



AFRL-RH-WP-TR-2019-0027

**THE CHARACTERIZATION OF A STANDARD BREATH
SAMPLING DEVICES FOR OFF-LINE TD-GC-MS ANALYSIS
(Briefing)**

**Jennifer A. Martin
Human Signatures Branch**

**APRIL 2019
Interim Report**

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13. SUPPLEMENTARY NOTES Report contains color. Case Number: MSC/PA-2019-0183 (original case number(s): 88ABW-2019-1935), cleared 24 April 2019					
14. ABSTRACT Due to several sources of potential variability associated with exhaled breath bag sampling procedures for off-line analysis, the Respiration Collector for In Vitro Analysis (ReCIVA) sampler was developed. Although designed to improve upon several pitfalls of sampling with exhaled breath bags, the ReCIVA remains a minimally studied research tool. In this manuscript, several attributes of the ReCIVA sampler are investigated, such as background contamination, performance of different adsorbent tubes, duplicate sample production, and comparison to exhaled breath bags. Initially, differences were observed in volatile organic compound (VOC) abundances due to flow rate variation between tube type, set point, and ReCIVA tube location. These effects were minimized when an unpublicized update was applied to the control software, however, tube types (Tenax TA & 5TD) still differed in VOC abundance, and Tenax TA VOC sampling was not reproducible between tube locations. The ReCIVA VOC abundances were similar to results obtained from exhaled breath bags, and siloxane contamination was reduced upon baking. Overall, the results suggest that ReCIVA is a feasible alternative to exhaled breath bags for application in breath research, but more research must be completed to develop methods and practical implementation approaches enabling human subjects studies.					
15. SUBJECT TERMS ReCIVA, exhaled breath, volatile organic compound (VOC), Tenax TA, 5TD, contamination, breath bags					
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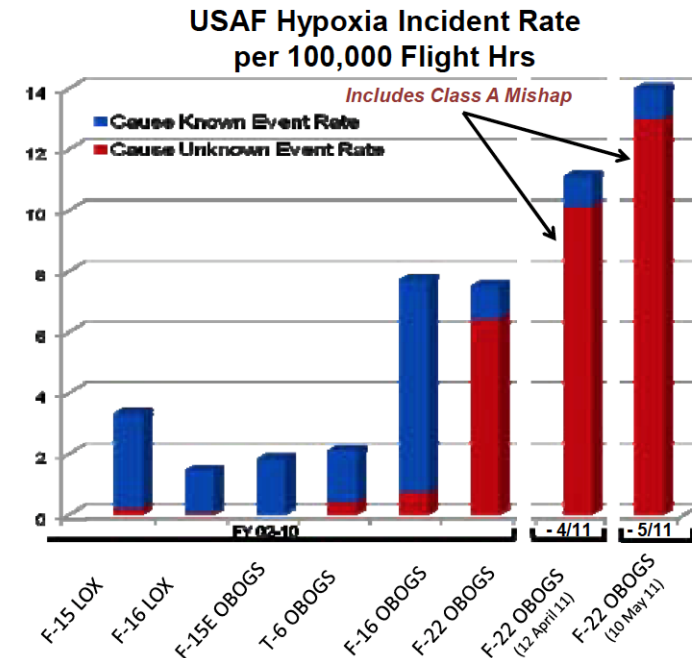
The Characterization of a Standard Breath Sampling Device for Off-line TD-GC-MS Analysis

Jennifer A. Martin, Ph.D.

711th Human Performance Wing, Airman Systems Directorate

US Air Force Training Aircraft

- Numerous reports by high performance aircraft pilots having unexplained physiological events (UPE) mid-flight.
- Examples include the recent grounding of the USAF training fleet in January 2018.
- Continued area of ongoing research within the US Air Force and US Navy.



United States Air Force Report on "Aircraft Oxygen Generation," 2012.
<http://www.af.mil/News/Photos/?igphoto=2000598771>

US Air Force Training Aircraft

- In response to the increase UPEs, the USAF has developed and deployed a response kit for diagnostic purposes.
 - Pilot exhaled breath.
 - Aircraft environmental monitoring.



- **However, exhaled breath collection, via bags, which requires compliance with exhalation protocols may not be an ideal strategy for those potentially impaired.**

Respiration Collector for In Vitro Analysis (ReCIVA)

- Improved Exhaled Breath Sampling:
 - Direct to adsorbent media sampling via real-time CO₂ monitoring. Removing the pitfalls of exhaled breath bags.
 - Versatile breath component collection.
 - Up to 4 breath samples per collection.
 - Replaceable masks.
- Very little published literature using the ReCIVA.
 - Unit Price: \$10,000-\$15,000
 - Consumables: Masks: \$50
 - TD Tubes: \$80 etc.
- Many questions still exist.



Journal of Breath Research

PAPER

Optimisation of sampling parameters for standardised exhaled breath sampling

Sophie L F Doran , Andrea Romano and George B Hanna
Department of Surgery and Cancer, Imperial College, London, United Kingdom

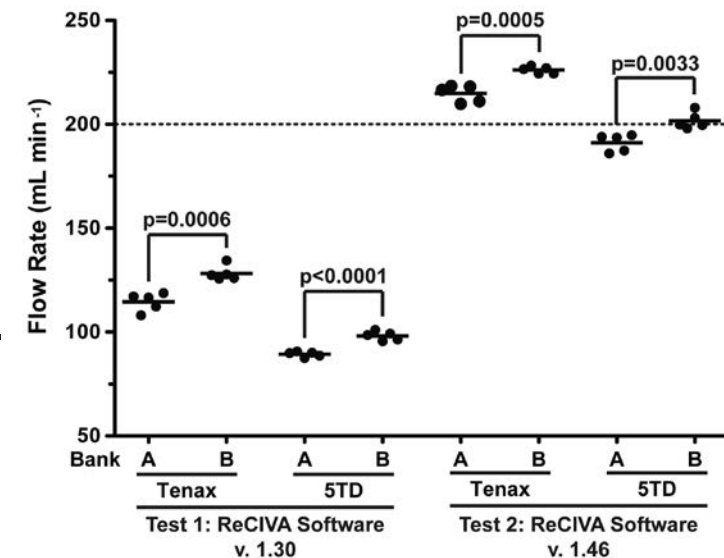
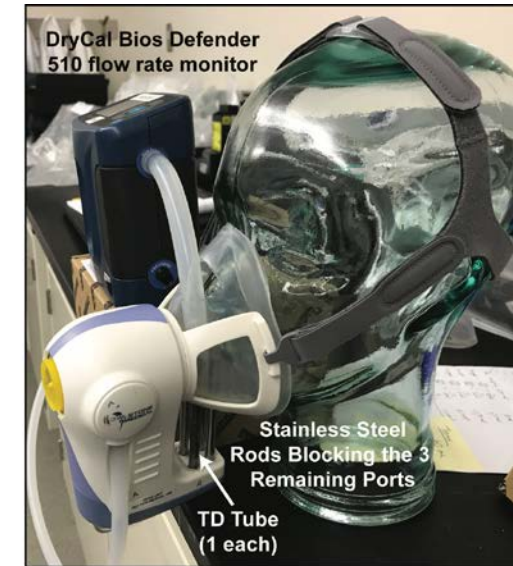
Research Questions

- Is there background associated with the ReCIVA sampler?
- Can the ReCIVA masks be cleaned?
- Can duplicate samples be acquired across ReCIVA banks?
- Does the ReCIVA sampler perform similarly to breath bags?
- Do different TD tubes perform similarly in the ReCIVA sampler?

The answer to these questions may provide justification for further research using ReCIVA device and for the investment of the ReCIVA sampler compared to breath bags.

