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Evaluation of a Novel Approach for the Collection and Analysis of Carbonyl Compounds

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for May 2017 to September 2019

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14. ABSTRACT

Pentafluorobenzylhyrdroxylamie (PFBHA) coated Tenax™ thermal desorption (TD) tubes were examined as a substitute for current media employed for the analysis of aldehydes and ketones (carbonyls). Current methods use Tenax™ thermal desorption (TD) tubes (uncoated) that do not account for the labile nature of these analytes. Typical approaches use media coated with various reagents that require an extraction step prior to analysis by liquid chromatography or gas chromatography (GC) combined with a various detectors. The PFBHA technique described here converts the carbonyls to their pentafluorophenyl oximes. These oximes can be analyzed by thermal desorption (TD) GC mass spectrometry (MS). The TD-GC-MS is fielded equipment so that the analysis can be conducted in near real-time. Additionally, since the TD technique does not require extraction, increased sensitivity can be obtained which may be required for air quality or breath analysis. Finally, the conversion of low molecular weight aldehydes and ketones such as formaldehyde to their PFBHA oximes enable better separation by GC and allow detection by MS. The unique fragmentation also avails this method to detect carbonyls that may be unique and not in established GCMS libraries.

15. SUBJECT TERMS
Acetone, Formaldehyde, Acrolein, Pentafluorobenzyl hydroxylamine, oximes

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1.0 SUMMARY

Literature describes a method for analyzing labile aldehydes and ketones via GC-MS by first stabilizing them through derivatization using O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA). For utilizing this approach, PFBHA was infused onto thermal desorption tubes in a collaborative effort with industry (Supelco). Conversion to the oxime facilitates analysis of aldehyde and ketones using field portable mass spectrometry.
This report examines data from analysis of a ketone (acetone) and two aldehydes (acrolein and formaldehyde) to determine the practicality of a commercially prepared derivatizing TD tube. We also examined if the technique could be automated by using thermal desorption autosamplers. The goal was to compare the results to OSHA Method 52. Another advantage of the PFBHA oxime tubes, as prepared, is that the literature indicates that non-carbonyl compounds (hydrocarbons, esters, etc.) can be collected and analyzed simultaneously.

2.0 INTRODUCTION

2.1 Rationale for Research

This research provides insight into the analysis of aldehydes and ketones ("carbonyls"), hazardous volatile organic compounds (VOCs) that are commonly found in environmental and industrial samples as well as breath. Aldehydes and ketones are compounds that are the product of respiration and incomplete fuel combustion and are possible contaminants of pilot breathing air. Current analyses utilize a sorbent tube combined with active sampling to entrap VOCs onto the sorbent bed for later analysis by TD-GC-MS. Similarly, the current protocols employed by AFRL use a TD tube containing the sobent Tenax^{TM} TA. This approach is not effective due to the labile nature of aldehydes which degrade easily and may not be captured by the sorbent due to the analytes' low molecular weight and degree of polarity. The literature describes an analysis of carbonyl compounds that is enabled by coupling the labile molecules to a pentafluorophenyl group using PFBHA reagent (Figure 1).This effort examines the development of a modified TD tube that incorporates the PFBHA into the sorbent bed to stabilize carbonyl compounds using in-situ derivatization upon collection[1]. The resulting increase in molecular weight of target analytes and enhances MS sensitivity to the parts per billion range. Additionally, the increase in structural complexity allows for greatly enhanced chromatographic separation. Another advantage of our approach is the added volatility of the derivative (oxime) compared to the starting compound. While other "gold standard" approaches currently employed require liquid chromatography[5,6], the oxime approach lends itself to the formation of derivatives that are sufficiently volatile to be analyzed by GCMS. Another advantage of the oxime is the presence of key ions that can be used to identify lesser known aldehydes and ketones that may be present.

The advantages of our technique are:

1. Volatization of aldehydes and ketomes by conversion to their fluorinated oximes;
2. Stabilization of the labile aldehydes;
3. Simultaneous collection of aromatic and aliphatic hydrocarbons with carbonyl compounds
4. Increased sensitivity over current methods by 100-fold

There has been two approaches described by Hang-Ho et al[2, 3] and this report focuses on O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) as the derivatizing agent. The agent is illustrated in Figure 1.

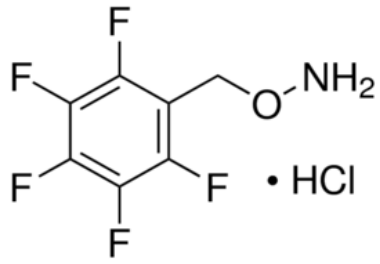

Figure 1: Derivatizing agent O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA)

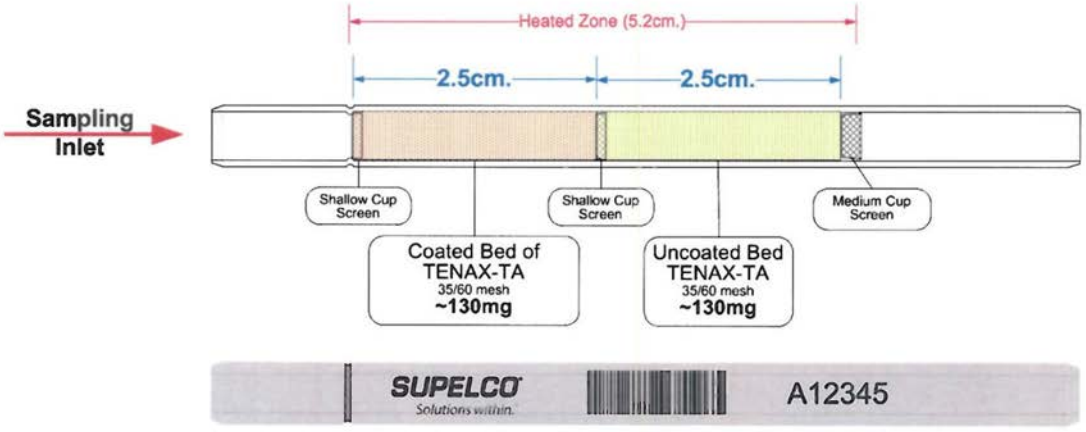

Figure 2: Design of the 2-bed tube (Artwork Supplied By Supelco Under Contract)

