

*Chapter 4*

ANALYSIS OF TITAN AEROSOL SIMULANTS THROUGH SOLID  
PHASE MICROEXTRACTION GAS-CHROMATOGRAPHY MASS  
SPECTROMETRY: A MISSION APPLICABLE ANALYTICAL  
METHOD FOR SPACE ENVIRONMENTS

**4.1 Abstract**

Saturn's moon Titan possesses a dense atmosphere comprising mostly nitrogen and methane, with a rich assortment of organic molecules and aerosols. Laboratory based synthesis of simulated Titan aerosols, called tholins, has produced molecules with rich chemical diversity and functionality over a wide range of molecular weights. This diversity has made the tholins difficult to characterize and the need to identify the specific structures of compounds remains. To allow for identification of compounds of various chemical functionalities and sizes, a solid phase microextraction gas chromatography mass spectrometry (SPME-GC-MS) method is proposed. The method allows for the preconcentration of semi-volatile compounds of both polar and non-polar functionalities, their analysis by GC, and subsequent structural identification by MS. This method is applied to a tholin sample generated by a hot plasma discharge in a nitrogen and methane atmosphere. Compounds with an array of molecular weights and structures are identified, spanning the major chemical functionalities expected on Titan. Many of the compounds identified are those seen in prior analyses, but some new compounds of astrobiological interest are also

seen. The large number of compounds successfully characterized suggests that this SPME-GC-MS method would be effective for inclusion on a future lander mission to Titan or other icy worlds.

## 4.2 Introduction

Due to the discoveries of the Cassini-Huygens mission to the Saturn system, the icy moon of Titan presents an astrochemically interesting target for further study.<sup>1-3</sup> The reasons for this interest are many, such as the nitrogen-methane atmosphere,<sup>4,5</sup> geographical features including lakes,<sup>6</sup> dunes,<sup>7</sup> and valleys indicative of flowing liquid,<sup>8</sup> and the proposed water-ammonia ocean under the surface.<sup>9</sup> The atmosphere has been one of the most studied aspects of Titan, since its composition may resemble that of an early Earth<sup>10, 11</sup> and is optically opaque due to an abundance of organic aerosols.<sup>10</sup> These aerosols are proposed to consist of diverse organic molecules with elemental compositions comprising carbon, nitrogen and hydrogen.<sup>12, 13</sup> Low molecular weight species such as hydrogen cyanide and acetylene are formed in abundance by various excitation processes in the upper atmosphere.<sup>10, 13-17</sup> The production of more complex species, such as benzene, require complicated reaction mechanisms.<sup>1, 2, 17</sup> With this diversity, precursors to molecules such as amino acids may be readily synthesized, increasing the interest in Titan's chemistry to the astrobiological community. Indeed, amino acid synthesis was observed in studies involving aged Titan aerosol simulants.<sup>18</sup> All of these factors make Titan an interesting target for the study of its atmospheric and surface chemistry.

Studies have been performed to replicate Titan aerosols in the hope of increasing understanding regarding their formation and chemistry. These studies take both Titan like conditions and experimental constraints into consideration when performing aerosol generation.<sup>19-24</sup> The atmosphere, consisting of 90-98% nitrogen and 2-10% methane, is the easiest to replicate.<sup>19</sup> While many different ratios have been tested, the 95% nitrogen and 5% methane ratio has been accepted as the most representative.<sup>25</sup> The low temperature conditions of 95-150K can be readily accommodated, but the lower pressure of  $10^{-6}$ - $10^{-7}$  mbar at 1000 km<sup>26</sup> where the solar photon flux peaks in Titan's atmosphere,<sup>27</sup> is much more difficult to implement. Low pressure increases timescales for reaction, so pressures are typically increased to allow for increased accumulation of aerosol. The energy sources used to produce the aerosol are also varied, with UV light,<sup>21</sup> cold plasma,<sup>28-30</sup> and hot plasma discharges<sup>31</sup> being the most common. While each method of excitation has benefits and detractions, hot plasma discharges produce a large amount of aerosol sufficient for evaluating the efficacy of different analytical methods. Studies on such aerosols will help to inform the choice of analytical methodologies and instrument design for future missions to examine the complex chemistry of the atmosphere and surface of Titan.

A range of different analyses have been performed on simulated Titan aerosols, or tholins, to better understand their chemical composition and the possible implications of that composition for interesting chemistry on Titan. These investigations have found a large number of compounds, with various degrees of nitrogen incorporation, and the expectation of multiple structural isomers of the same chemical composition.<sup>19, 20, 31-34</sup> It becomes highly probable that a subset of these isomers are astrobiologically significant. The large amount of complexity expected in these samples drives the need for analytical methods that can

separate different compounds, either by structure or chemical functionality. The use of derivatization is one such possibility, but it typically allows only for the analysis of a single chemical functionality.<sup>35, 36</sup> Additionally, the ability to detect other compounds of interest could be impaired if they are unintentionally changed during the derivatization process.

Prior analyses have encountered problems with differing solubilities of tholin components. Different solution phase extractions have been attempted, but there always remains an insoluble fraction.<sup>19, 31, 34, 37</sup> Prior analyses of tholins in the presence of water have found that some compounds readily react, producing amino acids.<sup>18</sup> While an interesting observation, chemical transformations that accompany solvent addition impair the ability to identify structures of nascent tholin components. Microfluidic based methods have been proposed for future Titan missions, but typically target a single chemical functionality out of the many expected within the aerosol.<sup>36</sup> Ideally, one of the analytical methods included in a Titan mission would allow for the analysis of multiple chemical functionalities simultaneously and without the use of solvents.

Since hydrocarbons and nitriles are expected to occur in abundance on Titan, gas chromatography (GC) has been considered the best at both simultaneous analysis of multiple functionalities and avoiding chemical change through solvation. Pyrolysis based GC has been the most widely studied solvent free method, even having been included on the Huygens lander.<sup>4, 5, 38-41</sup> While the chemical data obtained from that instrument was beneficial to understanding Titan, the instrument was designed primarily for the analysis of atmospheric gases, and was unable to provide chromatographic data due to valve failure during the mission. Additionally the GC method used by that instrument relied on heating the aerosol collected during descent with an aerosol pyrolyzer.<sup>41</sup> Several other methods have also

utilized pyrolysis for volatilization, with GC methods to determine which heavier compounds could be detected in Titan aerosols.<sup>39,40,42</sup> While these studies have successfully analyzed and identified a variety of organic molecules in different tholin samples, the compounds detected are rarely more complex than benzene. A difficulty with pyrolysis is that it can chemically change the sample, and other work has found that it both induces polymerization and releases hydrogen cyanide.<sup>43</sup> The atmospheric aerosols of Titan are expected to comprise mostly smaller molecules, with larger polymers being unlikely. So while GC enables the broad analysis desired in instrumentation for mission applications, pyrolysis presents difficulties.

A different GC method involves the use of solid phase microextraction (SPME) to preconcentrate gas or solution phase analytes prior to desorption in the GC inlet. SPME-GC has been attempted on tholin samples in the past, but those samples were obtained through discharges in a nitrogen-methane-hydrogen atmosphere in the presence of water.<sup>44, 45</sup> Additionally, the collected tholin was reacted to not only derivatize nascent molecules, but also to fractionate hydrophobic and hydrophilic components.<sup>35,46</sup> While the SPME analysis for these tholins concentrated on the identification of polyaromatic hydrocarbons (PAHs), neither fraction produced positive results with SPME extraction.<sup>44, 45</sup> Another study using SPME extraction of tholins synthesized in the same manner was able to identify multiple complex aromatics compounds, including PAHs, when extraction was performed after multiple freeze-thaw cycles.<sup>46</sup> Simpler compounds were not detected, as the study focused on the detection of substituted aromatics and PAHs with SPME. While relevant for the study of icy worlds, the majority of Titan tholins are produced with the exclusion of water. This is due to water ice not being present where photochemistry occurs, and thus it will have little if

any impact on the chemistry of Titan aerosols. Since more complex compounds are expected to be less abundant in these aerosols compared to smaller nitriles and hydrocarbons, the identification of only substituted aromatics and PAHs with this prior SPME work leads to the need to examine additional SPME extraction methods.

