhtar's research, upon what this paper is based, there were six target compounds of interest; including, 3-methyl-2-nitrophenol, 2-methyl-6-nitrophenol, 4-methyl-2-nitrophenol, 2-methyl-5-nitrophenol, 3-methyl-4-nitrophenol and 2-methyl-4-nitrophenol[6].

***Table 2** – Summary of Results from BAQS Campaign in both Harrow and Ridgetown Sites*

HARROW						
Filter Name	**From:**	**To:**	**2-me-4-nitrophenol (ng/m^3)**	**4-me-2-nitrophenol (ng/m^3)**	**2-me-6-nitrophenol (ng/m^3)**	**3-me-2-nitrophenol (ng/m^3)**
H190607	06/19/07	06/22/07	0.013	0.006	N/A	0.007
H220607	06/22/07	06/25/07	0.017	0.004	N/A	N/A
H250607	06/25/07	06/28/07	0.011	0.004	N/A	N/A
H280607	06/28/07	07/02/07	0.017	0.005	N/A	N/A
H050707	07/05/07	07/09/07	0.013	0.005	N/A	N/A
H090707	07/09/07	07/10/07	0.023	0.013	0.009	0.006
RIDGETOWN						
R200607	06/20/07	06/23/07	0.009	0.004	N/A	N/A
R230607	06/23/07	06/26/07	0.009	0.004	N/A	N/A
R260607	06/26/07	06/29/07	0.006	0.004	N/A	N/A
R290607	06/29/07	02/07/07	0.019	0.007	N/A	N/A
R020707	07/02/07	07/05/07	0.013	0.005	0.002	N/A
R050707	07/05/07	070/8/07	0.011	0.006	N/A	N/A
R080707	07/08/07	07/10/07	0.014	0.01	0.008	N/A

As found in the Border Air Quality Study (BAQS), which was a campaign that ran over a period of three weeks in Ridgetown and Harrow, Ontario, many of the target methylnitrophenols were not abundant in the atmosphere (*Table 2).* Some of the methylnitrophenols were below the detection limit of 0.004 ng/m^3 and were not detected

on any of the days that filters were sampled on[6]. Satoshi's flow reactor experiments were also consistent with results from BAQS[2].

It was shown that primarily, 2-methyl-4-nitrophenol was found most abundantly in both the Ridgetown and Harrow sites, although the concentrations were still very low. With regards to 4-methyl-2-nitrophenol, it was the second most abundant target compound found from BAQS. This made both of the methylnitrophenols potential target compounds.

The retention times of both of the methylnitrophenol compounds were tested separately, and then collectively; the retention times and peak widths can be seen in *Table 3.* The collection time window of the two target compounds was within a time range that was smaller than the previous time window, which would provide for a cleaner sample. The next step of the research is to test for internal standards.

Table 3 *– Retention times and peak widths of target compounds*

Standard	*Retention time (min)*	*Peak width (min)*
2-methyl-4-nitrophenol	11.858	1.2
4-methyl-2-nitrophenol	14.106	1.3
3-methyl-2-nitrophenol & 2-methyl-6-nitrophenol	13.037 & 14.953	3.0
2-methyl-3-nitrophenol	11.281	1.6
2-methyl-5-nitrophenol	13.606	1.4

3.2 HPLC – Determination of Internal Standards

When selecting an internal standard, there are two criteria that must be met. Firstly, one must find a compound that behaves similarly to the target compounds. Secondly, the chosen internal standard must not be found in significant concentrations in the atmosphere.

Several compounds were tested. These internal standard candidates were also methylnitrophenols to fulfill the first criteria for choosing an internal standard, and they were nitrophenols that were found in negligible concentrations in the atmosphere. From this, a new internal standard was chosen based on its retention time from the HPLC, since a short collection time window from this step was required to have a clean sample. 3-methyl-2-nitrophenol and 2-methyl-6-nitrophenol, 2-methyl-5-nitrophenol and 2-methyl-3-nitrophenol were the methylnitrophenols tested. The retention times and peak widths of various internal standards tested can be seen in *Table 3*.