Ratcliffe et al[4] have identified 1840 volatile organic compounds (VOCs) that are generated from healthy humans. Of these, there are 872 VOCs that are found in breath. A large percentage of these are ketones and aldehydes. This approach, with enhanced sensitivity and stabilization of aldehydes, has the potential to provide insight to biomarkers related to various ailments as part of our on-going studies. While this work will be focused on a specific set of compounds, the fact that the derivative yields a common mass fragment ion(s)[3] will allow for the tentative identification of unknown aldehydes and ketones. Carbonyl compounds are not only the product of biogenic processes but are also produced by incomplete combustion of fuels. It is currently unknown if these combustion by-products are removed by on-board oxygen generating systems installed onboard high-performance aircraft. Therefore, we suggest incorporation into an existing AFRL technology known as the real-time air quality sensor (RTAQS), a device intended for installation into high-performance aircraft for sampling and analysis of pilot breathing air in flight. RTAQS has completed Air Worthiness certification and been deployed for multiple sorties to date. Because the RTAQS device is also capable of collecting VOCs using an installed TD tube, an upgrade of that sorbent tube to the PFBHA tube would expand the range of analytes able to be analyzed The modified tubes could also be used in future Breathing Air Quality Studies .

Specific Study Goals:

a. Use commercially prepared standards to develop analysis methods for gas-chromatography mass-spectrometry that will resolve individual pentafluorobenzyl oxime species
b. Evaluation of tubes prepared by Supelco for ability to derivatize aldehydes and ketones
c. Demonstrate collection and in-situ derivatization of vapor phase carbonyl compounds using custom-prepared thermal desorption tubes

3.0 METHODS

3.1 Equipment and Supplies

TD tubes (Supelco, Bellefonte, PA) are glass (3.5" x 0.25") and contain two sections of Tenax™ TA. Section 1 contains ~130mg Tenax TA™ coated with ~32µg pentafluorbenzyl hydroxylamine (PFBHA) and section 2 contain ~130 mg of uncoated Tenax TA™. All Tenax TA™ was 35/60 mesh. The vapor generator unit was purchased from KIN-TEK Analytical, Inc. (La Marque, TX) and includes a base module, a secondary dilution module and humidity generator. Ultra-high purity N_2 is used as the diluent gas. All permeation tubes were sourced from KIN-TEK . Thermal desorption GC-MS is carried out using a TD-100xr automated thermal desorption system (Markes Int'l, Llantrisant, U.K.) equipped on an Agilent gas chromatograph/mass spectrometer (7890A/5975C). The GC column used was sourced from Restek (Bellefonte, PA) and was a Rxi®-624Sil MS of 60 m x 0.32 mm x 1.8 µm. The cold trap used in the Markes TD-100xr was manufactured by CAMSCO (Houston, TX) was their Air Toxics (SKU: CTM60401). Methanol was sourced from Fisher Chemical (Pittsburgh, PA). Acrolein (Sigma-Aldrich; Item#89116-1mL; Lot#BCBV1750), formaldehyde (Sigma-Aldrich; Item#252549-25mL; Lot#SHBG0805V), and acetone (Sigma-Aldrich; Item#650501-1L; Lot#MKBZ8392V) were also used. Additional TD tubes (Supelco, Bellefonte, PA) are stainless steel (3.5" x 0.25") and contain 1.0cm of Carbopack-Y (40/60mesh), 2.5cm of Graphsphere-2016 (60/80 mesh), and 1.5cm of Carboxen-1003 (40/60 mesh). Manual injection used the CSLR (Markes) and dry purged using the TC-20 (markes). Oxime derivatives of acetone (Santa Cruz Biotechnology; Item# sc-233804; Lot#J3017), formaldehyde (Santa Cruz Biotechnology; Item# sc-235211; Lot#E1519), and acrolein (Santa Cruz Biotechnology; Item# sc-233816; Lot#E1019), were purchased as neat standards. A Hazardous Air Pollutants On-Site Extended Range (HAPSITE-ER) GC-MS was purchased from Inficon (Syracuse, NY)

3.2 TD Tube Preparation

Based on literature published by Chien and Ko-Ghun (2009), a two bed thermal desorption tube was prepared. Because these TD tubes are not commercially available they were prepared by Supelco to contain a front section with the derivatizing agent and a back section with untreated Tenax™ TA (Figure 2). The first bed near the inlet side was coated Tenax®-TA and then uncoated bed of Tenax®-TA. In both cases the mesh size of the Tenax®-TA was 35/60. Incorporation of untreated sorbent at the back of the TD tube allows for collection of other VOCs in addition to aldehydes and ketones to provide a more complete analysis.The beds are of equal length (2.5cm) and entirely in the heated zone of the thermal desorber unit. One of the two beds

was coated with PFBHA (~32µg). To retain the packing material and to separate the beds, a shallow cup screen was used near the inlet size and between the coated and uncoated beds with a medium cup screen at the end.

Figure 3: Markes® International CLSR

Tubes were prepared by Supelco in the following manner. Sufficient Tenax®-TA 35/60 was heated in a purge bomb at 320 ºC under an inert nitrogen atmosphere for 24 hours. The derivatizing agent was dissolved in 25 mL of methanol and sonicated to ensure that the agent was completely dissolved. The mixture was then added to a 200 mL rotary evaporator with an additional 50mL of methanol. The rotovap was started and submerged into a water bath at 40 ºC for 1.5 hours under 28.5 in. Hg vacuum. The coated Tenax®-TA was removed from the rotovap and stored under inert atmosphere packing.

3.3 TD-GC-MS Parameters

All conditions and pertinent variables related to the analysis of carbonyls (except formaldehyde) are presented in tables 1-3.