The Backstory...

- Initial data acquired, as presented previously, was submitted for publication to the Journal of Breath Research.
- The major comment was to verify the functionality of the ReCIVA unit used for the data collection.
- Through communications with Owlstone Medical (ReCIVA manufacturer) it was determined that the ReCIVA control software contained a bug that caused incorrect flow rates to be applied to the TD tubes.
- Therefore, we repeated the overall experiment with updated ReCIVA control software and newly manufactured masks.
- I will focus on this new data collected with the updated software rather than data acquired using the outdated software.

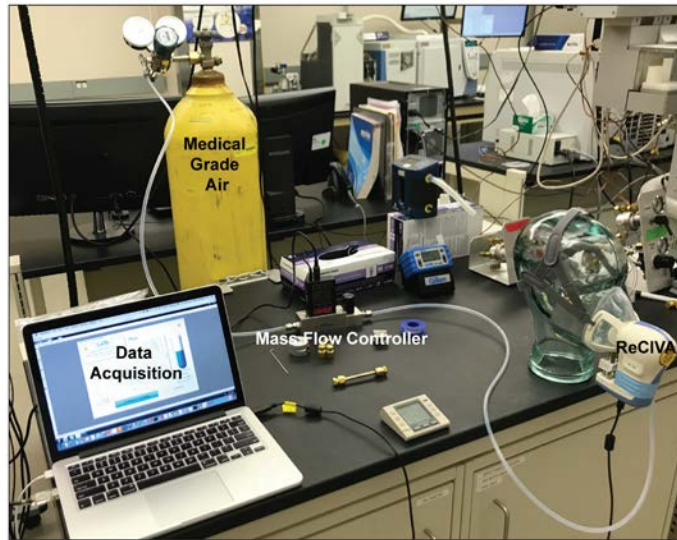


Research Questions

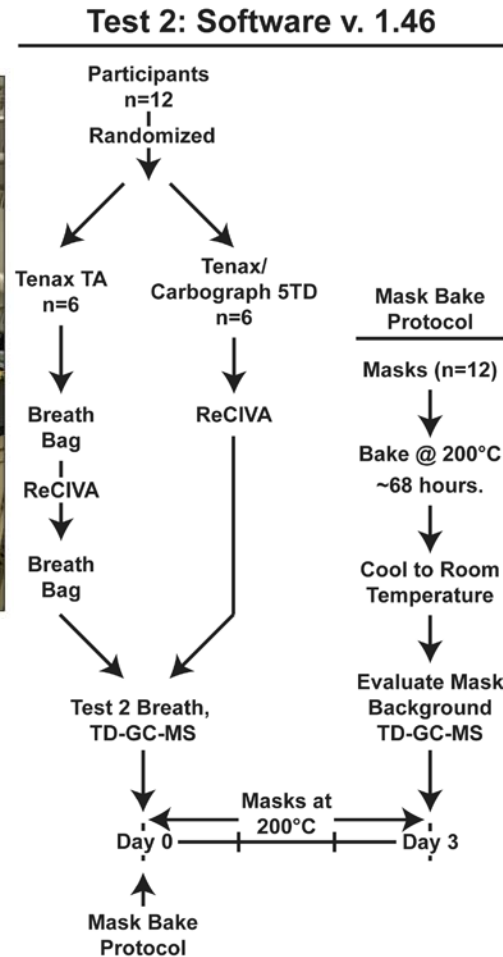
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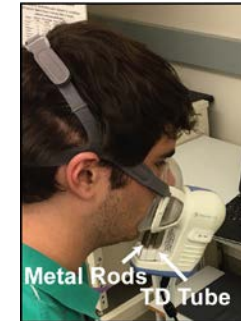
Experimental Design



- 12 male, non-smoking, fasting (1h), volunteer participants were randomized for order and adsorbent material, Tenax TA and Tenax TA/Carbograph 5TD (5TD).
- Each participant sat in a relaxed position for at least 5 minutes prior to sampling.
- All sampling was performed at 200ml min^{-1} for 550mL total.
- Tenax TA participants provided pre/post exhaled breath bags in addition to the ReCIVA collection. 5TD participants only provided ReCIVA samples.

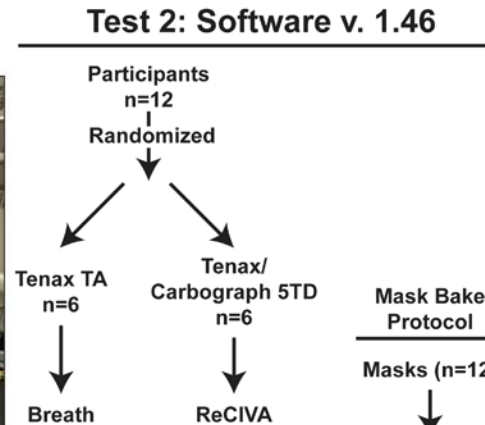
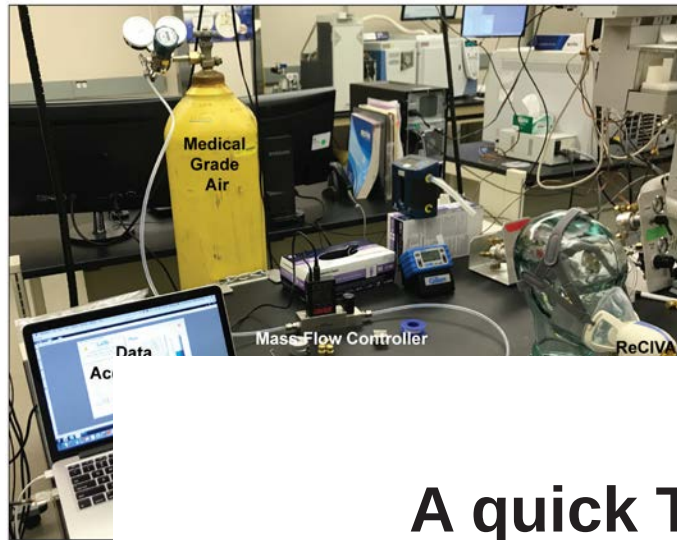


Test 2 (v. 1.46)	
Adsorbent Tube Group	Breath
-	-
-	-
-	-
5TD	R
5TD	R
-	-
Tenax TA	B ₁ , R, B ₂
-	-
Tenax TA	B ₁ , R, B ₂
Tenax TA	B ₁ , R, B ₂
-	-
-	-
5TD	R
5TD	R
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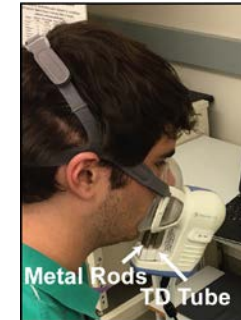


- ReCIVA was operated as recommended by the manufacturer for lower airway breath on both banks (A and B) with the collection ports closest to the participants mouth blocked.

Experimental Design

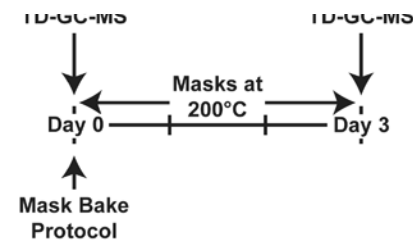


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Adsorbent Tube Group	Breath
-	-
-	-
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5TD	R
5TD	R
-	-
Tenax TA	B ₁ , R, B ₂
-	-



A quick TD-GC-MS primer...