Other SPME studies have been applied to solid phase samples for the identification of small volatile organic compounds (VOCs). Most of these studies concentrate on VOCs released from plant matter, such as leaves and flowers.<sup>47, 48</sup> Additional studies have been performed to examine different volatile products from gas phase reactions simulating those seen in Earth's atmosphere contributing to the production of aerosols.<sup>49-52</sup> These extractions use both high temperature and room temperature conditions, depending on the sample and VOC of interest. These studies report not only great success in the detection of simple VOCs, but also that some of the compounds detected are different from those seen with direct gas phase analysis alone. The success of these studies supports the conjecture that SPME-GC can detect small organics simultaneously with larger more complex molecules from samples such as tholins.

To accomplish the desired analysis of a diverse range of compounds, a study was performed on two different tholin samples considered representative of Titan, using SPME-GC-MS with the goal of assessing and developing a possible mission applicable analytical method. Additionally the study is designed to allow for the analysis of tholins without solvent extraction or excessive heating. The SPME extraction was performed with two different fiber media to better understand its impact on the molecules detected. The results presented in this study show that a wide variety of molecules can be readily detected with SPME extraction and that the fiber media greatly impact the compounds seen in the resultant

chromatogram. Both simple and complex molecules are identified with fewer hydrocarbons detected than with other GC studies. The ability to perform GC on less volatile compounds without pyrolysis or solvent extraction, especially using water, make this SPME method one that should be considered for inclusion in future missions to icy worlds, particularly Titan.

## **4.3 Experimental Methods**

### **4.3.1 Production of Tholins**

The two tholin samples examined in this study were produced in a similar manner. The synthesis of T1 performed by Smith and associates at the University of Arizona, and described in detail in prior publications,<sup>23, 31</sup> is summarized here. A gas mixture of 95% nitrogen and 5% methane is flowed inside a high vacuum stainless steel and glass reaction chamber at a temperature of 195K. The flow rate maintained a constant pressure of 850 Pa. An AC electrical discharge, with a current of approximately 100 mA was applied with an estimated discharge exposure time of 2 seconds. Synthesis was continued for 72 hours, after which the vessel was warmed to room temperature under vacuum for 24 hours, and the solid then collected under a nitrogen atmosphere. This sample has been exposed to ambient atmosphere prior to testing. It was stored under ambient atmosphere, but in the dark and frozen, for two years prior to testing.

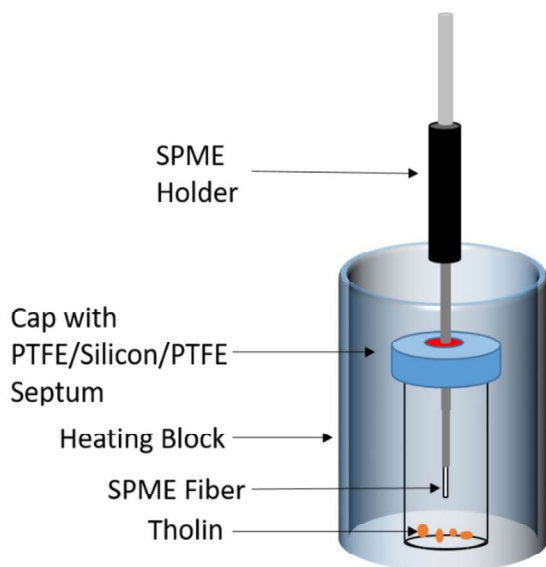
While T2 has only just recently been investigated, it was synthesized by Hörst and associates at John's Hopkins University in a similar manner.<sup>53</sup> The 95% nitrogen and 5% methane gas mixture was exposed to an AC glow plasma in a ultra-high vacuum reaction

chamber at room temperature under a consistent gas flow maintaining a chamber pressure of 400 Pa. After synthesis the reaction chamber was evacuated for 24 hours at room temperature, and the solid was then collected under ambient atmosphere. The samples have since only been exposed to nitrogen atmosphere and stored in the dark at room temperature prior to testing.

#### **4.3.2 Extraction and analysis by SPME-GC-MS**

Each extraction, a graphic for which is shown in figure 4.1, was performed using a separate vial for each tholin. All tholins were weighed and placed into 2 mL GC autosampler vial and sealed with a Teflon-faced silicone rubber septum and screw cap. Both samples of T2 were placed in vials while under a nitrogen atmosphere in a glove-bag. Approximately 4 mg were used for each aliquot to allow for comparison. The 100  $\mu\text{m}$  PDMS fiber (Supleco, Bellafonte PA) was held in a manual sample holder during use and the 75  $\mu\text{m}$  CAR/PDMS field sampler assembly (Sigma Aldrich, St. Louis, MO) was used as provided. Each fiber was conditioned prior to use and cleaned between extractions as specified in their respective manuals. All extractions were performed at 60°C with the fiber inserted into the vial headspace. The temperature was held constant during extraction by placing the sample vial in a temperature controlled Teflon coated graphite SCP Science Digi heating block (SCP Science, Champlain, NY). The PDMS extractions were performed for 1 hour and the CAR/PDMS extractions for 15 minutes. Temperature was monitored for the duration of the extraction, with the vial being heated only during the extraction process.





### Experimental Steps

If tholin is unexposed to atmosphere:

1. Place tholin in 2 mL vial inside nitrogen filled glove bag

If tholin is exposed, place in vial.

Next steps apply to all tholins

1. Seal 2mL vial with cap and use Teflon tap around cap-vial seal
2. Place SPME holder needle into vial
3. Place vial with SPME needle into heating block
4. Dispense SPME fiber into headspace
5. Allow for adsorption
6. Return fiber to SPME holder
7. Remove sample from heating block

Figure 4.1. A graphic, along with experimental steps, for the extraction process. Extra steps for unexposed samples are specified.

Immediately following extraction, the fiber was inserted into the split-splitless GC inlet at 200°C for 3 minutes under splitless inlet conditions. A new, deactivated, 2mm ID liner was used (Sky liner, Restek Corp., Bellefonte PA). Helium was used for the inlet purge and carrier gas. A 30m x 0.25mm ID x 0.25  $\mu\text{m}$  film thickness 5%-phenyl-arylene-95% dimethylpolysiloxane stationary phase column (Zebron ZB-5MS, Phenomenex, Torrance, CA) was used. For the PDMS extractions the column oven temperature program began at -55°C for 3 minutes followed by ramps to 65°C at 20°C/min, to 130°C at 1.5°C/min, and finally to 200°C at 30°C/min with a final 1 minute hold. With CAR/PDMS extractions the temperature program began at -55°C for 3 minutes, with a ramp to 150°C at 3°C/min, a 3 minute hold at 150°C, a ramp to 200°C at 8°C/min with a final 1 minute hold. Cooling was

accomplished by liquid nitrogen. A 5 minute solvent delay was used for all analyses to mitigate effects of the large air peak desorbed from the fibers. The GC-MS instrument used for this study was a Waters Micromass GCT Premier (Waters Corp., Milford, MA) with a mass resolution of 7000 at 219 m/z, from a lock mass peak obtained with heptacosafuorotributylamine, and a mass accuracy of  $\pm 5$  mDa. Spectral accumulation was performed in centroid mode.