The methylnitrophenols were ran through the HPLC instrument separately to determine their respective retention times. Each of the internal standard candidates were then ran through the HPLC together with 2-methyl-4-nitrophenol. This was done in order to monitor any shifts in retention time due to interactions between the methylnitrophenols. The collection time window that would be used for each of the internal standards can be seen in *Table 4*.

Table 4 *– Collection time window range of candidate internal standards with 2-methyl-4-nitrophenol*

Internal Standard with 2-methyl-4-nitrophenol	*Collection time window range (min)*
3-methyl-2-nitrophenol & 2-methyl-6-nitrophenol	12.0 – 15.3
2-methyl-3-nitrophenol	11.5 – 13.5
2-methyl-5-nitrophenol*	12.0 – 15.5

*2-methyl-5-nitrophenol was tested with both 2-methyl-4-nitrophenol and 4-methyl-2-nitrophenol

As seen in *Table 4*, 2-methyl-3-nitrophenol is an exceptional internal standard as it overlaps with 2-methyl-4-nitrophenol when detected from the HPLC instrument.

Furthermore, as seen in *Table 2*, it was undetectable in most of the samples and in the two samples that it was detected, it was in very low concentrations.

The improvement in the duration of the time collection widow can be seen in *Figures 5 & 6. Figure 5* depicts the chromatogram which shows each of the initial target compounds, as determined by Dr. Sophie Moukhtar. The eluted solution from the HPLC was collected from 11 to 19.5 windows, which was 8.5 minutes. *Figure 6* illustrates the chromatogram containing the newly selected target compounds and the internal standards – 2-methyl-3-nitrophenol and 2-methyl-5-nitrophenol. With this change, the solution was collected from 12 to 15.5 minutes, which had a collection window of only 3.5 minutes.