Table 1: GC oven ramp program

Start	Ramp Rate (ºC/Min)	Ramp Time (Min)	Temperature (ºC)	Hold Time (Min)
00:00			40	02:00
31:00	10.0	24.00	280	05:00
35:00	05.0	4.00	300	00:00

Table 2: GC column and flow parameters

Column Information	Rxi-624Sil MS Column: Restek#13872

Temperature Range	50 °C – 320 °C (320 °C)
Dimensions	60m X 320µm X 1.8µm
In	Front SS Inlet He
Out	MSD
Initial	40ºC
Pressure	8.7465 psi
Flow	1.5 mL/min
Average Velocity	31.404 cm/sec
Holdup Time	3.1843 min
Flow Setpoint	On
(Initial)	1.5 mL/min
Post Run	1mL/min

Table 3: Markes XR-100 thermal desorption conditions

Name	**Markes TD**		**PurgeTrapFlow**	**68**
Mode	**TD 100 Internal Std. 2-3 Stage Desorb**		**PrepurgeSplit**	**0**
CarrierGas	**He**		**PrepurgeSplitFlow**	**50**
TD100Type	**Series 2 Advanced**		**DryPurgeTime**	**1.0**
LoadTemp	**40**		**DryPurgeFlow**	**50**
UltrAUnload Temp	**100**		**InternalStandard**	**0**
StandbySplit	**1**		**AddISTo**	**Tube**
FlowPathTemp	**10**		**DesorbTime1**	**5**
Overlap	**1**		**DesorbTemp1**	**300**
GCCycle Time	**45**		**TrapInLineDesorb1**	**1**
MinCarrierPressurePSI	**5**		**TrapDesorbFlow1**	**68**
StdInjectType	**Prepurge time (min)**		**DesorbSplit1**	**0**
PrepurgeTime	**1**		**DesorbSplitFlow1**	**50**
PrepurgeTrapInLine	**1**		**TubeDesorb2**	**0**
DesorbTime2	**10.0**		**TrapPurgeFlow**	**50**
DesorbTemp2	**250**		**TrapLow**	**30**
TrapInLineDesorb2	**1**		**TrapHeatingRate**	**MAX**
DesorbFlow2	**50**		**TrapHigh**	**300**
DesorSplit2	**0**		**TrapDesorbTime**	**3.0**
DesorbSplitFlow2	**50**		**TrapDesorbSplit**	**1**
TrapPurgeTime	**1**		**TrapDesorbFlow**	**41.75**

3.4 Sample Preparation – Manual Injection Method

Standards of the underivatized aldehydes/ketones were prepared in methanol and dilute solutions were injected onto TD tubes. The commercially prepared oximes were injected onto Tenax™ tubes at varying concentrations to prepare calibration curves and discussed under results. Standards acquired from Supelco were diluted and varying concentrations were injected onto the commercially prepared PFBHA tubes in comparison to the commercially prepared oximes and is discussed under results. All liquid injections onto TD tubes were performed using a compact syringe loading rig (CSLR; Markes Int'l, Llantrisant, U.K.) shown in Figure 3.

3.5 Sample Preparation – Vapor Infusion Method

The permeation tubes were inserted into the permeation device (Kin-Tek) and vapor was generated as described in Tables 5 and 6. The data is discussed under Results.

Table 4: Vapor generator parameters for acetone infusion

KIN-TEK Parameters	
Dilution Flow=1.625	Component Flow=0.0
Temperature=30°C	HG Flow=0.143
2° Dilution Flow=0.685	PSIG=0.11
Concentration=100 ppb	
Collected w/GilAir @ 50 mL/min	
Molecular Weight of Acetone=58.08 g/mol	

Table 5: Vapor generator parameters for formaldehyde infusion

KIN-TEK Parameters	
Dilution Flow=1.910	Component Flow=0.0
Temperature=80°C	HG Flow=0.143
2° Dilution Flow=0.833	PSIG=0.11
Concentration=100 ppb	
Collected w/GilAir @ 50 mL/min	
Molecular Weight of Formaldehyde=30.03 g/mol	

Table 6: Vapor generator parameters for acrolein infusion

KIN-TEK Parameters	
Dilution Flow=0.240	Component Flow=0.0
Temperature=30°C	HG Flow=0.143
2° Dilution Flow=0.018	PSIG=0.11
Concentration=35 ppb	
Collected w/GilAir @ 20 mL/min	
Molecular Weight of Acrolein=56.06 g/mol	

3.6 Breath Analysis

Breath samples were collected onto PFBHA derivatization tubes by using Tedlar breath bags (1L) to capture the sample and immediately pulling exhaled breath samples onto the TD tubes using a GilAir sampling pump. A total of 0.5 L of breath was loaded to the TD tube at a flow rate of 100 ml/min. TD tubes were analysed as described using GC-MS with the exception of column stationary phase, which had a DB-5 chemistry and was 30m in length.

4.0 RESULTS

4.1 Derivatization Reaction

The PFBHA reaction with aldehydes and ketones is depicted in Figure 4. This example illustrates the reaction of an aldehyde with the derivatization reagent. In the case of formaldehyde, R would be hydrogen. For acrolein, R would be substituted with C_2H_3. In the case of acetone, both the H and R would be substituted with CH_3. It should be noted that, with the exception of formaldehyde and acetone, all aldehydes and ketones will result in 2 enantiomers. This was observed for acrolein.

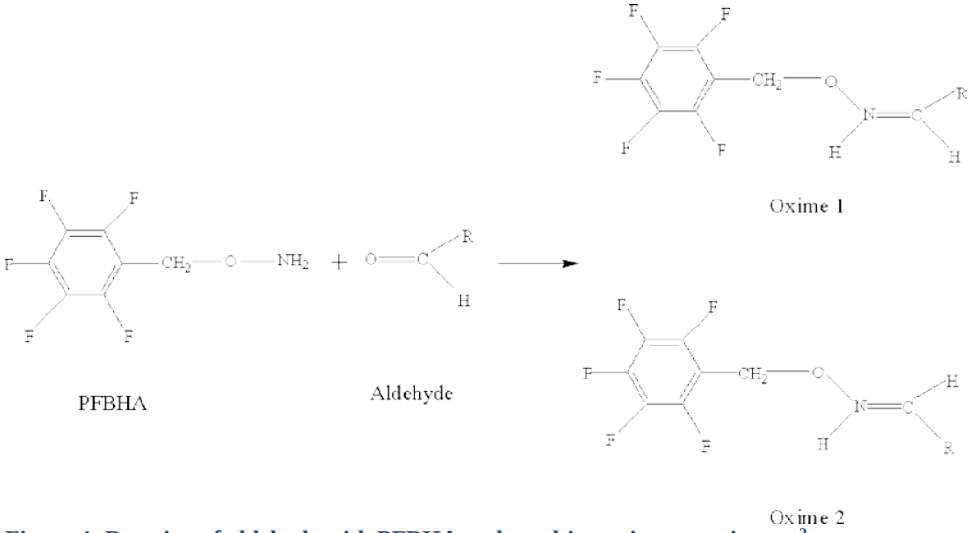

Figure 4: Reaction of aldehyde with PFBHA and resulting oxime enantiomers[2]

4.2 Commercially Prepared Oximes

The results for the commercially available standards provided linear curves with an R^2 value ≥ 0.99 (Table 7-9). Table 7 and Figure 6 illustrate that the commercially prepared oxime for formaldehyde generates a linear response over a wide range of concentrations.