#### 4.3.3 Standards and Identification

Standards obtained for this study included benzene (HPLC grade), pyrimidine (98%), adiponitrile (99%), succinonitrile (99%), methenamine (99%), dimethylaminoacetonitrile (97%), 2-methylglutaronitrile (99%), propionitrile (99%), 3-pyridinecarbonitrile (98%), tertbutylnitrile (98%), benzonitrile (99%), isobutyronitrile (99%), glutaronitrile (99%), pentanenitrile (99%), and 4-methylpentanenitrile. All were obtained from Sigma Aldrich. Standard solutions were made in milliQ water at 200  $\mu\text{g/mL}$  to 50  $\mu\text{g/mL}$  in 2 mL GC autosampler vials. Headspace extraction was performed for 1 minute to 1 hour depending on the fiber and the standard tested. Compounds not identified by standards were matched used the NIST 2005 EI mass spectrometry library.

#### 4.4 Results and Discussion

The overall chromatogram for the PDMS extraction of both tholins is shown in figures 4.2a and 4.2b, with the CAR/PDMS extraction for T1 shown in figure 4.2c. Retention times are labeled and all identified compounds are tabulated in Table 4.1. In all of these

chromatograms peaks corresponding to siloxanes are seen as major peaks. The prevalence of these species is due in part to the nature of the SPME fibers containing siloxanes. A larger contributing factor is the extraction method. These extractions were performed in headspace for longer timescales compared to most other work performed with SPME. Even though T2 was sealed under nitrogen, there is atmospheric leaking over the extraction timescale. Furthermore, the septum is formed from silicone rubber, presenting a large possible source of siloxanes. This contamination is easily removed between sampling by baking out the fibers and ensuring no siloxanes are observed prior to extracting a new tholin sample. Presence of the siloxanes was also confirmed in “blank” extractions done in new, clean, empty vials. As such, the observation of siloxanes is not a major concern and within expectations for this method.

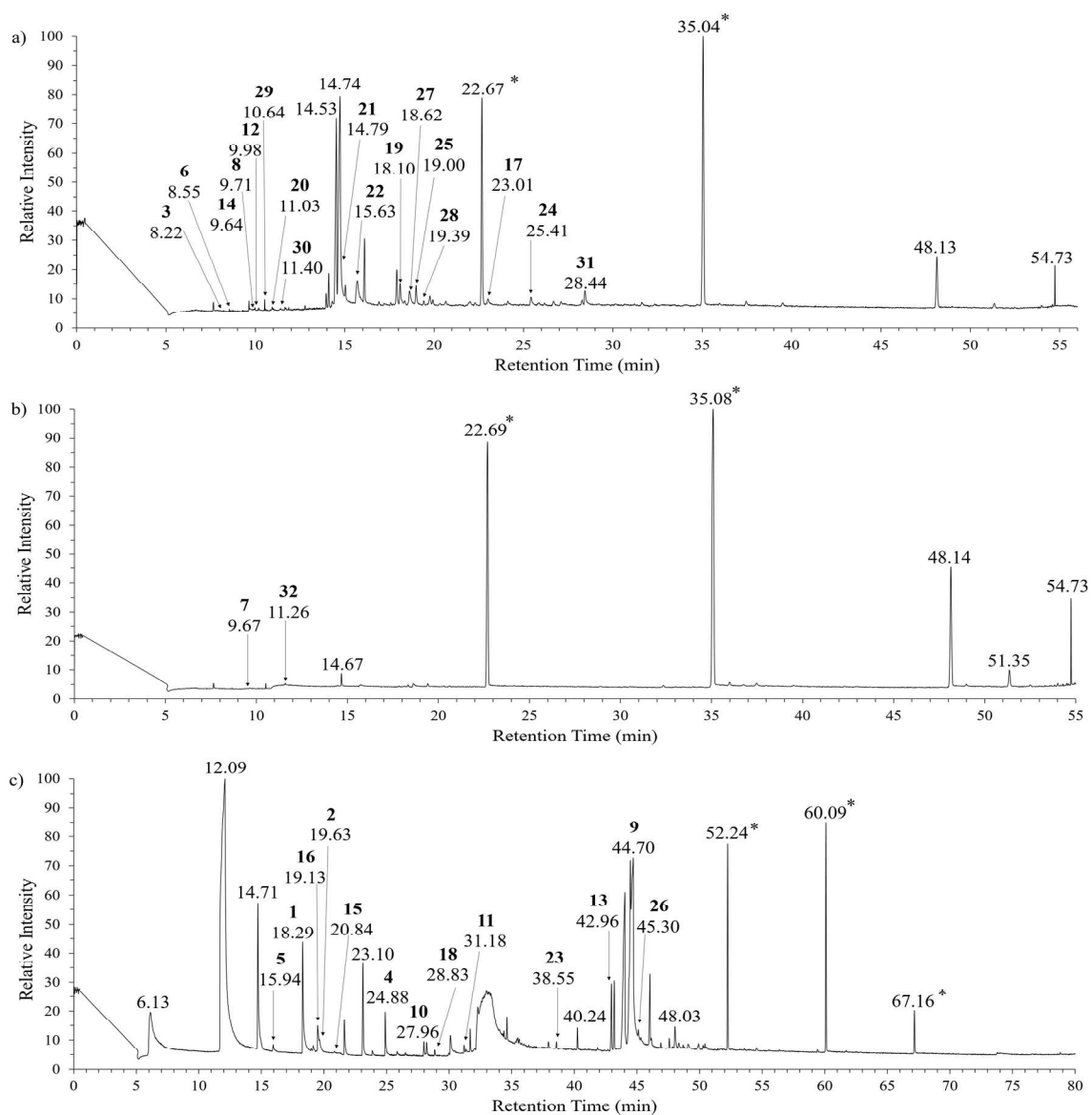


Figure 4.2. a) Total ion chromatogram (TIC) for the PDMS extraction of T1 b) TIC for the PDMS extraction of T2 c) TIC for the CAR/PDMS extraction of T1. Bold numbers refer to the peak number value in Table 4.1. Siloxanes are labeled with an asterisk.

Peak Number	Compound Name	T1		T2
		CAR/PDMS RT (min)	PDMS RT (min)	PDMS RT (if different)
1	propionitrile	18.29	n.d.	n.d.
2	2-methyl-2-propenenitrile	19.63	n.d.	n.d.
3	isobutyronitrile	21.62	8.22	n.d.
4	butanenitrile	24.88	n.d.	n.d.
5	cyclopentane	15.94	n.d.	n.d.
6	benzene	23.10	8.55	X
7	pyridine	n.d.	n.d.	9.67
8	pyrimidine	n.d.	9.71	n.d.
9	succinonitrile	44.70	n.d.	n.d.
10	2-methyl-2-butenitrile	27.96	n.d.	n.d.
11	methylallylcyanoide	31.18	n.d.	n.d.
12	pentanenitrile	31.67	9.976	X
13	tertbutylnitrile	42.96	n.d.	n.d.
14	dimethylaminoacetonitrile	30.08	9.64	n.d.
15	methylcyclopentane	20.84	n.d.	n.d.
16	hexane	19.13	n.d.	n.d.
17	1H-pyrrole-3-carbonitrile	n.d.	23.01	X
18	3-methyl-butanenitrile	28.83	n.d.	n.d.
19	glutaronitrile	43.86	18.10	X
20	4-methylpentanenitrile	32.98	11.03	n.d.
21	benzonitrile	43.78	14.79	n.d.
22	3-pyridinecarbonitrile	45.10	15.63	n.d.

23	1-methylethylidene propanedinitrile	38.55	n.d.	n.d.
24	adiponitrile	49.09	25.41	X
25	2-methylglutaronitrile	44.46	19.00	n.d.
26	cyclohexanecarbonitrile	45.30	n.d.	n.d.
27	2,4-diaminopyrimidine	n.d	18.62	X
28	alpha-methylstyrene	49.29	19.39	X
29	2,4-dimethylheptane	n.d	10.64	X
30	2,3,4 trimethylhexane	n.d	11.40	X
31	hexamethylenetetramine	56.37	28.43	n.d.
32	4,5-diethyloctane	n.d	n.d	11.256

Table 4.1. Compounds Identified in SPME Extraction of Tholins. All compounds identified by either standards or library matches and their retention times are reported. All retention times (RT) are reported in minutes corresponding to the specified extraction. If the compound was not detected no detection (n.d.) is reported. For the T2 column an X indicates the same RT as observed for the PDMS extraction for T1. The peak number refers to the bold peak labels in figure 4.2.