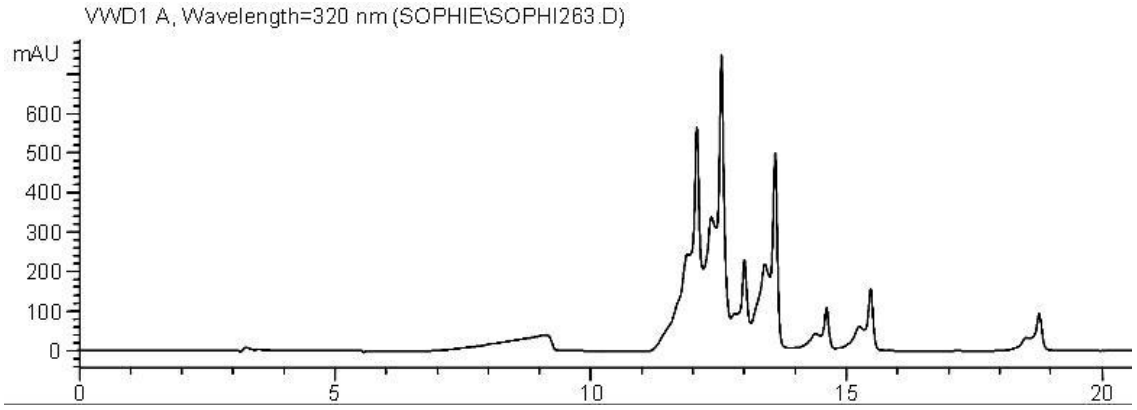

***Figure 5** – HPLC chromatogram of Target Compounds and Internal Standards using the unchanged method.*

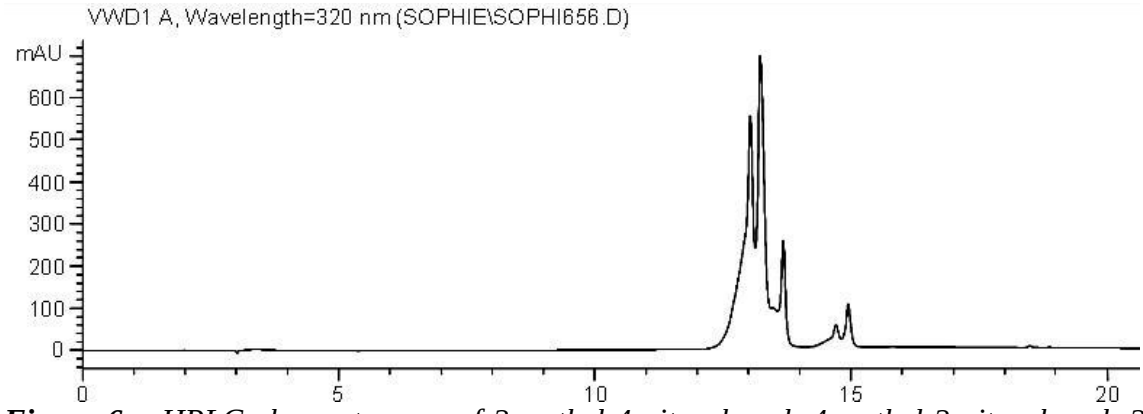

***Figure 6** – HPLC chromatogram of 2-methyl-4-nitrophenol, 4-methyl-2-nitrophenol, 2-methyl-3-nitrophenol and 2-methyl-5-nitrophenol*

It was also tested if the recovery of 2-methyl-3-nitrophenol is comparable to the recoveries of the target compounds. This was tested by spiking known amounts of internal standards and target compounds on a filter that was collected during winter - Q180108A. A winter filter is used to measure recoveries because the target compounds are the products of the photo-oxidation reaction of toluene, therefore during winter, they should be present in very small, often undetectable, concentrations in the atmosphere. The results of the recovery tests can be seen in *Table 5*.

However, for a filter (Q061208A) that was stored for 3 weeks in the refrigerator between extraction and HPLC collection, it was found that 2-methyl-3-nitrophenol was not stable when stored for such periods of time, although this could be due to the uncertainty. The results can be seen in *Table 5*.

Table 5 *– Recovery of 2-methyl-3-nitrophenol and 2 Target Compounds with filter Q180108A and with filter Q061208A*

Compound	***Filter Q180108A***	***Filter Q061208A***
	Recovery (%)	*Recovery (%)*
2-methyl-4-nitrophenol	23 ± 15	22 ± 15
4-methyl-2-nitrophenol	26 ± 15	30 ± 15
2-methyl-3-nitrophenol	20 ± 15	14 ± 15

At this point, it was decided that an additional internal standard should be chosen. Based on results, presented in *Tables 3 & 4*, it was decided to use 2-methyl-5-nitrophenol as a second internal standard. In order to confirm that this was a suitable internal standard, the two internal standards as well as the two target compounds were spiked onto two atmospheric winter filters and their recovery was determined using the modified method. The recoveries can be seen in *Table 6*.

***Table 6** – Recoveries using TIC of 2 target compounds and 2 internal standards on filters Q261007B and Q011107A*

Standard	*Filter Q261007B*	*Filter Q011107A*
	Recovery (%)	*Recovery (%)*
2-methyl-4-nitrophenol	31 ± 15	31 ± 15
4-methyl-2-nitrophenol	33 ± 15	25 ± 15
2-methyl-3-nitrophenol	39 ± 15	30 ± 15
2-methyl-5-nitrophenol	40 ± 15	28 ± 15

3.3 GC - Column

From analysis of GC-MS chromatograms, it was noticed that there was a compound that was overlapping with 2-methyl-4-nitrophenol. It was discovered that the overlapping compound came from the solid phase extraction step. In order to eliminate the overlap of 2-methyl-4-nitrophenol with the SPE contaminant, the column of the GC was changed. The intention was to increase the polarity of the column, which would change the separation of the compounds.

Figure 7 shows the TIC of 2-methyl-4-nitrophenol (approximately 12 ng/µL). As seen, its retention time in the GC is 32.23 minutes. It is confirmed to be 2-methyl-4-nitrophenol by the characteristic nitrophenol derivative peaks in the mass spectrum at *m/z* 225, 210 and 165. *Figure 8* is the GC chromatogram obtained for the extract of a blank filter that was spiked with only the internal standards, and ran through the whole procedure. As seen in *Figure 8*, there is a peak at 32.24, which is very close to a 2-methyl-4-nitrophenol. Comparison of the mass spectrum of the interfering peak with the mass spectra library on the computer showed that this unknown compound is most likely eicosane.