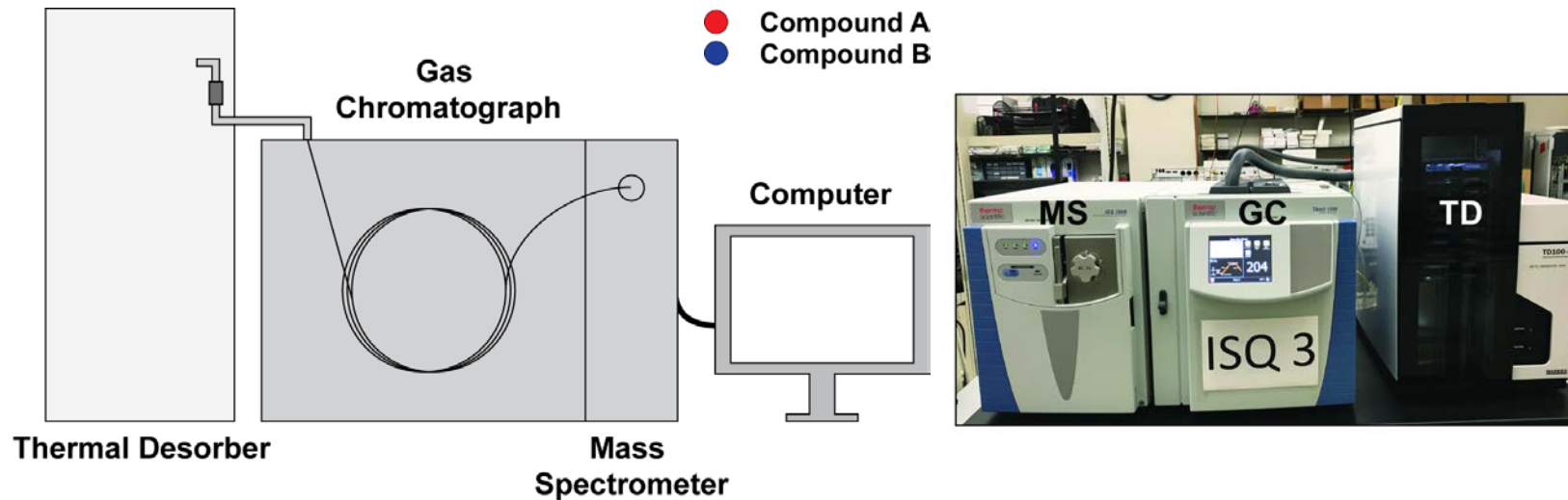
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5TD	R
Tenax TA	B ₁ , R, B ₂

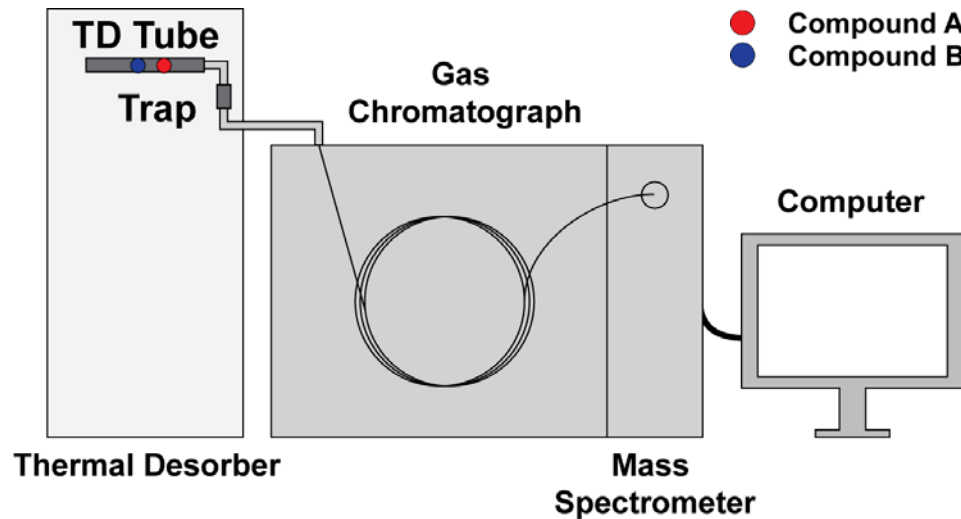
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TD-GC-MS Analysis



- Thermal Desorption (TD):
 - Volatile organic compounds off TD tubes
- Gas Chromatography (GC):
 - Separation of compounds
- Mass Spectrometry (MS):
 - Detection of volatile organic compound fragments for identification and relative quantitation

TD-GC-MS Analysis

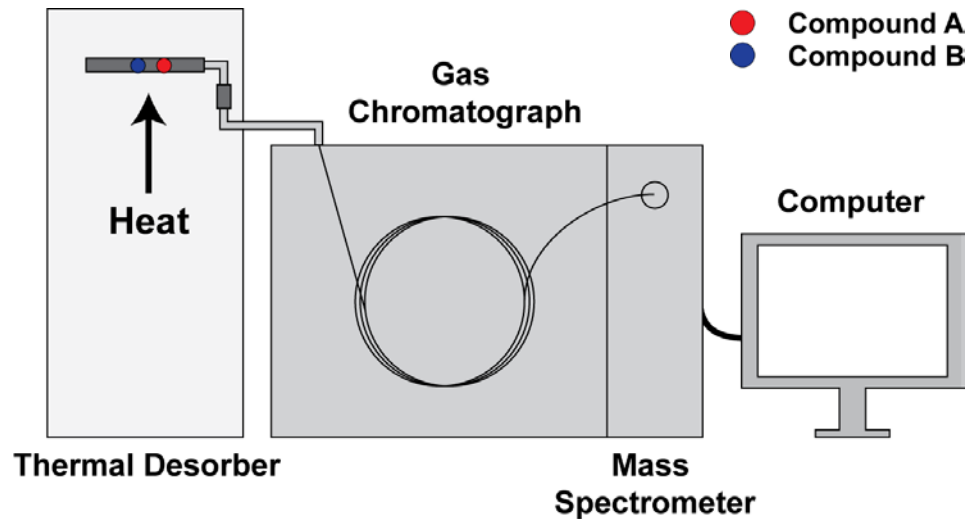


- **Thermal Desorption:**

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TD-GC-MS Analysis



- **Thermal Desorption:**

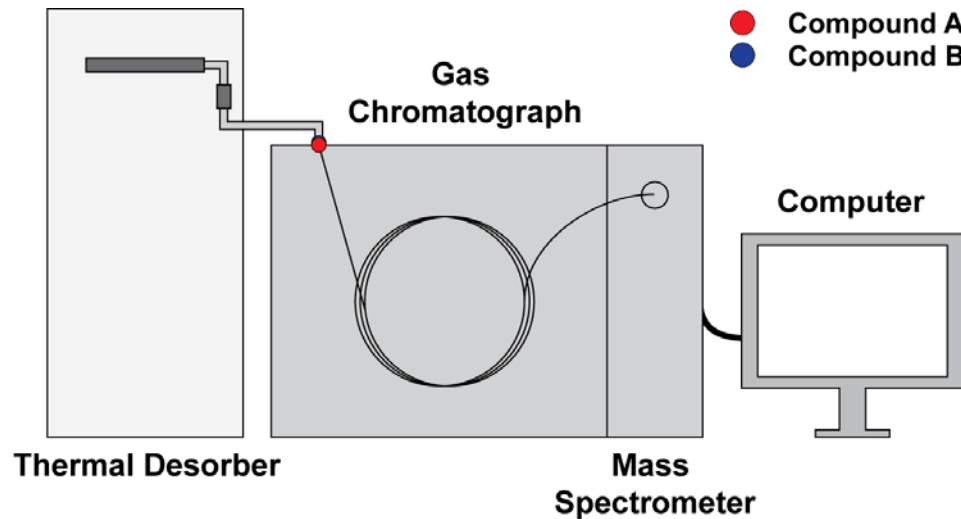
- **Primary Desorption (TD Tube):**
 - 310 °C for 10 minutes.