Table 7: Calibration gradient for formaldehyde oxime injection

Manual Injection of Formaldehyde Oxime on Tenax®-TA 35/60	**Area Response** (Formaldehyde Oxime) RT=16.94 min; Q_{ion}=181
47.2 ng	89,872
94.4 ng	149,646
118 ng	157,499
236 ng	390,422
354 ng	619,374
472 ng	859,596
590 ng	922,231
1,180 ng	2,265,906
R^2 = 0.9914	

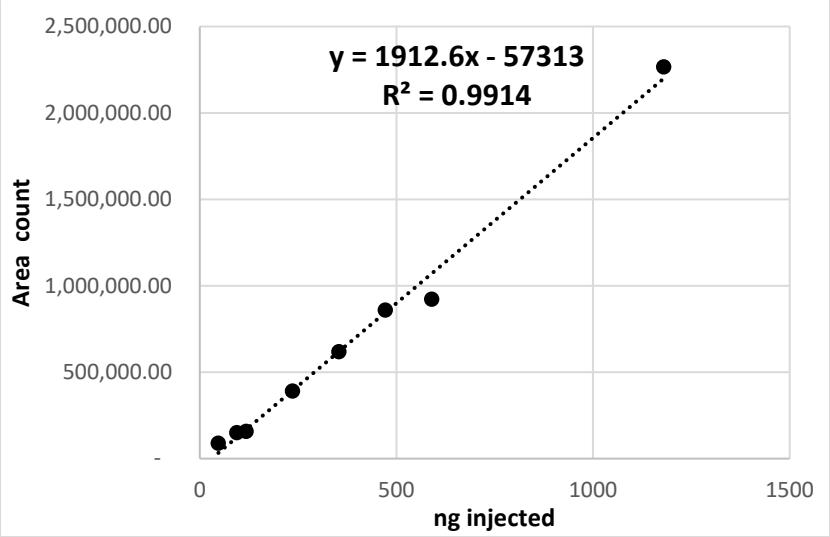

Figure 5: Linear regression of formaldehyde oxide standard titration

Table 8 and Figure 7 show the same for acetone oxime.

Table 8: Calibration gradient for acetone oxime injection

Manual Injection of Acetone Oxime on Tenax®-TA 35/60	**Area Response** (Acetone Oxime) RT=19.49 min; Q_{ion}=181
1 ng	4,096
2 ng	6,956
10 ng	52,667
40 ng	278,045
200 ng	555,340
1,000 ng	2,429,755
R^2 = 0.9948	