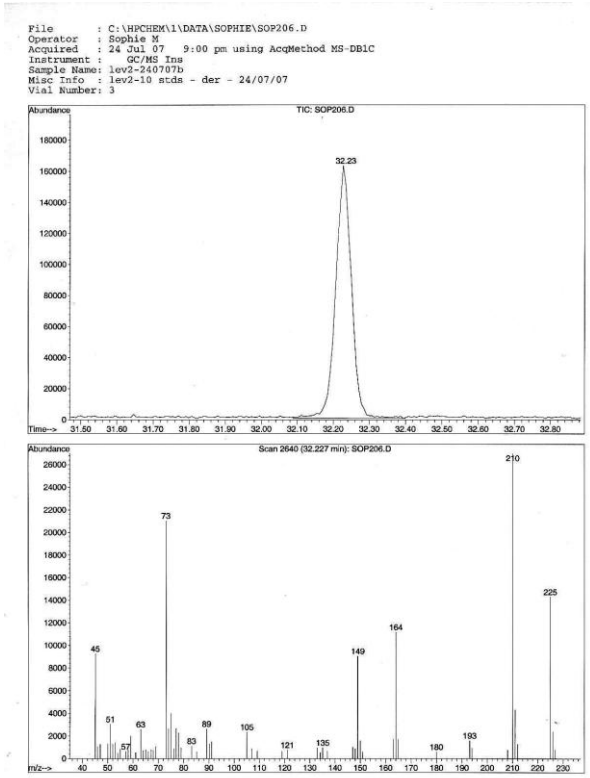

Figure 7 *– TIC (top) and MS spectrum (bottom) of 2-methyl-4-nitrophenol (40uL)*

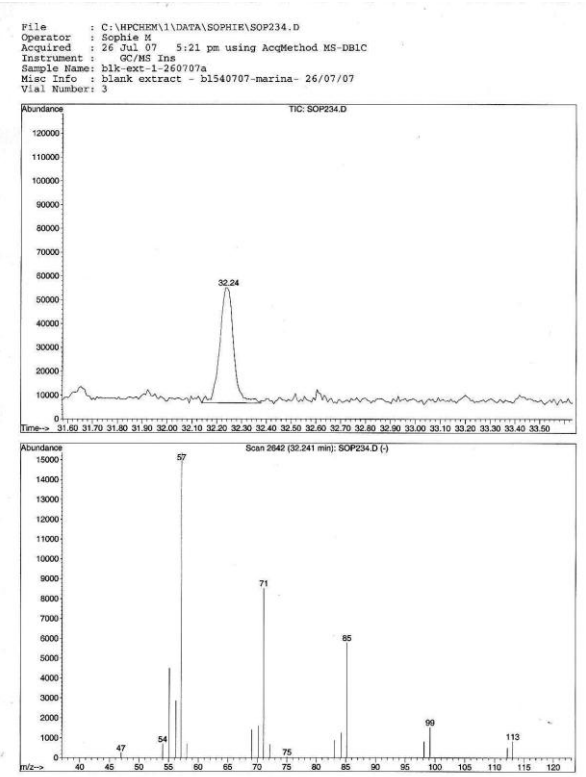

Figure 8 *– TIC (top) and MS spectrum (bottom) of unknown SPE contaminant*

The chromatograms shown were obtained using a DB-1 column in the GC-MS system to separate the compounds. Provided that the Rtx-5 column will eliminate the overlap of 2-methyl-4-nitrophenol and the unknown compound from the SPE test, and that no further overlap of any of the compounds of interest, the problem will be solved. In fact, through rigorous analysis, there was found to be no overlap between any of the internal standards or the target compounds.

Figure 9 is an overlay chromatogram of two separate chromatograms. One chromatogram is the result of using a blank for the SPE stage, and the second is the two target compounds, and 2-methyl-3-nitrophneol. As seen in *Figure 12*, there is no longer an overlap of the compound eluted during the SPE step, and 2-methyl-4-nitrophenol

(circled in *Figure 12*). The concentrations of both of the target compounds and both of the internal standards can be determined in a quantitative fashion.