- Thermal Desorption (TD):
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Tenax TA Thermal Desorption Tube

TD-GC-MS Analysis



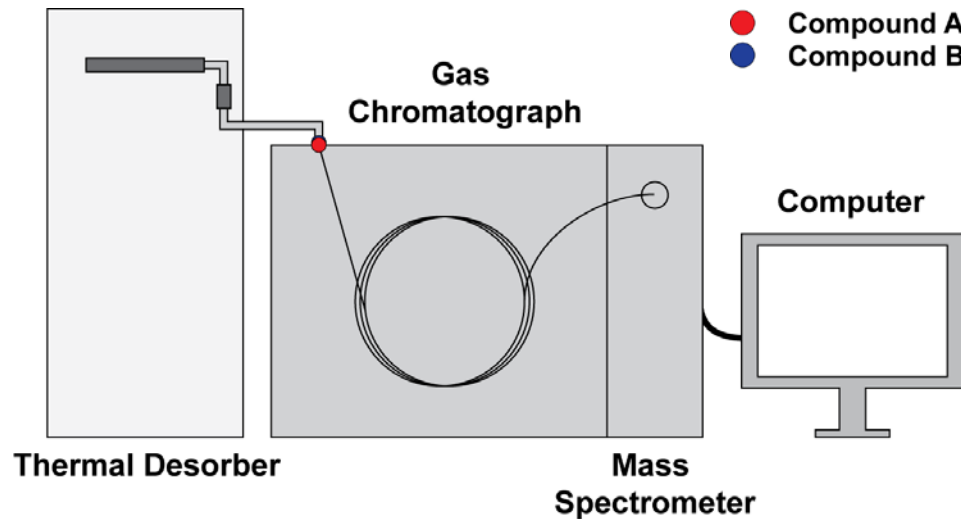
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• Thermal Desorption:

- **Primary Desorption (TD Tube):**
 - 310 °C for 10 minutes.
- **Secondary Desorption (Trap):**
 - 315 °C at 50 °C/s hold for 5 minutes
 - Output Split Ratio: 4.3:1
- **Result:**
 - Analytes removed from the TD tube and transferred to GC via heated transfer line for separation.



TD-GC-MS Analysis



- Thermal Desorption (TD):
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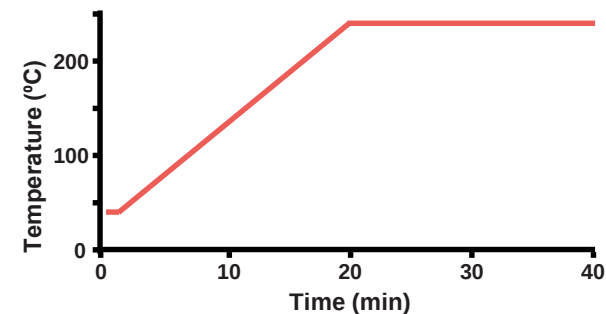
• Gas Chromatography:

• Column:

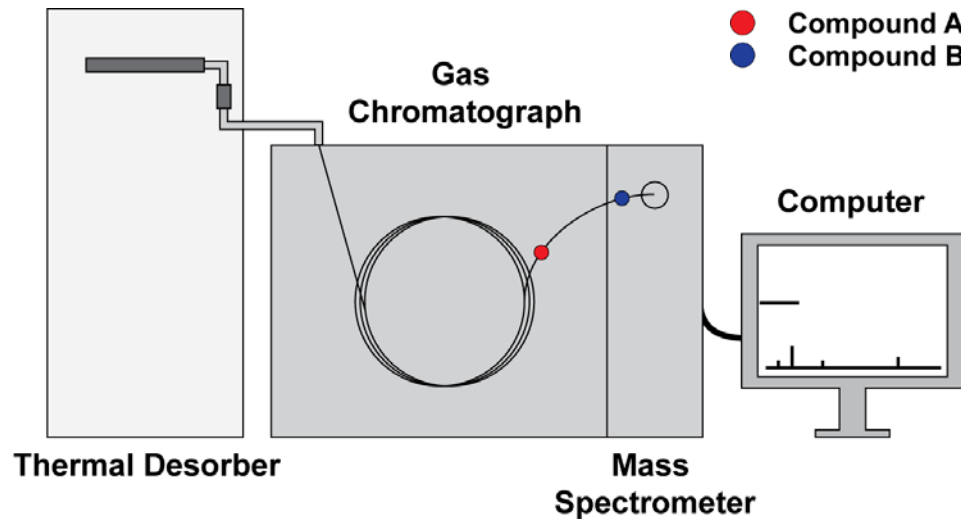
- Mid-Polar, Polysiloxane
- Restek Rxi-624 Sil
- 60m x 0.32mmID, 1.8 μ m df

• Separation

- 2mL/min He carrier
- 40 min gradient from 40-240 °C



TD-GC-MS Analysis



- Thermal Desorption (TD):
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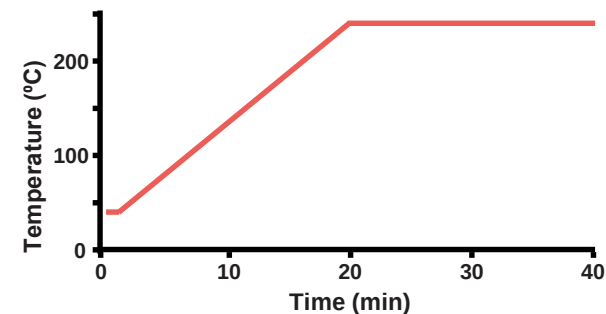
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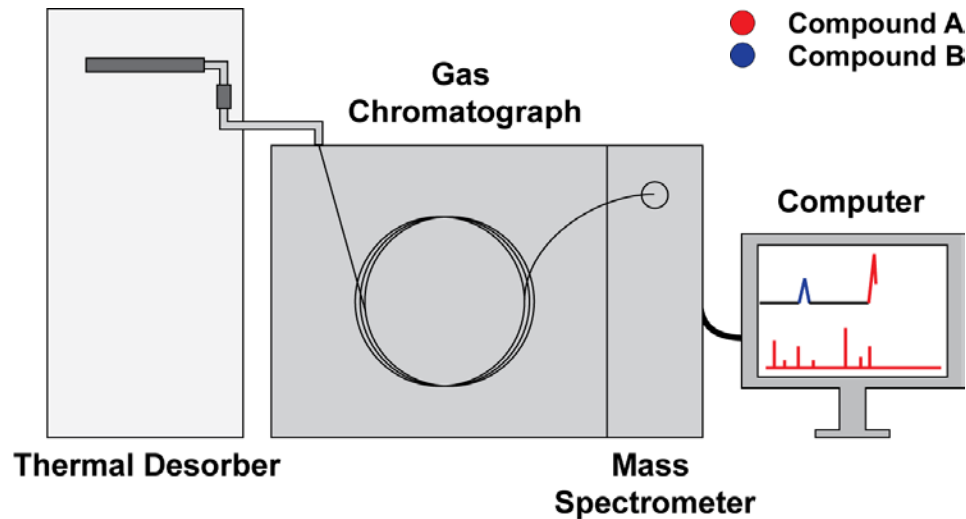
- 2mL/min He carrier
- 40 min gradient from 40-240 °C

• Result:

- Compound separation by roughly boiling point for MS detection.