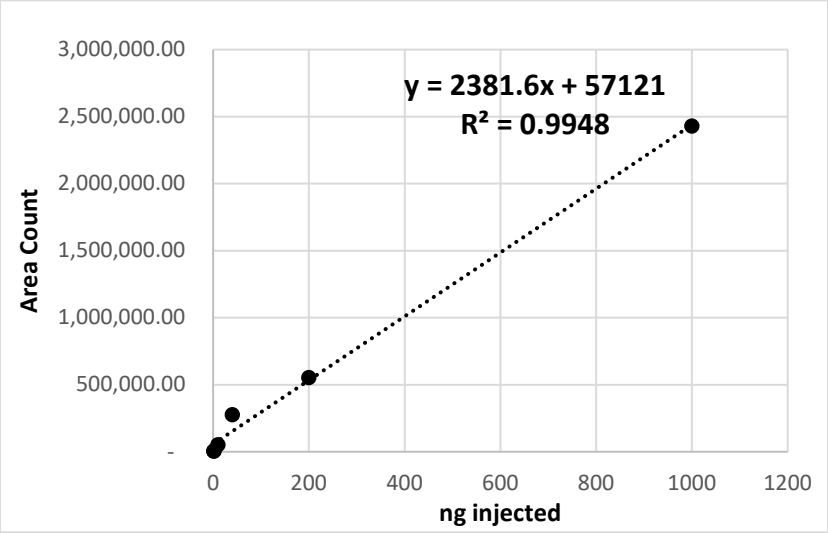

Figure 6: Linear regression of acetone oxime standard titration

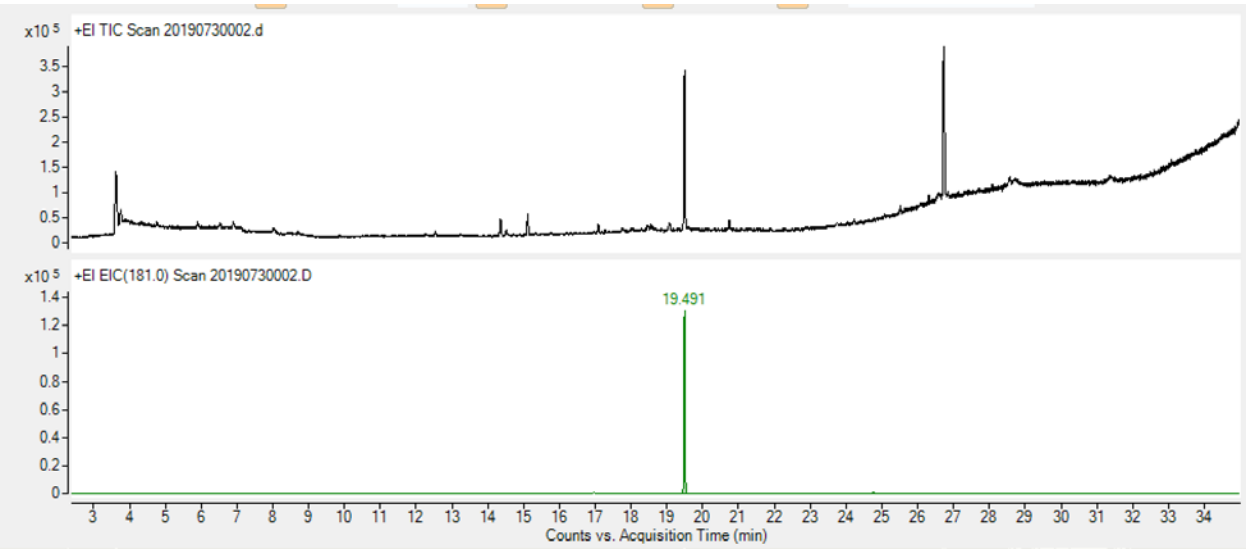

Figure 7: Acetone oxime chromomatogram on Agilent GCMS

The mass spectrum also illustrate the unique characteristics of the oximes. The oximes are easily separated by gas chromatography. Additionally, the aliphatic oxime derivatives have a unique fragment at *m/z* 181, which represents the pentafluoro benzyl hydroxylamine moiety. The signal from this ion is represented in Figure 7 showing the ease of identifying the target oximes in this analysis.

Table 9 and Figure 8 refer to the calibration curve generated with commercially prepared acrolein oxime. Table 9 shows amounts used for acrolein oxime as well as the sum peak area values from both enantiomers. Figure 8 illustrates linear regression analysis of both enantiomers individually. Both showed good linearity across the range of tested amounts. Figure 9 represents the GC-MS data for this compound and exhibits separation of enantiomers of the analyte.

Table 9: Calibration gradient for acrolein oxime injection

Manual Injection of Acrolein Oxime on Tenax®-TA 35/60	**Area Response** (Acrolein Oxime) RT=19.96 min; Q_{ion}=181
21 ng	40,298
42 ng	81,195
100 ng	126,462
210 ng	280,566
315 ng	463,228
525 ng	762,592
735 ng	1,303,684
1,050 ng	1,544,093
R^2 = 0.9923	

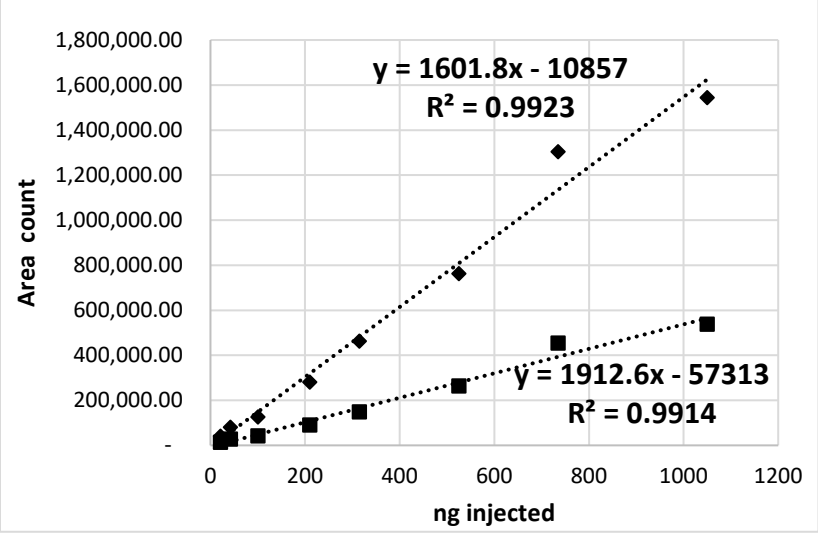

Figure 8: Linear regression of acrolein oxime standard titration showing values from both enantiomers

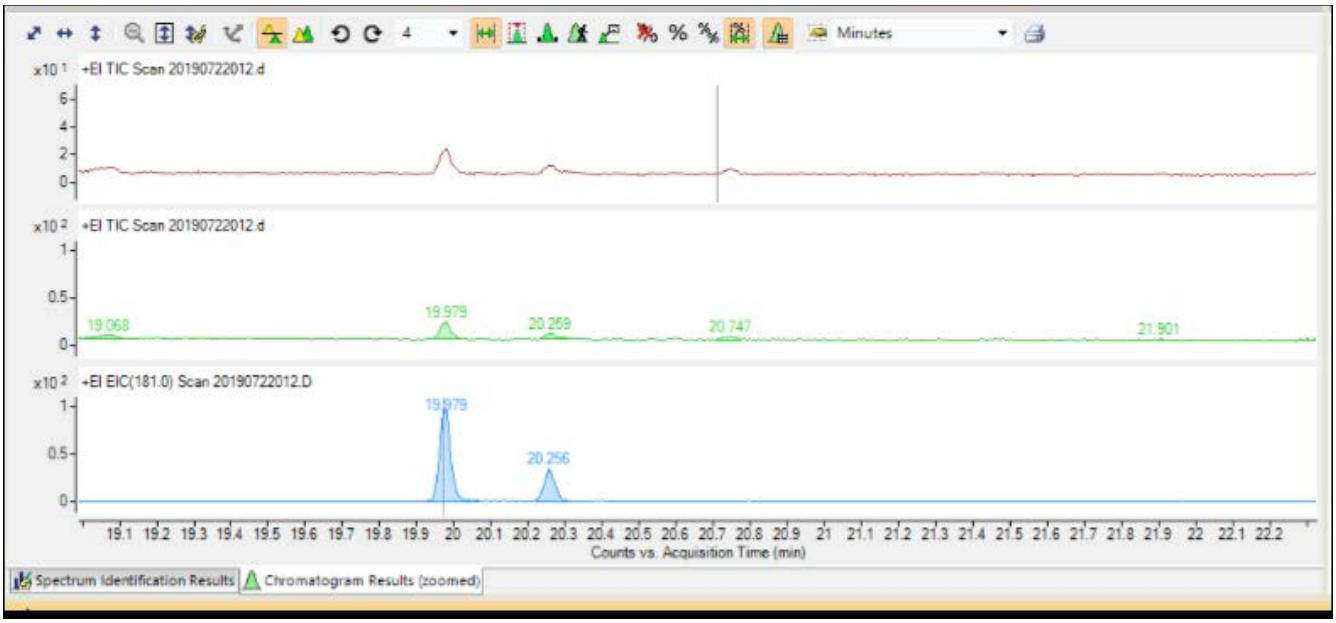

Figure 9: Acrolein oxime chromomatogram on Agilent GCMS showing both enantiomers (20.0' and 20.3').