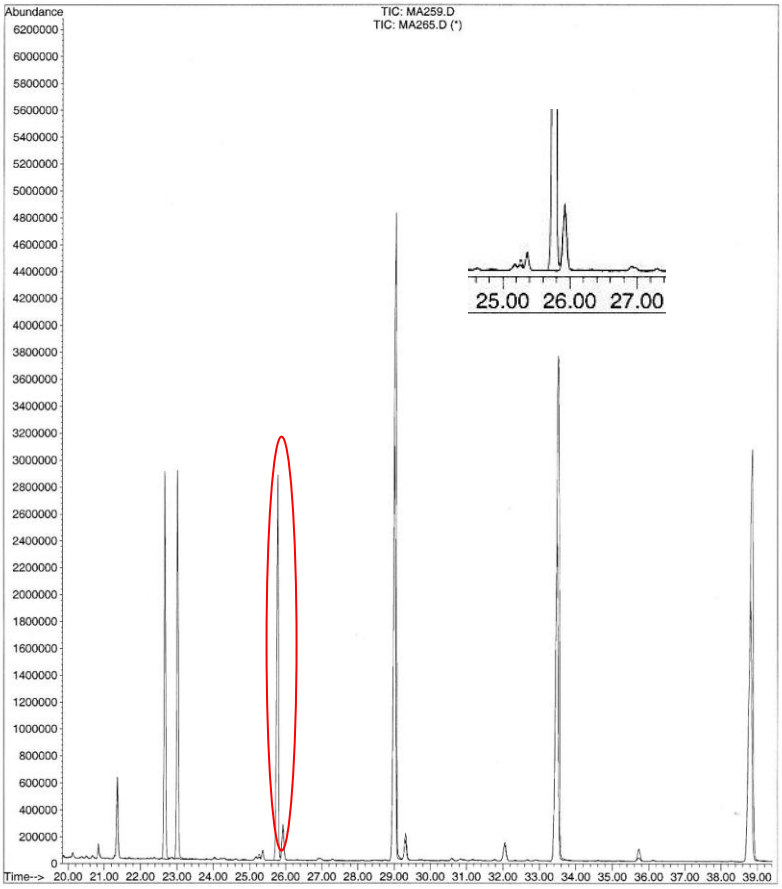

Figure 9*- Overlay GC chromatogram of a blank SPE collection with the chromatogram of SPE collection spiked with 2-methyl-4-nitrophenol, 4-methyl-2-nitrophenol and 2-methyl-3-nitrophenol*

3.4 GC – Internal Standard Reference Compound (ISRC)

Initially, in Dr. Moukhtar's global procedure, 4-ethyl resorcinol was used as a reference standard for the GC-MS portion of the experiment. That is, it was spiked quantitatively to the sample solution before derivatization, for this was also derivatized. 4-ethyl resorcinol is used as a reference to determine the volume of each standard that is representing the signal of each peak. This allows one to determine the recovery of the internal standards, as well as the concentration of the methylnitrophenols in the

atmosphere. However, when determining the isotope ratio of the compounds, there was overlap between 4-ethyl-resorcinol and 2-methyl-3-nitrophenol. Furthermore, Satoshi conducted an experiment in which a sample was stored with 4-ethyl resorcinol for a period of two months. At the end of the two months, it was found that the 4-ethyl resorcinol evaporated and was no longer in solution. With heptadecane, octadecane and nonadecane as ISRC's, they did not evaporate and were stable in solution.

When measuring the isotope ratio of the methylnitrophenols, the ISRC that was used was a combination of more or less the same concentrations of heptadecane, octadecane and nonadecane. In order to keep the ISRC constant for both the concentration and isotope ratio measurements, it was decided to switch from 4-ethyl resorcinol to heptadecane, octadecane and nonadecane. In order to ensure that there was no overlap of the new ISRC in the Rtx-5 column, standards of the target compounds and internal standards were tested with both 4-ethyl resorcinol and the 3 alkanes. Since there was no overlap with either of the compounds, it was decided that the 3 alkanes will be used as the ISRC.