The first thing to note for the chromatograms is the large number of eluted species for the PDMS extraction of T1. There is a tight clustering of peaks between 13 and 20 minutes, with other clearly evident peaks at other retention times. Many of these peaks are broad or display shoulders, which suggests the possibility of co-elution of multiple components. Since the detection method used in this study is electron impact mass spectrometry it allows for identification of co-eluting species. Electron impact produces characteristic mass fragments for different compounds, dependent on both chemical functionality and compound size. This combined with the exact mass resolution of the mass

spectrometer allows for an extracted ion chromatogram (EIC) to be obtained. An EIC shows peaks for a particular mass, which can then be used to make identifications of compounds with a desired chemical functionality easier even in the presence of multiple co-eluted species. Some of these EIC and representative mass spectra are shown in the subsequent analysis of different functional groups below.

The second overall observation from these chromatograms is how different the CAR/PDMS extraction for T1 is to the PDMS extractions for both tholins. While both display a large number of peaks, those identified in the CAR/PDMS extraction are much more separated. The broad nature of many peaks in this chromatogram is suggestive of compounds that are not easily resolved, which likely correspond to highly polar compounds. The implications of this are discussed below, but it makes those regions of the chromatogram difficult to use for identification. As such the majority of the identifications discussed below correspond to either retention times outside of those peaks, or species that can be readily resolved with an EIC. A large number of peaks have these specifications, and multiple compounds are identified that are not seen on the PDMS fiber extraction (figure 4.2a). Using a combination of EICs for both fibers along with comparison to compounds found in prior analyses, some of the organic compounds of interest in the material are identified and described below.

#### **4.4.1 Analysis of Organic molecules**

##### *4.4.1.1 Amines*

Amines are of interest due to both their likelihood on Titan and their biological significance. While the detection of primary amines is desirable for their astrobiological

significance, they are difficult to detect with most GC columns without significant care.

Simple primary amines do produce a distinctive fragment in EI,  $\text{CH}_2\text{NH}_2^{\bullet+}$  with a mass of 30.034 Da. This fragment, the EIC for which is shown in figure 4.10, is seen in the CAR/PDMS extraction chromatogram for both tholins, but the peak is too broad to enable analysis. As such simple primary amines cannot be readily analyzed with this SPME-GC method, which is to be expected considering the difficulty of their analysis with GC and possible losses due to volatility. A more complex primary amine can be detected by examining different distinctive fragments. This includes 2,4-diaminopyrimidine seen on the PDMS fiber for both tholin samples at 18.62 min and identified based on library matching. While a complex amine is interesting, finding a compound containing four nitrogen atoms and multiple primary amines shows that a large amount of chemical complexity is possible for these samples, even in the smaller molecules produced. This shows that this method can detect more complex primary amines within these tholins.



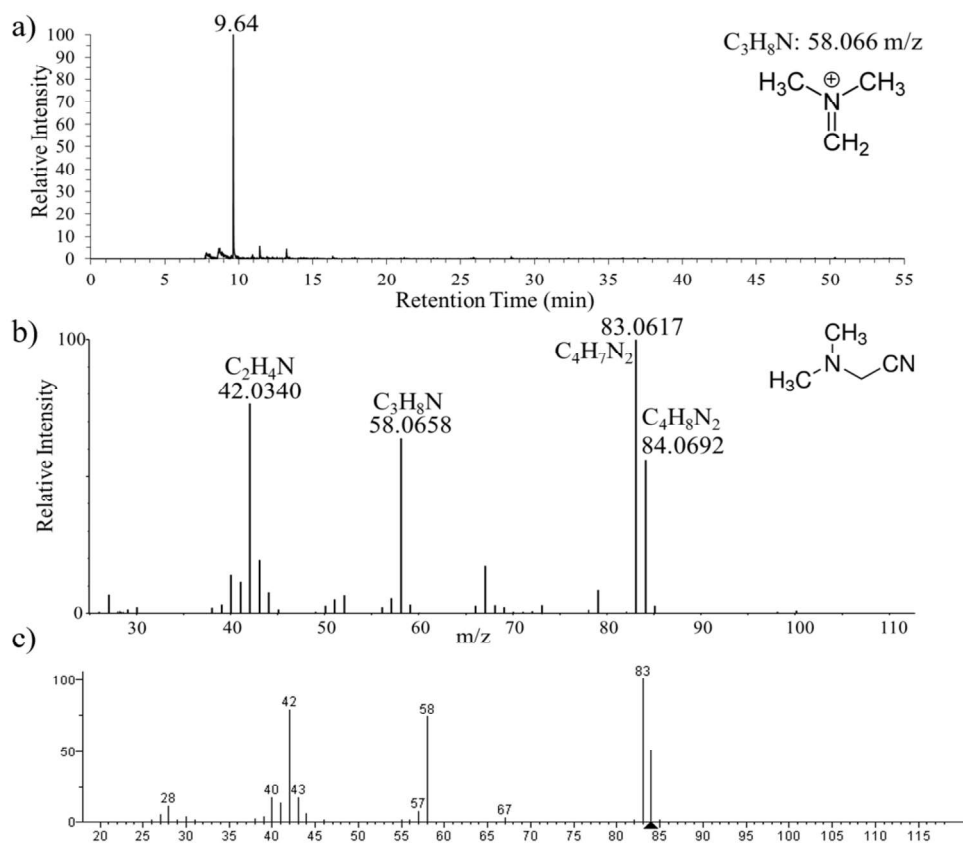


Figure 4.3. a) EIC of 58.066 m/z for the entire chromatographic run with the structure of the targeted ion shown. b) The EI mass spectrum for the 9.64 minute peak with relevant fragments labeled and the compound structure shown. Both are from the T1 PDMS extraction. c) EI library match spectrum from NIST library.

More highly substituted amines become more difficult to identify, since there are fewer distinctive EI fragments as the complexity of the substitution increases. One or two methyl groups on a secondary or tertiary amine respectively do produce identifiable fragments, even if the other sidechain is more complex. The EIC for the simple tertiary amine fragment from the PDMS fiber extraction for T1 is shown in figure 4.3. The single large peak was identified as dimethylaminoacetonitrile by comparison with a standard. This peak was seen in T1 only and extracted by both fibers, showing SPME can detect small

tertiary amines. The detection of this amine is of astrobiological interest since it is the doubly methylated form of aminoacetonitrile. That small primary amine is considered a prebiotic molecule since it reacts with water to produce glycine, the simplest amino acid.<sup>18</sup> The production of dimethylaminoacetonitrile in one of these tholins increases the likelihood that the simpler precursor is also made, and is either undetectable with this method or has undergone further reaction during the synthesis process.