In most cases the molecular ion is also observable. Acrolein undergoes an asymmetrical reaction yielding 2 enatiomers, which are visible as two distinct peaks in Figure 9 (though the current analysis does not provide the resolution to distinguish between enantiomers of acrolein oxime). This is typical for all compounds other than formaldehyde and acetone. The data reinforces the idea that the oximes can be used for discovery work in breath analysis and other studies involved with oxidation products of hydrocarbons.

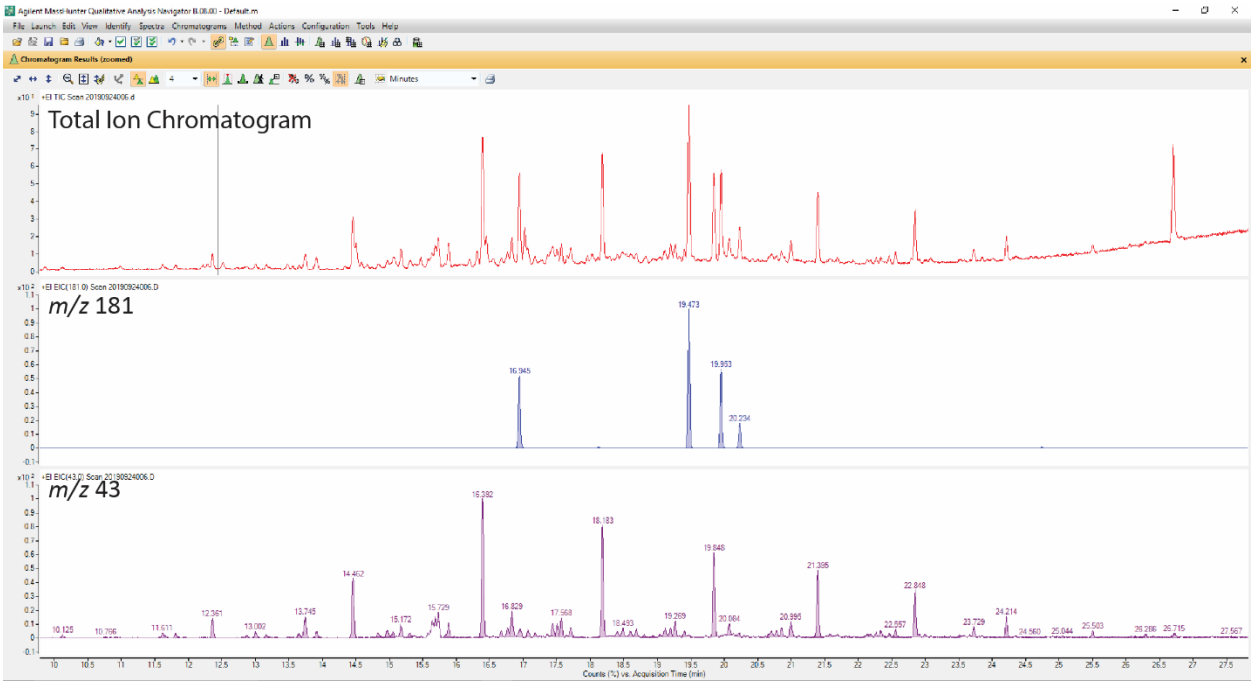

Figure 10: Detection of oximes in the presence of JP-8

4.3 Other Sample Types

In effort to explore all modes of potential utility for this technology a series of more challenging sample types were generated that simulate anticipated uses in the field. Because the aerospace environment frequently coexists with high concentrations of fuel vapor, analysis of oximes in the presence of JP-8 fuel was done. At m/z 43, which is commony used for aldehydes and ketones, the target (underivatized) compounds would be masked by the hydrocarbons. They are easily detected as the PFBHA derivatives at *m/z* 181.

To demonstrate the utility of this method for field analysis, acetone oxime standard was loaded onto a standard TD tube and analyzed by the HAPSITE-ER instrument at varied concentrations (Figures 11 and 12). These analyses show that using standard, fielded instrumentation, PFBHA-treated TD tubes will likely provide quantitative analysis of a subset of volatile toxins that would be undetectable by the legacy methods, as acetone oxime provided a linear response (R^2 value $\geq$ 0.9983) with the HAPSITE-ER.