3.5 GC – TIC versus SIM

Now that nearly all of the problems associated with the previous method have been overcome, what is left now to solve is the detection problem. Since concentrations of nitrophenols in the atmosphere are found in such low quantities, a detector that is sensitive enough to detect these small concentrations is needed. The combination of a GC instrument coupled with a mass spectrometer can provide for a very powerful system, known as GC-MS.

There were three possibilities in how to evaluate and detect the data from the GC-MS. The first way is to use a scan mode, in which all masses are detected. They then can be evaluated using a total ion chromatogram (TIC) (*Figure 10)*. This is a chromatogram that is plotted as a function of time, and all ion abundances throughout the entire duration of the run are detected[4]. Each peak will have its own mass spectrum, which can verify peak identity. This method would detect all compounds, and would therefore increase the noise, and therefore would decrease the signal to noise ratio. In fact, when the detection limit was calculated, it was found to be 7 x 10^{-4} ng/uL (1 x 10^{-5} ng/m^3). The accuracy of this method was within 1%.

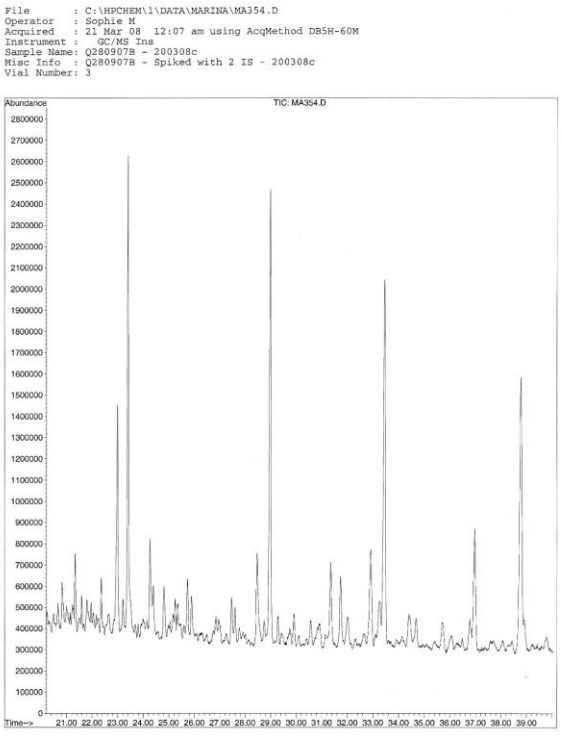

Figure 10 *– TIC of atmospheric sample Q280907B*

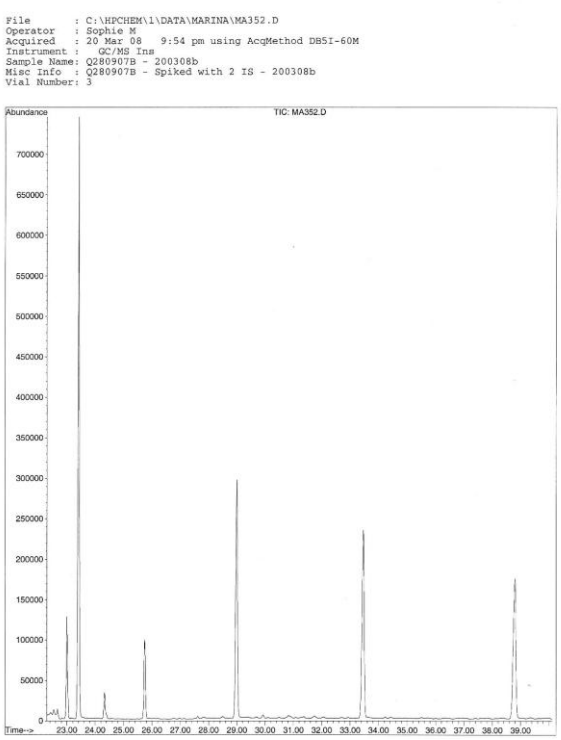

Figure 11 *– SIM chromatogram of atmospheric sample Q280907B*

The second method would still include a scan mode. However, once data is collected, specific masses can be chosen, and the peaks of only these mass fragments can

be evaluated. With this method, a TIC is still available for peak identification or other evaluations.