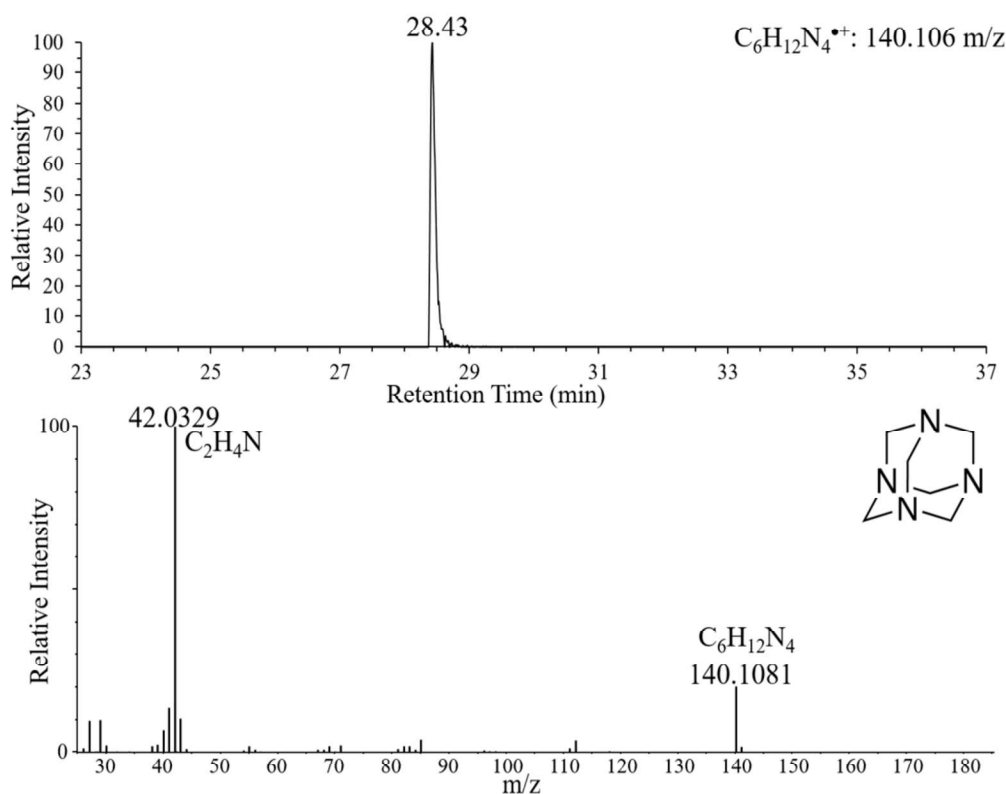


Figure 4.4. Top: EIC for 140.106 m/z for the time range of interest to hexamethylenetetramine from the T1 PDMS chromatogram. Bottom: EI mass spectrum for 28.43 minutes with relevant fragments labeled.

The most unique molecule detected within T1 was hexamethylenetetramine, a four nitrogen molecule with a structure resembling a cage. It was detected on both fibers for T1 with the longest retention time for any identified compound. It was also one of the few compounds with more than two nitrogen atoms, with 2,4-diaminopyrimidine being the only other four nitrogen compound detected. Hexamethylenetetramine was also seen in nuclear magnetic resonance (NMR) studies of T1,<sup>34</sup> indicating the ability of this SPME-GC method to detect compounds found with other analytical methods. It also shows that SPME-GC enables the simultaneous detection of both small and complex molecules. This simultaneous detection is important since the majority of GC methods in prior tholin sample analysis have concentrated on the identification of small molecules, and any larger molecules detected have been mostly hydrocarbons without the high nitrogen incorporation seen in hexamethylenetetramine. While this amount of nitrogen incorporation may not readily occur on Titan, the ability to detect such molecules in mission applications is important in case they are present. In this way this SPME-GC method presents an analytical possibility for much broader identification than could be accomplished with other prior studied GC-MS methods.

#### *4.4.1.2 Aromatics*

For aromatic molecules identifying simple, substituted, and heterocyclic compounds is important. Aromatic compounds can be easier to identify with EI due to their stability. They do not readily fragment and can be identified based off of EICs for the molecular ion as opposed to a fragment ion. Benzene is one of the simplest, and the EIC of a characteristic benzene fragment is shown in figure 4.5 for the PDMS extraction of T1. There are a variety

of peaks, some of which are background ions and are labeled accordingly. The identification of benzene at 8.55 minutes is important since it was confirmed in Titan's atmosphere through the Cassini NIMS and any analytical method proposed for the identification of diverse compound types should be able to detect it along with other more complex molecules.

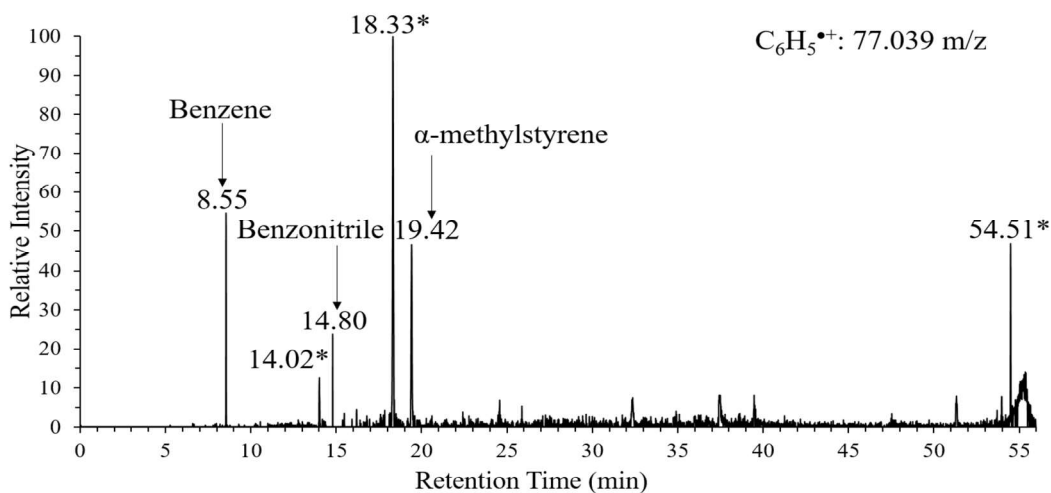


Figure 4.5. EIC for 77.039 m/z from the PDMS chromatogram for T1 with identified peaks labeled and background contaminants marked with an asterisk.

Other more substituted benzene molecules can also be identified, including  $\alpha$ -methylstyrene and benzonitrile. While the former is a common background ion, it was not seen in the background chromatogram and must originate from the tholins themselves.  $\alpha$ -Methylstyrene is unique in that it only contains carbon and hydrogen, while also containing one of the few nonaromatic alkenes identified. Since acetylene has also been seen in Titan's atmosphere, benzene molecules substituted with unsaturated hydrocarbons are expected, and

the detection of  $\alpha$ -methylstyrene fits with those expectations. Benzonitrile is a simple substituted benzene and one of the most likely substituted aromatics on Titan considering the prevalence of hydrogen cyanide and benzene in the photochemically active atmospheric regions. Additionally it has been seen in other analyses of tholins and thus is an expected observation.<sup>38, 40, 42</sup> Other substituted benzene molecules could contribute even more to Titan's chemistry and the detection of simpler variants indicates that more complex benzene molecules could possibly be detected.

Heterocyclic aromatic compounds are more common in the tholin samples compared to homocyclic aromatics due to the abundance of nitrogen and the high energy synthetic methods used. Some of these heterocyclic aromatics are expected to occur in Titan's atmosphere, making their detection important. The identified compounds of this type were all on the PDMS extractions and include 3-pyridinecarbonitrile, 2,4-diaminopyrimidine, and 1-H-pyrrole-3-carbonitrile detected in both tholin samples, pyrimidine detected in T1, and pyridine detected in T2. The variety in complexity is an expected observation since reactive nitrogen is abundant in these synthetic methods, but the different chemistry seen is interesting. This variability showcases the need for comparative studies when developing a suitable analytical method for tholin characterization.