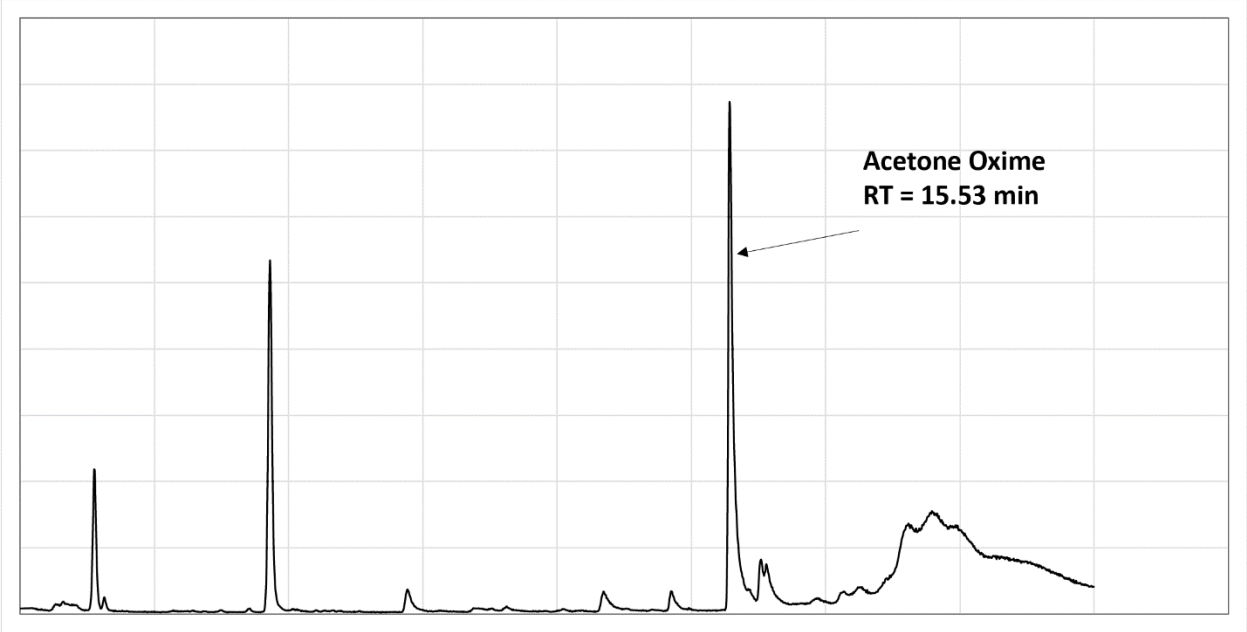

Figure 11: Acetone oxime total ion chromomatogram on HAPSITE-ER GCMS

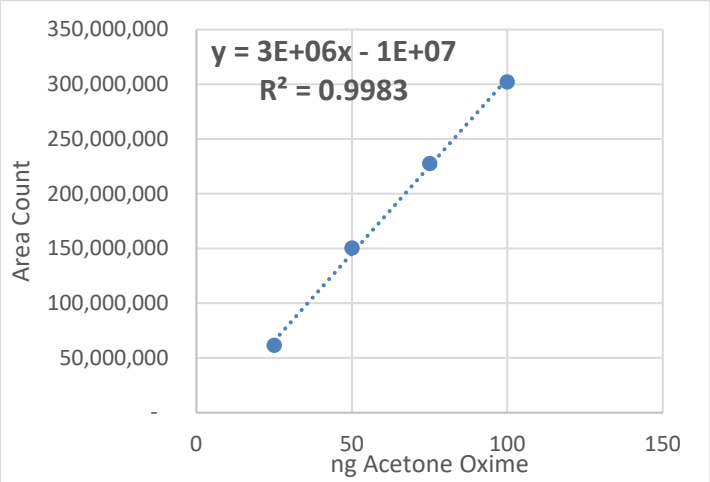

Figure 12: HAPSITE-ER analysis of acetone oxime standard at varied concentrations

In-situ derivatization of carbonyl compounds also offers significant advances toward breath analysis[9]. To date, analysis of breath compounds is done by TD-GC-MS but uses collection media composed primarily of Tenax-TA or a mixture of Tenax and graphite-based sorbents and offers little stabilization for labile aldehydes and ketones known to be found in breath. Only recently (following proposal and funding for the current work) has our approach been discussed as a method for breath analysis[9]. Figure 13 is an annotated chromatogram (extracted ion of *m/z* 181) from a breath sample collected using a PFBHA TD tube and analyzed by TD-GC-MS. Identifications of the peaks by NIST database correlate well with compounds previously discovered in breath samples. As discussed previously, nearly all of the carbonyl compounds will exhibit stereochemistry upon derivatization however it is likely that the chromatographic

conditions used to generate the analysis in Figure 13 (DB-5 GC column) were unable to resolve them apart for each compound. Nevertheless, biomarker discovery in breath samples would be greatly accelerated by the stabilizing effect and increase in sensitivity offered by PFBHA derivatization of carbonyls.

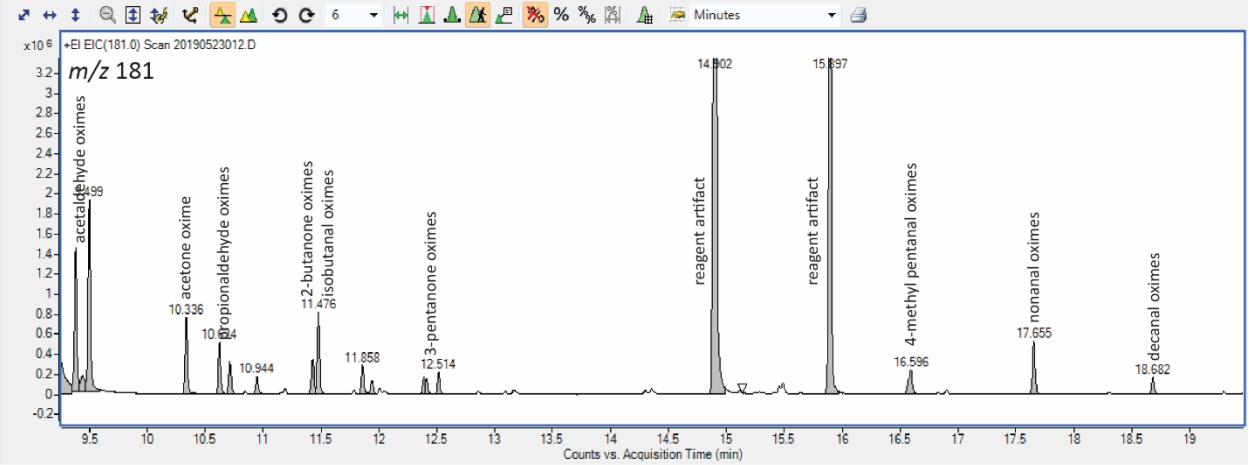

Figure 13: Representative PFBHA-derivatized breath sample

4.4. Vapor Generations of Aldehydes and Ketones onto PFBHA Tubes

The Kin-Tek vapor generator was used to provide vapor directly to the tubes prepared by Supelco. Multi-point calibrations were attempted for acetone and acrolein to demonstrate the feasibility of capturing carbonyl compounds from gaseous samples using PFBHA tubes. The calibration parameters and peak area response data is shown in Table 11. Figures 14 and 15 show linear regression analysis for calibrations of acetone and acrolein, respectively. While acetone was able to more or less generate a stable and linear calibration curve across five points, acrolein did not provide as reliable of a response, possibly due to the unstable nature of permeation tubes containing acrolein due to polymerization reactions that are known to occur with this somewhat unstable compound. While frustrating for the purpose of this experiment, the unstable nature of acrolein highlights the utility of our method.

Table 10: Calibration parameters and responses for vapor generation of acetone and acrolein standards.