The third method, which is selected ion monitoring (SIM), was considered (*Figure 11)*. This analyzes specific mass fragments, as chosen by the operator, at selected retention times[4]. When using this technique, some results are not detected, and the sensitivity of the acquisition increases. When comparing *Figures 10 and 11*, one can notice the obvious change in baseline. Because a TIC detects everything in the sample, and an atmospheric sample is being analyzed, there will be plenty of noise. The baseline in the SIM method is considerably lower, and therefore will decrease the detection limit considerably. In fact, the detection limit with this method was found to be 2×10^{-5} ng/µL (4×10^{-7} ng/m^3). The accuracy of this method was within 1% for 2-methyl-4-nitrophenol, but was approximately 10% for 4-methyl-2-nitrophenol. The retention times and selected mass to charge (m/z) values can be seen in *Table 7*.

***Table 7** – Selected retention times and m/z values chosen for target compounds, internal standards and ISRC for SIM*

Compound	*Retention Time (min)*	*m/z value(s)*
4-methyl-2-nitrophenol	22.67	225, 210, 165
2-methyl-3-nitrophenol	23.03	225, 210, 165
2-methyl-5-nitrophenol	23.43	225, 210, 165
2-methyl-4-nitrophenol	25.79	225, 210, 165
heptadecane	29.02	85
octadecane	33.49	85
nonadecane	38.82	85

3.6 Final Results with all Method Changes

With all of the method adjustments made, two filters collected in the summer were analyzed. The GC-MS measurements were conducted in the TIC as well as in the SIM mode. The results for both of the filters using SIM mode can be seen in *Table 8*. With reference to filter Q280907B in *Table 8*, it can be seen that relatively high concentrations are seen of 2-methyl-4-nitrophenol.

Table 8 *– Results of two summer atmospheric filters analyzed with all method adjustments using selective ion monitoring*

Filter	*Target Compound*			
	2-methyl-4-nitrophenol (ng/m^3)		**4-methyl-2-nitrophenol (ng/m^3)**	
	ng/m^3	**ng/µL**	**ng/m^3**	**ng/µL**
Q100807A	**0.006 ± 0.001**	**0.40 ± 0.02**	**0.004 ± 0.001**	**0.3 ± 0.1**
Q280907B	**0.065 ± 0.003**	**3.0 ± 0.2**	**0.011 ± 0.001**	**0.5 ± 0.1**

Figure 12 shows a total ion chromatogram of an atmospheric filter (Q110907A) prior to any method adjustments, and *Figure 13* shows a total ion chromatogram of an atmospheric filter (Q280907B) subsequent to all method adjustments. The circled portion on each chromatogram is the 2-methyl-4-nitrophenol peak. Although there is not a clear improvement by examining the TICs of each of the filters, it is important to note that by using TIC with the modifications that have been made, there is no longer overlap. When deriving concentrations of methylnitrophenols, SIM should be used. This is demonstrated by chromatogram shown in *Figure 11*. By changing from a TIC to an SIM chromatogram, fewer peaks are detected, which thereby increases the signal to noise ratio.