TD-GC-MS Analysis



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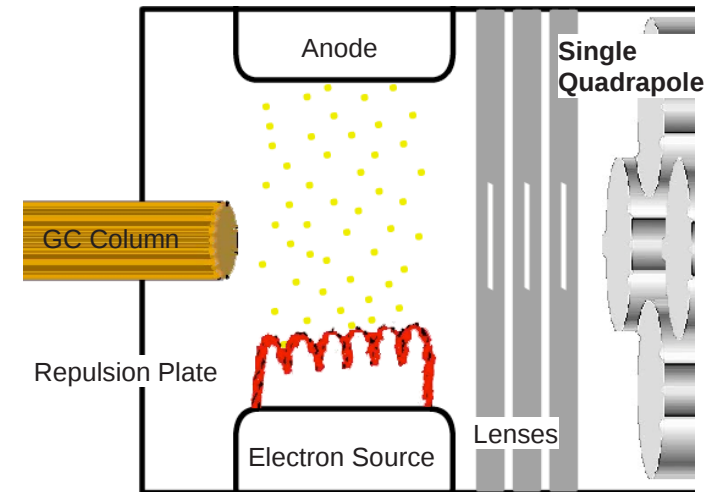
• Mass Spectrometry:

• Electron Impact Ionization:

- 70 keV
- $M^+ \rightarrow X^+ + Y$

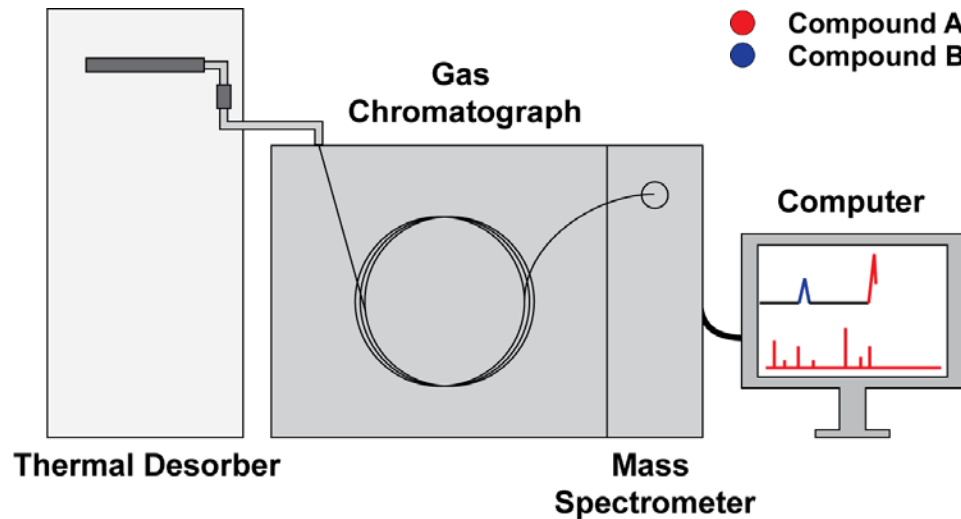
• Single Quadrapole Mass Filter:

- 35-300 m/z, 0.154 scan/s



Dunnivant & Ginsbach, Gas Chromatography, Liquid Chromatography, Capillary Electrophoresis - Mass Spectrometry, A Basic Introduction. 2011.

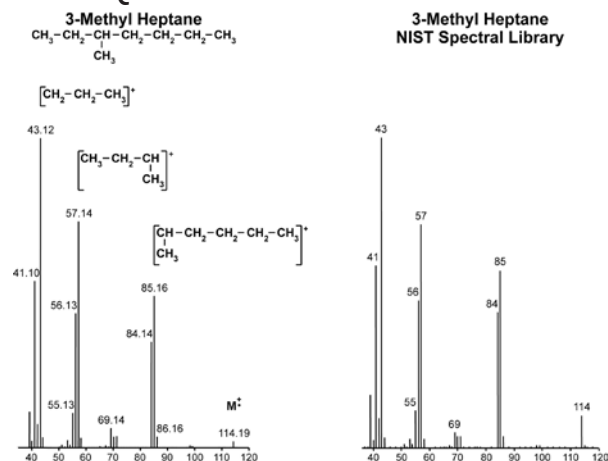
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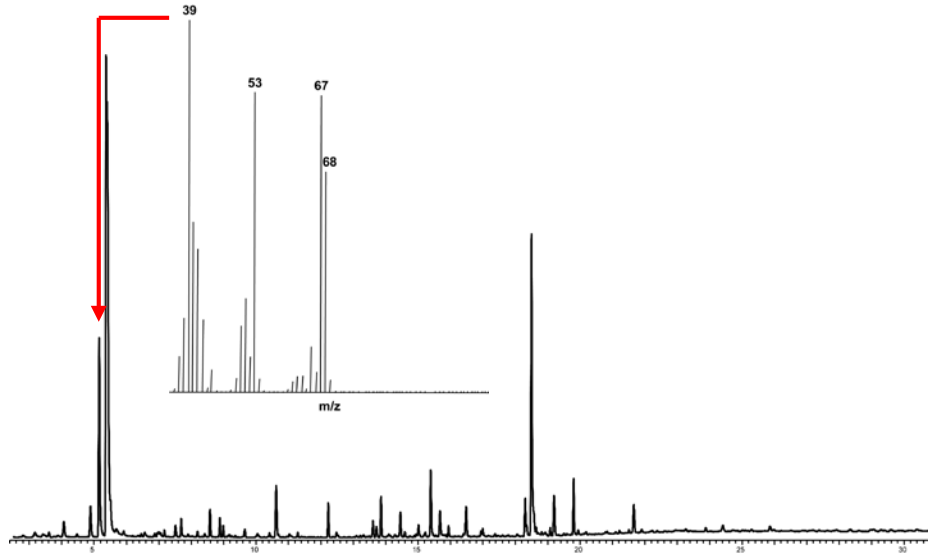
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• Mass Spectrometry:

- Electron Impact Ionization:
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- Single Quadrapole Mass Filter:
 - 35-300 m/z, 0.154 scan/s
- Result:
 - Detection of compound fragments for ID & Relative Quantitation.