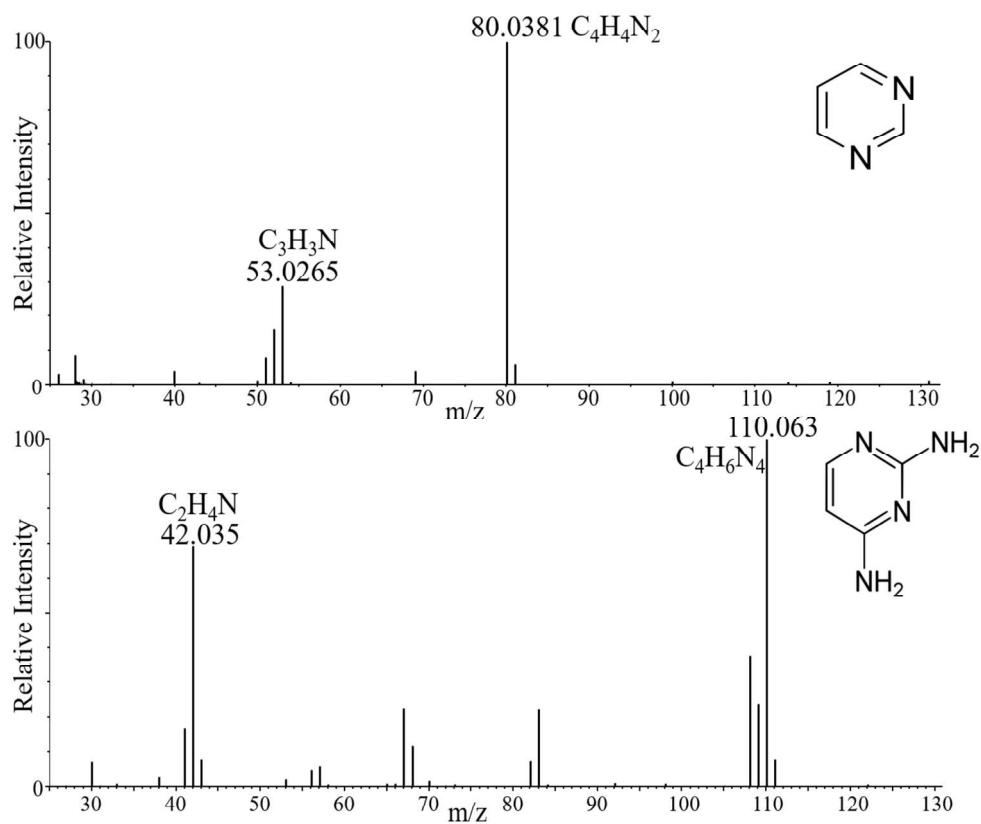


Figure 4.6. Both EI mass spectra are taken from T1 PDMS chromatogram. Top: EI mass spectrum for pyrimidine with relevant fragments labeled. Bottom: EI mass spectrum for 2,4-diaminopyrimidine with relevant fragments labeled.

The detection of 2,4-diaminopyrimidine in both tholins increases the likelihood that the precursor pyrimidine is produced in T2 as well. The difficulty in the identification of pyrimidine likely stems from the pyridine present in T2, which has a retention time close to pyrimidine, but elutes as broad peak with high intensity. Pyrimidine and dimethylaminoacetonitrile both display retention times very close to that seen for pyridine, but elute in much narrower peaks. This presents a possible concern for application of this GC method, since the aerosols on Titan are expected to contain similar molecules. Once

molecules of concern, such as pyridine, are identified the oven temperature ramps can be modified in subsequent experimental studies to account for closely eluting species, allowing for optimization of GC oven methods prior a Titan mission. The mass spectra for both pyrimidine and 2,4-diaminopyridimidine, shown in figure 4.6, also display why identification of aromatics can be difficult. Even though both compounds have the same heterocyclic base, they do not display the same characteristic fragment for pyrimidine alone. As such, the identification of substituted heterocycles cannot be based off extracting the mass of the common base alone, and so each aromatic would need to be identified individually.

The detection of pyridine and pyrimidine molecules within the tholins presents promising possibilities for astrobiology. While both have been identified in tholins with prior analyses,<sup>38-40, 42</sup> the identification of substituted pyridine and pyrimidine deviates from those prior data. Substituted pyrimidines comprise three of five nucleic bases present in DNA and RNA. Additionally pyridine forms the basis for multiple biological molecules such as alkaloids. The presence of substituted versions of these heterocycles indicates their possible inclusion in more advanced chemistry in Titan's atmosphere.

The last heterocycle detected, 1-H-pyrrole-3-carbonitrile, displays similar chemistry to the 3-pyridinecarbonitrile, and the precursor pyrrole was not detected in either tholin. The reason may be similar to pyridine, considering their similar chemistry. They are both single nitrogen aromatics and thus pyrrole is likely to elute broadly as well. The detection of the substituted molecule means pyrrole was likely produced during the synthesis of the tholins and either lost to evaporation or subsequent reaction. This assumption is further supported with the detection of 1H-pyrrole in other tholin samples analyzed with pyrolysis-GC.<sup>39, 40</sup> To confirm the retention time for this molecule in future analyses, standards and possibly doping

the tholins with pyrrole would be required. Finding 1-H-pyrrole-3-carbonitrile does indicate that smaller molecules readily react to produce more complex versions. Such reactions could also be expected in Titan's atmosphere, especially considering the likely production of cyanide radicals in large scale.

#### *4.4.1.3 Nitriles*

The nitrile functionality is found in the largest number of molecules within T1, with many of the most intense chromatographic peaks displaying nitrile functionality. Nitriles are also one of the most readily identified fragments in the EICs. This is because the McLafferty rearrangement for alkyl nitriles produces a distinctive fragment with a monoisotopic mass of 41.027 Da. Since this fragment is 12 mDa larger than C<sub>3</sub>H<sub>5</sub> it can be selected for in an EIC without isolating the hydrocarbon fragment, which significantly simplifies analysis and identification. The EIC for 41.027 Da is shown in figure 4.7 for part of the total chromatogram of the PDMS extraction of T1. In this EIC, species other than nitriles can be seen due to some compounds, such as nitrogenated aromatics, also producing a 41.027 Da fragment, including aromatic nitriles. While many of these compounds have been discussed in detail above, it shows how common nitrile chemistry is in these tholin samples. The commonality of nitrile substitution on compounds of multiple additional functionalities supports the idea that nitrile containing isomers will be common.



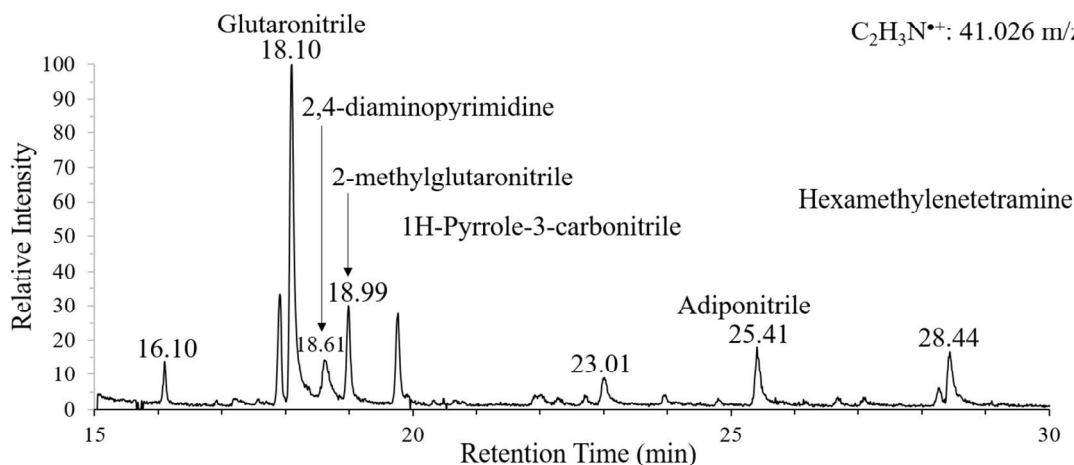


Figure 4.7. EIC for 41.027 m/z for the T1 PDMS extraction for the retention times of 15-30 minutes. The numbers refer to the compounds in Table 4.1. More than just nitriles are seen due to the mass also matching other possible fragments. An isolation window of 0.01 Da was used to improve chromatographic resolution while still eliminating the hydrocarbon fragment.