Vapor Generation of Acetone on PFBHA thermal desorption tubes	**Area Response** (Acetone Oxime) RT=19.49 min; Q_{ion}=181
25 ng	644,136
100 ng	686,599
150 ng	942,794
250 ng	1,001,018
350 ng	1,083,692
R^2 = 0.8724	

Vapor Generation of Acrolein on PFBHA thermal desorption tubes	**Area Response** (Acrolein Oxime) RT=19.96 min; Q_{ion}=181
40 ng	379,179

100 ng	387,783
140 ng	430,021
R^2 = 0.7869	

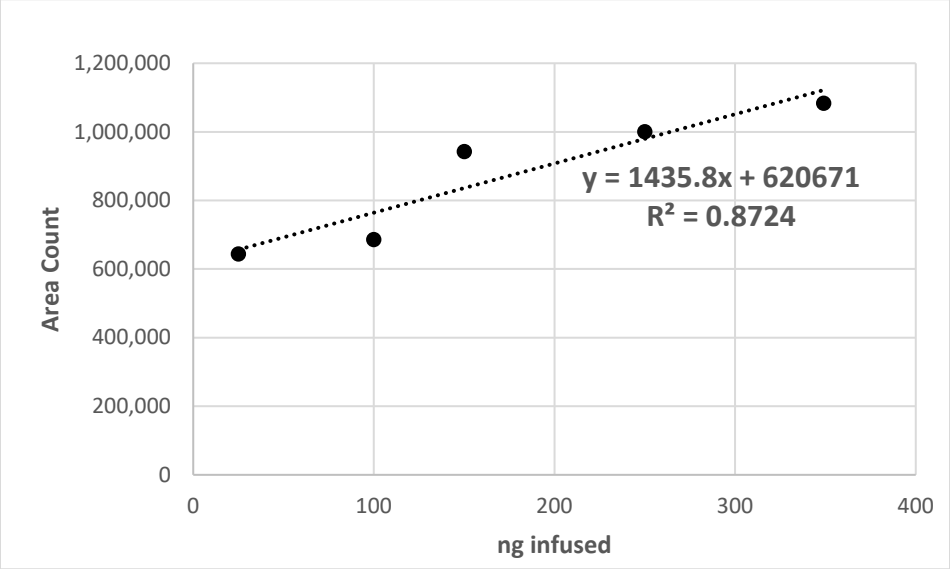

Figure 14: Regression analysis of vapor-phase acetone collected on PFBHA TD tubes

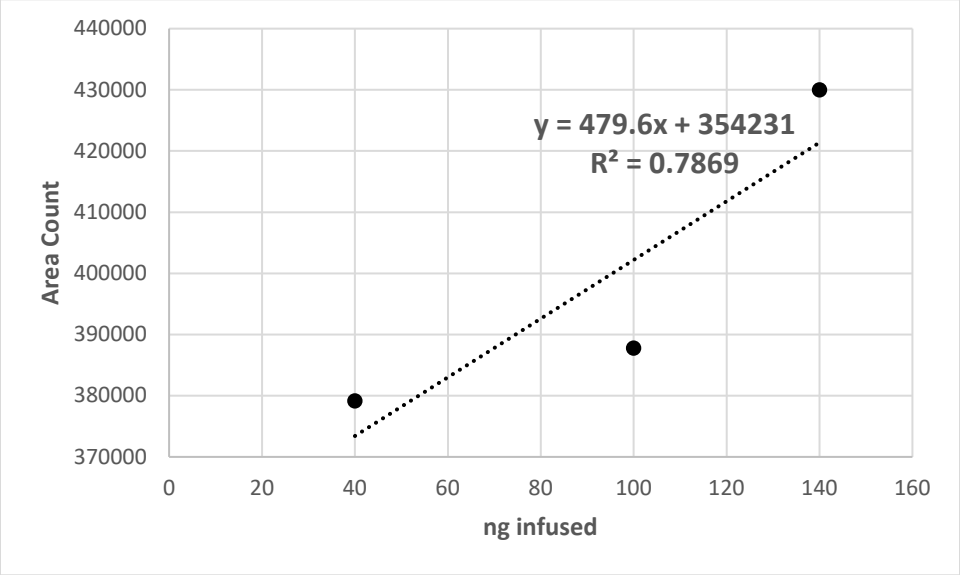

Figure 15: Regression analysis of vapor-phase acrolein collected on PFBHA TD tubes

Though a similar calibration experiment was attempted with formaldehyde, the results demonstrated very little or no separation based on concentration. We attribute this to either poor volatilization of this compound or interference with breakdown products. Literature[8] has shown that permeation may not be suitable with formaldehyde.

5.0 Discussion

The experiments demonstrated great reproducibility with the commercially available oximes, confirming that GC-MS analysis of carbonyls derivatized with PFBHA is appropriate. However, despite apparent success with acetone, the derivatization of gas-phase acrolein and formaldelhyde yielded inconsistent results. Due to difficulties in logistics, the PFBHA-loaded TD tubes were in refrigerated storage for longer than anticipated (> 1 year), leading to the hypothesis that this may have resulted in some loss of efficacy in the derivatization reaction. While the tubes were stored at 4˚C, we still suspect that aging may have been a factor. Because this is a new approach there are no guidelines set for storage conditions or duration of these materials. For proper implementation of this method it is reasonable to conclude that an "expiration date" should be experimentally established and that these types of derivatizing TD tube should be either generated just prior to use or stored for a minimal amount of time after manufacture. Another factor may have been the rate of sampling of the vapor generated standards, but this was done consistent with literature[8,9]. Another consideration that differs from the literature is the use of an autosampler that employs a cold trap concentrator. However, since we obtained reasonable results with the commercially prepared standards this does not fully explain the variable data obtained with the tubes.

While some unanticipated challenges were observed in this research, a significant number of successes resulted as well. First, our GC-MS analysis of pentafluorobenzyl oximes was successful in separating individual oximes and provided mass spectra that are contained in commercial databases. Second, across the range of concentrations tested, it is possible to obtain linear response when calibrating instrumentation for quantitative analysis of the oximes. Third, capture and analysis of carbonyl compounds in exhaled breath offers a promising way to achieve more complete qualitative coverage and increasingly accurate quantitative analysis in biomarker discovery efforts. Finally, the commercially prepared oxime for acetone was shown to chromatograph successfully on the HAPSITE-ER.

6.0 REFERENCES

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