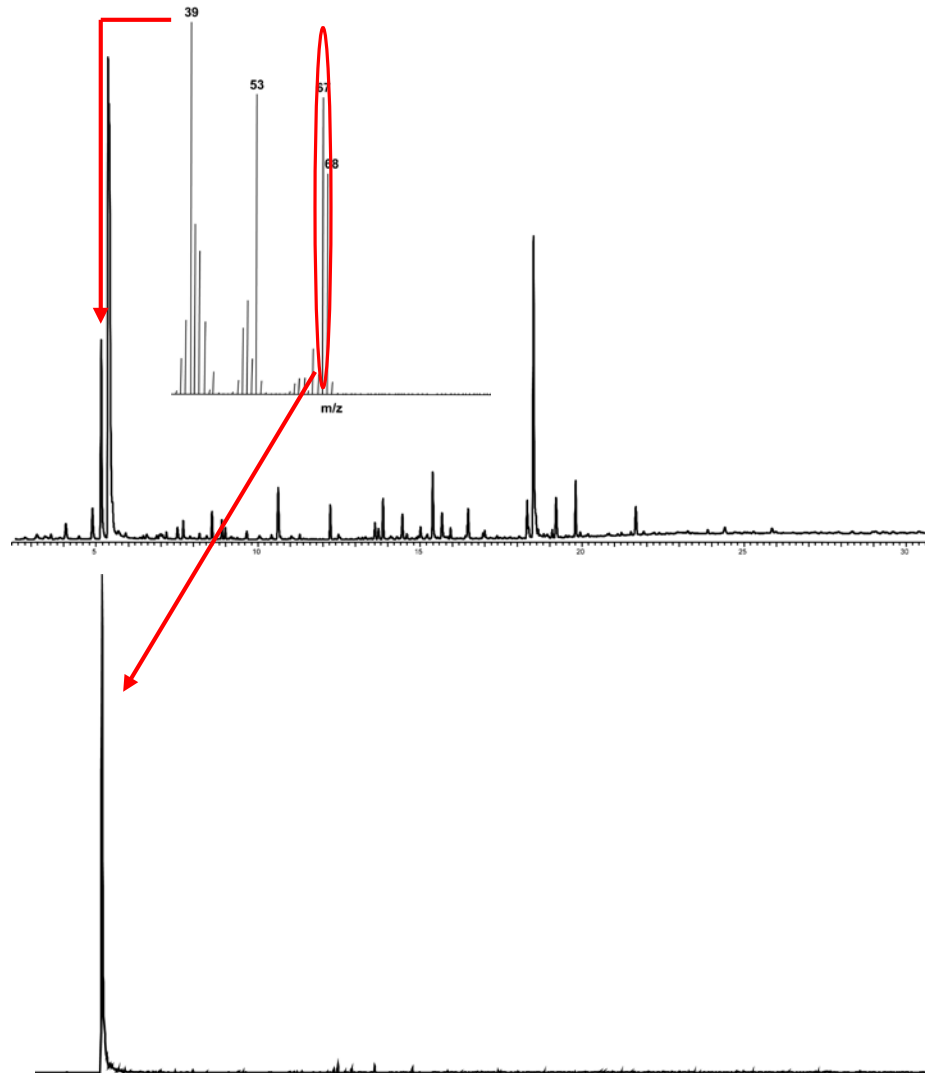
TD-GC-MS Analysis



- **Compound Abundance:**

- Abundances from repetitive MS scans over time are summed and plotted (total ion chromatogram).

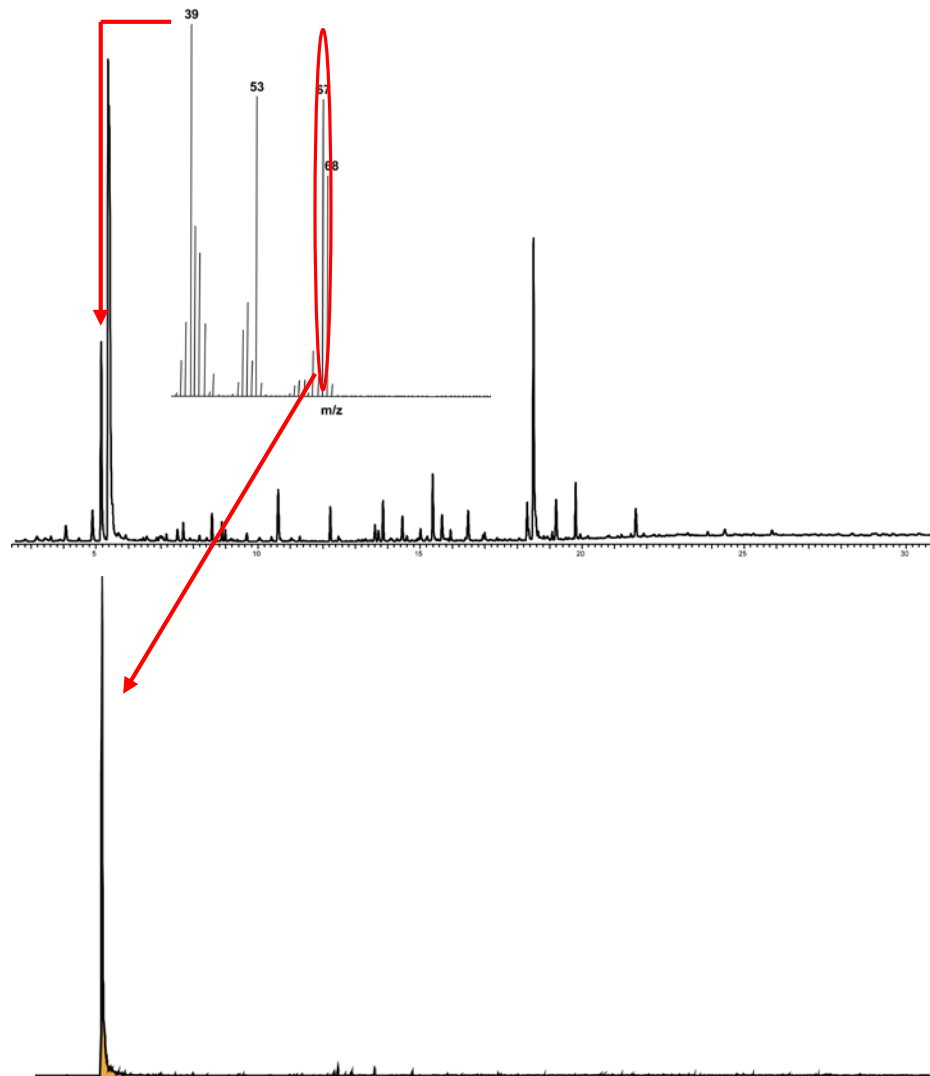
TD-GC-MS Analysis



• Compound Abundance:

- Abundances from repetitive MS scans over time are summed and plotted (total ion chromatogram).
- Specificity is added by plotting a single ion corresponding to a compound of interest (single reaction monitoring or SRM).
- Peak areas of SRM plots are directly related to the compound abundance.