The other mononitriles identified in the material are similar to those identified in prior pyrolysis-GC studies of other tholins.<sup>38-40, 54</sup> They also comprise the largest diversity of identified compounds, including small and large alkyl chains. Alkenes are also identified in methylallylcyanide and 2-methyl-2-propenenitrile, both only seen on the CAR/PDMS extraction for T1. Other mononitriles identified include propionitrile, isobutyronitrile, tertbutylnitrile, 3-methyl-butanenitrile, pentanenitrile, and 4-methylpentanenitrile. These were identified by a mixture of standards and library matching and the conditions for their detection are shown in Table 4.1. Some of these nitriles comprise the smallest compounds seen in the extraction, with the next smallest non-nitrile being cyclopentane. These small nitriles could be indicative of smaller hydrocarbon precursors that were not identified with this method but seen with prior GC analyses.<sup>38,40,54</sup> This also supports the presence of nitriles

as the most common functionality in these tholin samples, and their contributions to more complex chemistry. The identification of multiple nitriles of various sizes is well within expectations for this sample, but also supports the use of SPME-GC for the identification of both small and large compounds in the same extraction.

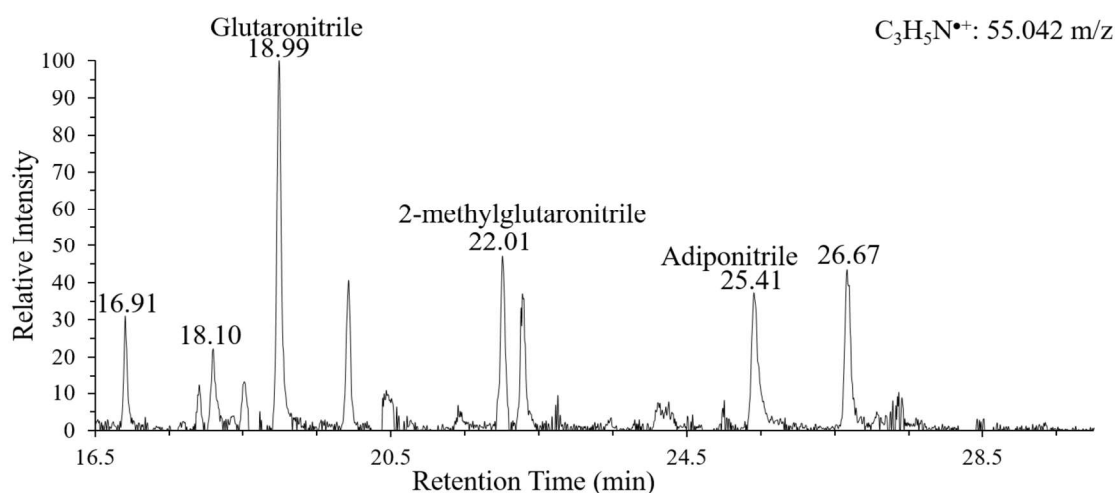


Figure 4.8. EIC for 55.042 m/z from the T1 PDMS extraction. The numbers refer to the compounds in Table 4.1.

Dinitriles are also seen in both fiber extractions and both tholin samples as shown in Table 4.1. The dinitriles seen also include the other alkene observed in the sample, 1-methylethylidene propanedinitrile, also only detected in the CAR/PDMS extraction. The identification of alkene containing nitriles on the CAR/PDMS fibers suggests that media is more effective at extracting alkenes. This may also be complicated by the smaller size of these alkene containing nitriles. The other dinitriles identified in this material include succinonitrile, glutaronitrile, and adiponitrile. These are all alkyl dinitriles and much larger,

being four, five, and six carbons respectively. These dinitriles are more difficult to identify, since the McLafferty rearrangement is also seen but does not unambiguously identify dinitriles. The alkyl dinitriles typically produce fragments for  $C_3H_5N$  at 55.042 Da, and for  $C_3H_4N$  at 54.034 Da. These fragments correspond to breaking along the alkyl chain, and the EIC for the former is shown in figure 8. There are multiple other compounds with this fragment seen in the EIC, but the similarity of the fragments in these chromatographic peaks likely makes the identification difficult. It is most likely that these unidentified peaks correspond to isomers of other alkyl dinitriles, the confirmation of which would require testing with additional standards. This data shows that SPME-GC allows for the extraction of nitriles with various sizes and polarities, with the actual number being extensive. Since nitriles are the most commonly expected functional group in Titan aerosols, the effectiveness of this SPME-GC method for their extraction suggests it would be well suited for their *in-situ* analysis.

#### 4.4.1.4 Hydrocarbons

Hydrocarbons produced in the tholin samples are difficult to detect. This is due to multiple factors, including the lack of solubility in solvents used for analyses such as electrospray ionization, high volatility, and the lack of distinctive chemical functional groups that can be derivatized or preselected for using specialized analytical conditions. The analysis of hydrocarbons is typically performed by GC, since the technique can analyze nonpolar compounds effectively. One of the difficulties with the analysis of hydrocarbons by the GC-MS used in this work is the high amount of co-elution with aliphatic nitrile

species. Many aliphatic nitriles contain similar ion fragments to those seen in hydrocarbons which, combined with the high amount of co-elution observed for the molecules, minimizes the usefulness of an EIC for identification.

Compared to the nitriles there are relatively few hydrocarbons detected, even though they are expected to be the next most abundant type of compound due to the GC column selection and comparison with prior pyrolysis-GC analyses of tholin samples.<sup>39,42</sup> There are many possibilities behind this observation. One is that the hydrocarbons are readily lost from the tholins during collection due to their volatility. Another is that the hydrocarbons may not adhere as readily to the fibers as the more polar compounds, or may adhere extremely well and not readily desorb in the GC inlet. Two different fibers were tested in this study, the PDMS and CAR/PDMS, the latter of which is known to be much more adsorbent for small volatiles. When testing each fiber with T1, more hydrocarbons were seen using the CAR/PDMS compared to the PDMS alone. This seems to suggest that a more adsorbent media may be necessary to see hydrocarbons from tholins.

The observation of a hydrocarbon in T2 on the PDMS fiber not seen in T1, and little else other than broadly eluted amines in the CAR/PDMS extraction of T2 (shown in figure 4.9) suggests another possibility. The complexity of the tholin samples could lead to oversaturation of the SPME fiber media, lowering the dynamic range of the method for less abundant species or those with lower specificity of binding for the fiber media. This could be readily addressed through optimization of the SPME fiber media. While 100  $\mu\text{m}$  PDMS and CAR/PDMS SPME fibers are well suited for volatiles, 7 or 3  $\mu\text{m}$  PDMS are better suited for semi-volatile non-polar compounds, providing a ready point for future optimization of hydrocarbon detection. Some inferences can be drawn from the hydrocarbons identified in

the sample. Few hydrocarbons overall are identified for T1, with the most abundant peaks occurring for the CAR/PDMS extraction. In that extraction the only hydrocarbons readily identified are cyclopentane, methylcyclopentane, and hexane. Not being able to detect these hydrocarbons on the PDMS fiber extraction does indicate that a more adsorbent fiber media is necessary to detect small hydrocarbons, which fits with the CAR/PDMS fiber being well suited to gases and low molecular weight compounds. The larger hydrocarbons identified in T2 include 2,3,4 trimethylhexane, 2,4-dimethylheptane, and 4,5-diethyloctane, all of which are only seen on the PDMS extraction and the first two of which are also seen in T1 in very small quantities. This may be due to lower production, unsuitability of the SPME fiber media, or losses over time due to volatility or atmospheric exposure since T1 is older and has been exposed to air multiple times prior to this study, compared to the newer T2 that has only been exposed to atmosphere during initial collection. Modeling of mechanisms that enable the synthesis of these hydrocarbon products or their precursors under Titan conditions may enable anticipation of smaller hydrocarbons to target in future studies.