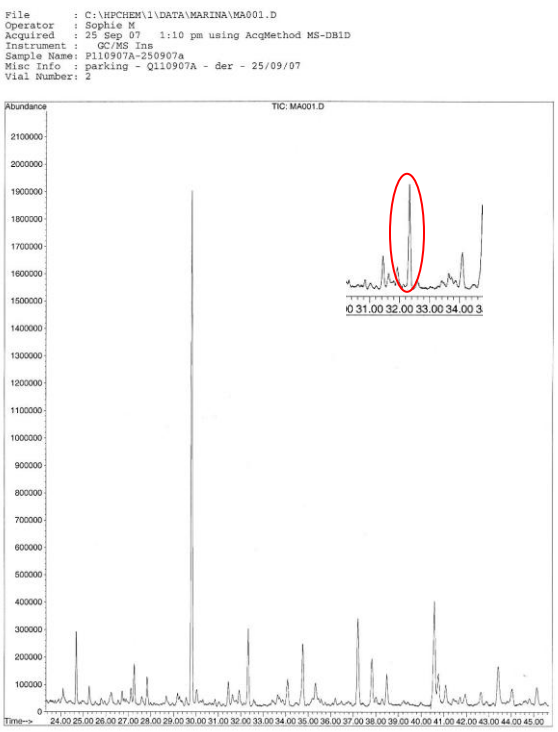

Figure 12 – *TIC of atmospheric filter Q11090A prior to method changes*

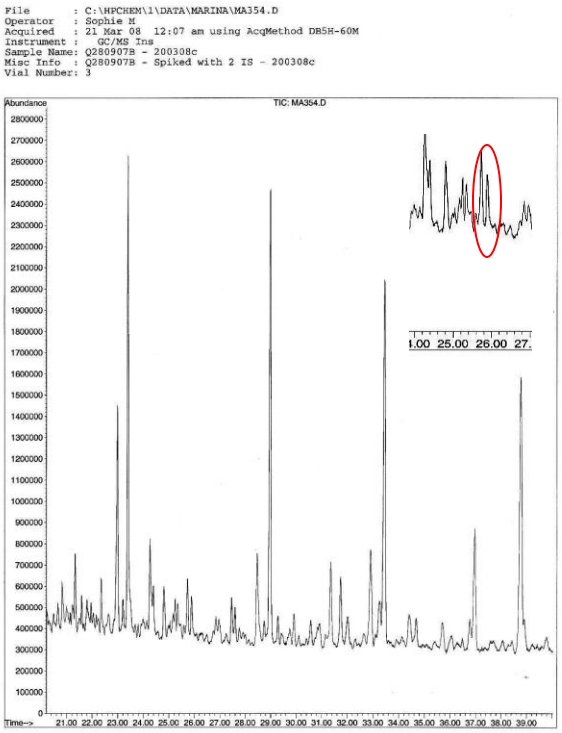

Figure 13 *–TIC chromatogram of atmospheric filter Q280907B with all method changes*

4. Conclusion

The main purpose of this project was to develop a sample preparation method for stable isotope ratio measurements of particulate organic matter and more specifically that of nitrophenols. In order to do this, there were two main problems to overcome. The first was to obtain concentrations of nitrophenols that would be high enough that an isotope ratio measurement would be possible. The second was to eliminate overlap in both the GC-MS chromatogram and the GC-IRMS chromatogram.

By reducing the amount of target compounds to an amount that was based on nitrophenols that were found in the atmosphere during the BAQS campaign, the HPLC collection window could be reduced. This allowed for a “cleaner sample”. That is, fewer compounds were analyzed by the GC-MS instrument, thereby reducing interference. To

eliminate the overlap caused by an SPE contaminant, the GC column was changed to a slightly more polar column.

Although several parameters, including the target compounds, internal standards and GC column were altered, it was observed that the concentration of nitrophenols detected was highly sample dependent. Their concentration depends on wind patterns, precipitation and other meteorological aspects. With that said, an accomplishment achieved by the adjustments made is that when there is a sample that has nitrophenols in reasonable concentrations, isotope ratio measurements will be possible. This is specifically shown by filter Q280907B, in which 2-methyl-4-nitrophenol is detected in a concentration of 3.0 ng/μL, and a peak for 2-methyl-4-nitrophenol is clearly seen in the SIM chromatogram (*Figure 11*). Although 3.0 ng/μL is not the ideal concentration to derive an isotope ratio, it is close to the target concentration of 10 ng/μL.

There are some problems that still exist, and can be further examined for future work. Methylnitrophenol concentrations found in atmospheric samples are very low, and are significantly lower than expected from laboratory experiments, which often suggests concentrations of several ng/m^3 [2]. These discrepancies could be further studied. Also, the findings in this report are based on a small number of sample measurements, and the meteorological data has not been analyzed. This can be improved in order to better understand the chemistry of methylnitrophenols. The analysis of the concentration and isotope ratio of methylnitrophenols is of great importance due to its toxicity towards both humans and plants[6].

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