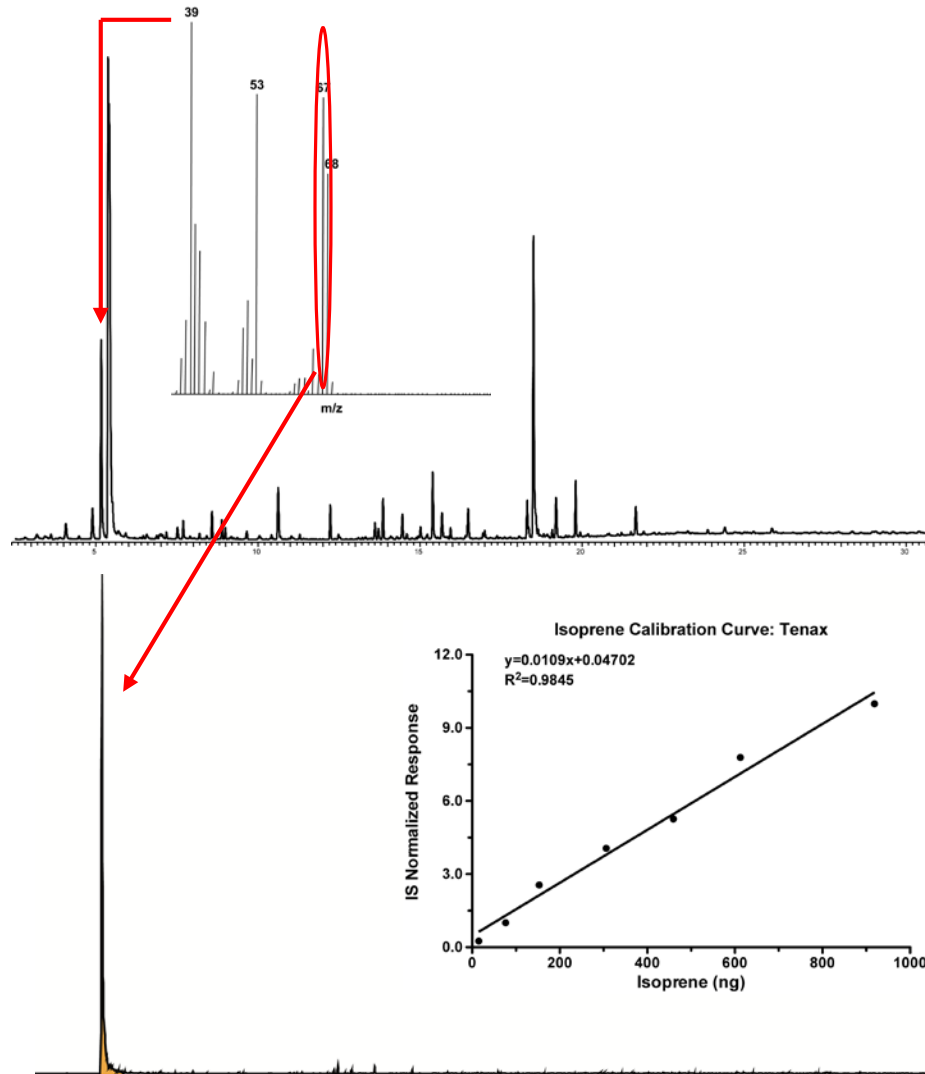
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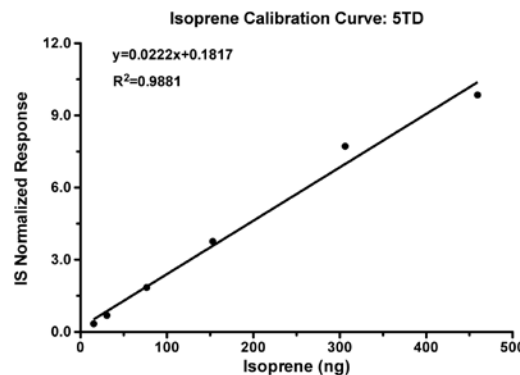
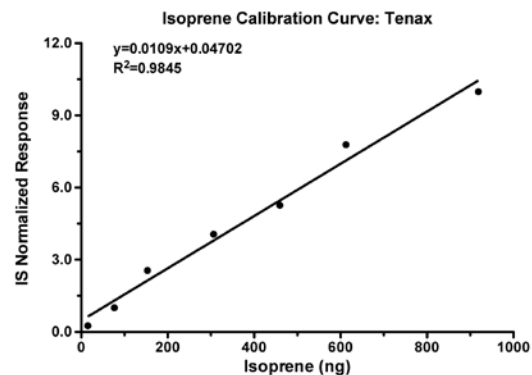
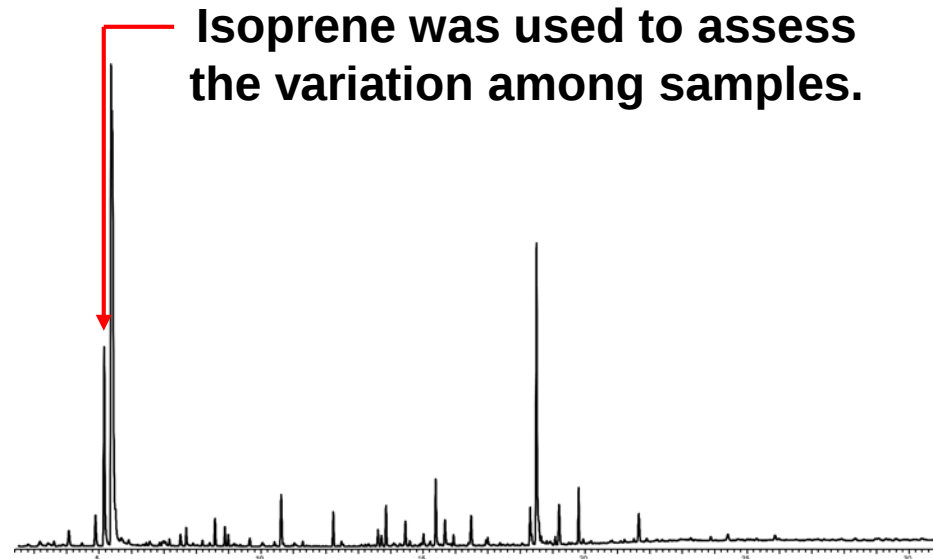
TD-GC-MS Analysis



• Compound Abundance:

- Abundances from repetitive MS scans over time are summed and plotted (total ion chromatogram).
- Specificity is added by plotting a single ion corresponding to a compound of interest (single reaction monitoring or SRM).
- Peak **areas** of SRM plots are directly related to the compound abundance.
- Using neat standards, a calibration curve can be generated for compounds of interest based on known concentrations and instrument response.

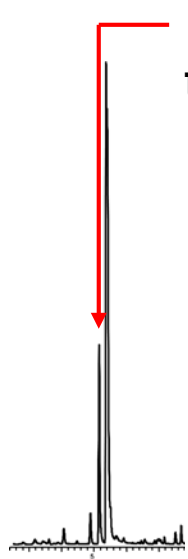
TD-GC-MS Analysis



• Isoprene Calibration:

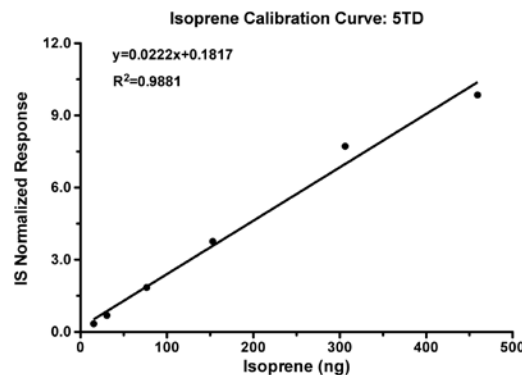
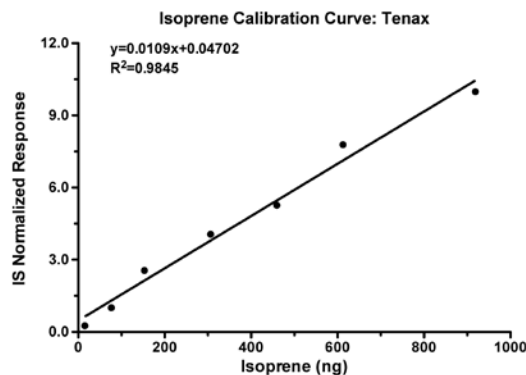
- Linear isoprene calibration curves were generated using a custom standard canister.
- Calibration curves were generated via normalized response to internal standard for each TD tube adsorbent material.
 - Tenax TA Calibration
Range: 15.3ng to 918.8ng
 - 5TD Calibration Range:
15.3ng to 459.4ng
- Additional results, where calibration was not applicable, were analyzed via peak area or internal standard normalized peak area.

TD-GC-MS Analysis



Isoprene was used to assess the variation among samples.

What does the raw data from the ReCIVA look like?