#### **4.5 Conclusion**

A SPME-GC method has been presented for the extraction and analysis of tholins considered representative of Saturn's moon Titan. Two different SPME fiber types have been tested on two different tholin samples and found to allow for the successful identification of a wide range of compounds with various sizes and chemical functionalities. The majority of the compounds detected are larger semi-volatiles, filling a gap in prior pyrolysis-GC analyses which focused on smaller compounds. Functionalities identified

comprised mostly nitriles, as expected from these earlier studies. Amines, hydrocarbons, and aromatics were also identified. While many of these compounds have been identified in prior work some new compounds, such as 2,4-diaminopyrimidine, have been identified that are of particular astrobiological interest. The additional identification of hexamethylenetetramine, seen in prior NMR studies, solidifies the ability of this method to identify a wide variety of compounds. These characteristics makes this SPME-GC method worthy of consideration for inclusion in future missions to study the aerosols of Titan's atmosphere and surface.

The present study is exploratory and as such there are factors impacting the effectiveness of the method for the identification of molecules such as amines and high sensitivity analysis. These factors would need to be addressed prior to mission application. Major issues include oversaturation of the SPME fiber media and subsequent lower dynamic range, the specificity of binding for both the fiber media and the GC column for analytes of interest, large headspace volume, and long extraction timescales. The impact of oversaturation on the dynamic range for some compounds and ways to mitigate it have been addressed above. To address specificity of binding for the fiber media, a variety of fiber media could improve both sensitivity and detection, while also positively impacting dynamic range. While 2D-GC could be considered as a way to address the specificity of binding for the GC-column, considering it has been used in successful analysis of tholins by pyrolysis-GC<sup>39</sup> and petroleum sample characterization,<sup>55</sup> it would be extremely difficult to implement during a lander mission. An alternative would be the inclusion of ancillary columns optimized for compounds for which the primary column had lower binding specificity. The final issues of headspace volume and extraction timescales could be readily mitigated with

engineering controls and increased optimization. Since the issues of this exploratory study can be readily addressed with further work, SPME should be considered for inclusion on any future lander mission to Titan considering its success with the analysis of these samples.

If the SPME method proposed here was to be included on a future mission to Titan, the overall protocol could be easily adapted. An aerosol collection media, such as a Teflon filter, could be loaded onto a sample carousel, allowing for sequential exposure during descent into Titan's atmosphere. Upon landing, the collection media would be heated in the presence of the fiber media of choice. Here, the headspace volume would be minimized while the fiber media surface area maximized to ensure maximum absorption of the analytes. From there, the fiber media could readily be desorbed in the inlet area of a mission GC-MS. Analysis of aerosols on the surface would necessitate a modified setup. The Huygens probe detected volatiles from the surface after landing due to either heating of the probe itself during descent or heating of aerosol collected on the outside of the GC inlet.<sup>5</sup> Any volatiles released from heating due to descent could be measured by exposing the fiber media to the heated volatiles. Detecting less volatile molecules would require an IR source for heating the targeted sampling area, such as an IR bulb with an appropriate reflector, combined with a camera to monitor any physical changes to the surface. The fiber media could then be exposed to the heated surface in a similar manner as discussed above. These methods could be modified further for application of this SPME method to other icy world missions, such as investigations of Europa or Enceladus. There, SPME would enable the detection of volatile organic compounds dissolved in ice or liquid water, considering that many other SPME methods have been designed for the detection of trace organics in water.<sup>56-57</sup>

## 4.6 Acknowledgements

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## 4.8 Supplemental Information

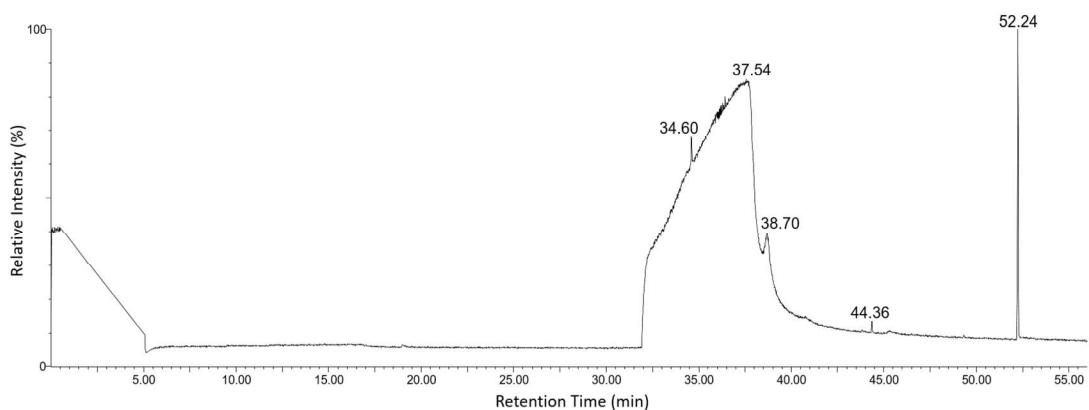


Figure 4.9. CAR/PDMS extraction of T2 showing a large siloxane peak at 52.24 and a very large peak that cannot be identified.

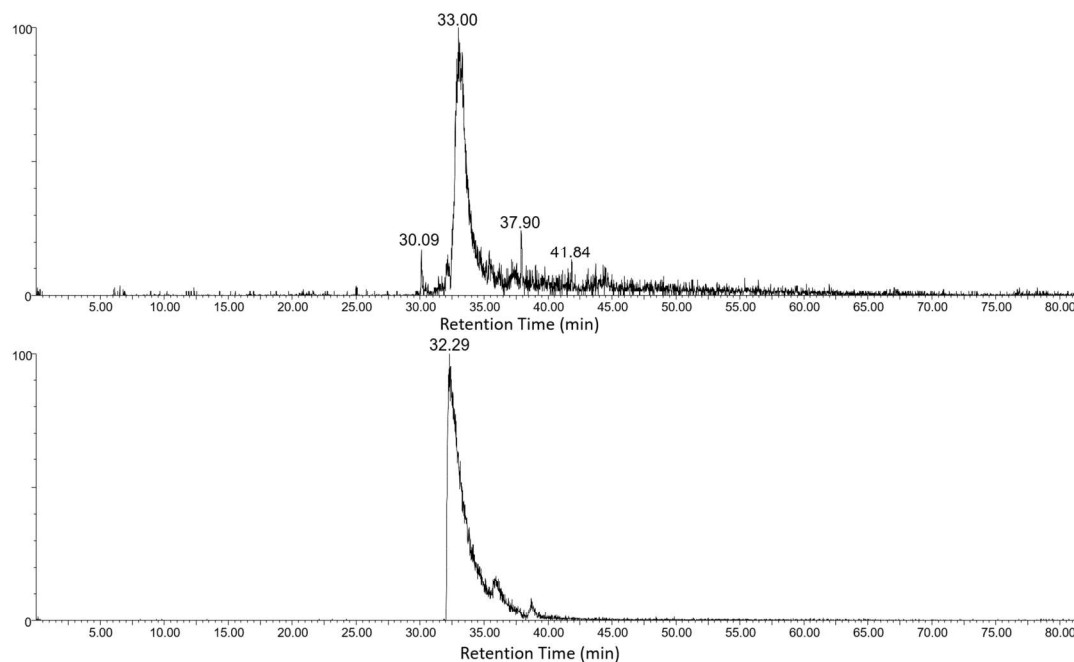


Figure 4.10. The EIC of 30.034 m/z from the CAR/PDMS extraction of each tholin. This fragment is characteristic for a primary amine. Top: EIC of 30.034 m/z for T1 Bottom: EIC of 30.034 m/z for T2.