• Isoprene Calibration:

- Linear isoprene calibration curves were generated using a custom standard canister.
- Calibration curves were generated via normalized

standard for
material.

on
918.8ng

range:

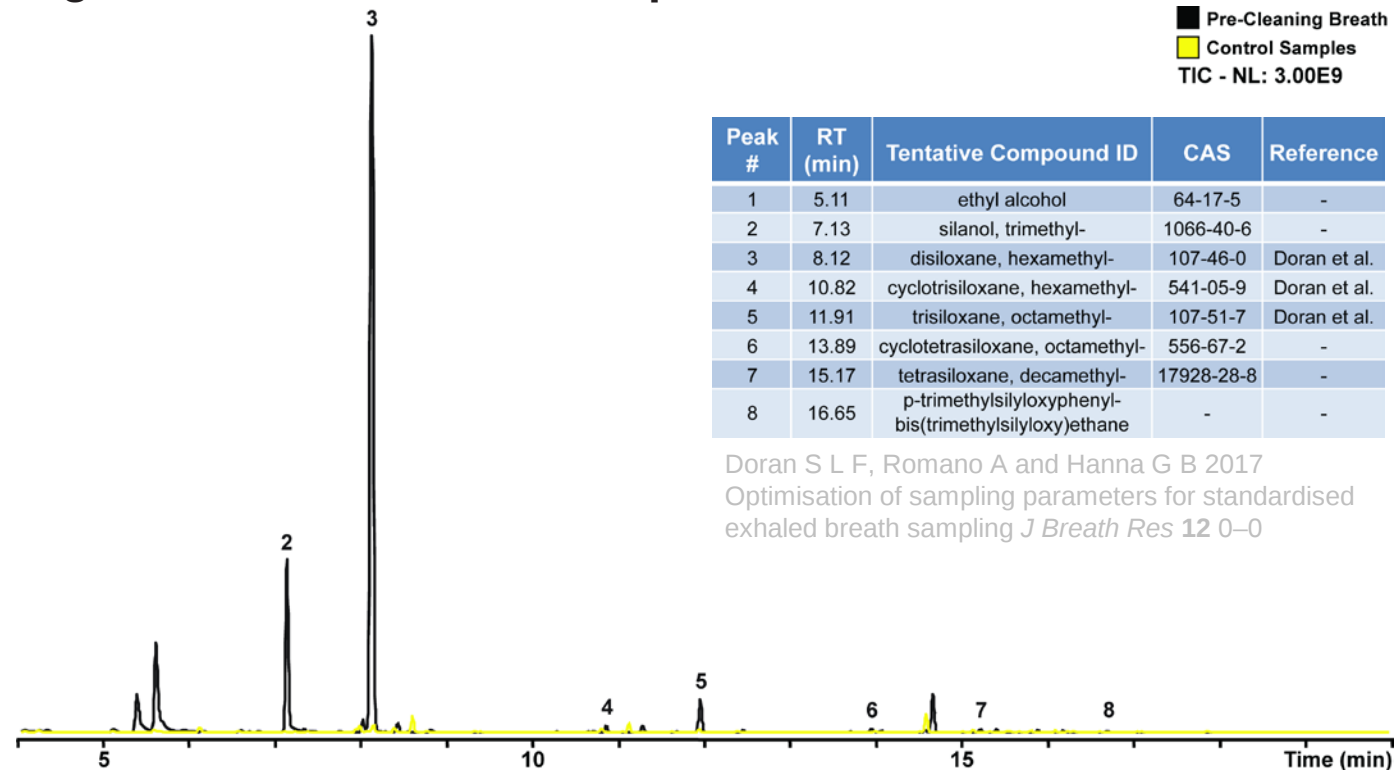
J

- Additional results, where calibration was not applicable, were analyzed via peak area or internal standard normalized peak area.

Results

Is there background associated with the ReCIVA sampler? **Yes.**
Can the ReCIVA masks be cleaned?

- Analysis:
 - Data suggest abundant siloxane background in the ReCIVA samples.

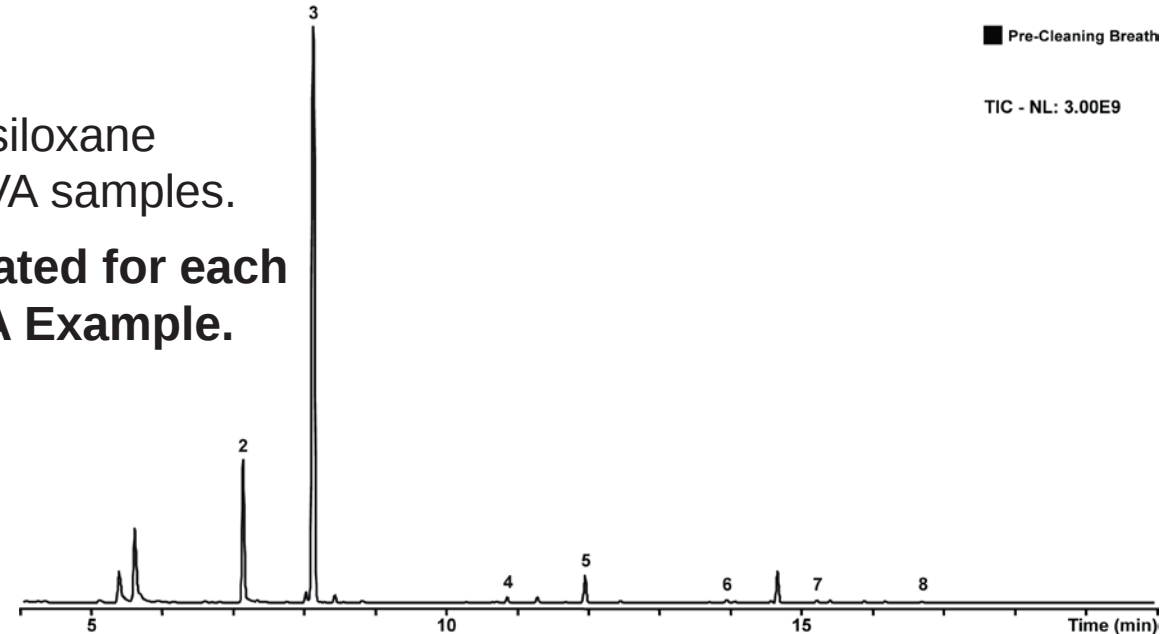
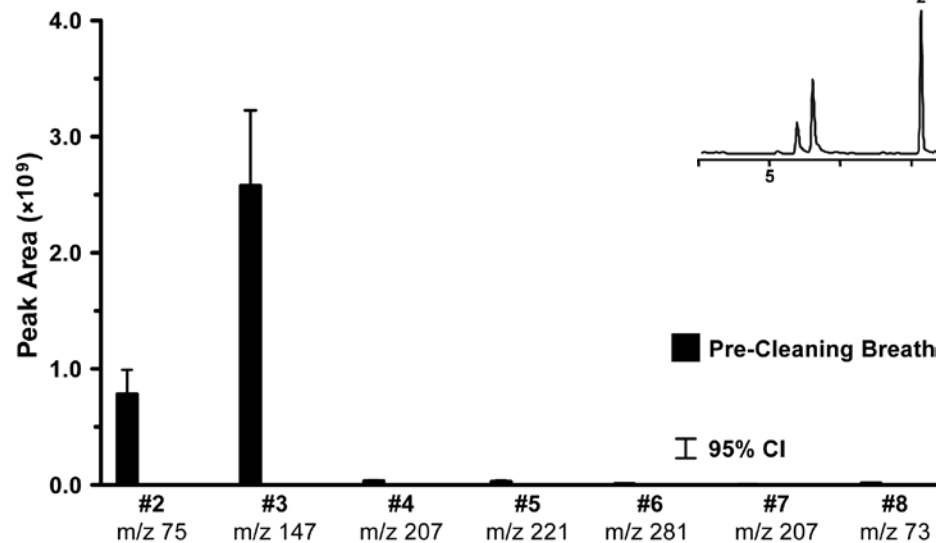


Results

Is there background associated with the ReCIVA sampler? **Yes.**
Can the ReCIVA masks be cleaned?

- **Analysis:**

- Data suggest abundant siloxane background in the ReCIVA samples.
- **Peak areas were tabulated for each contaminant: Tenax TA Example.**



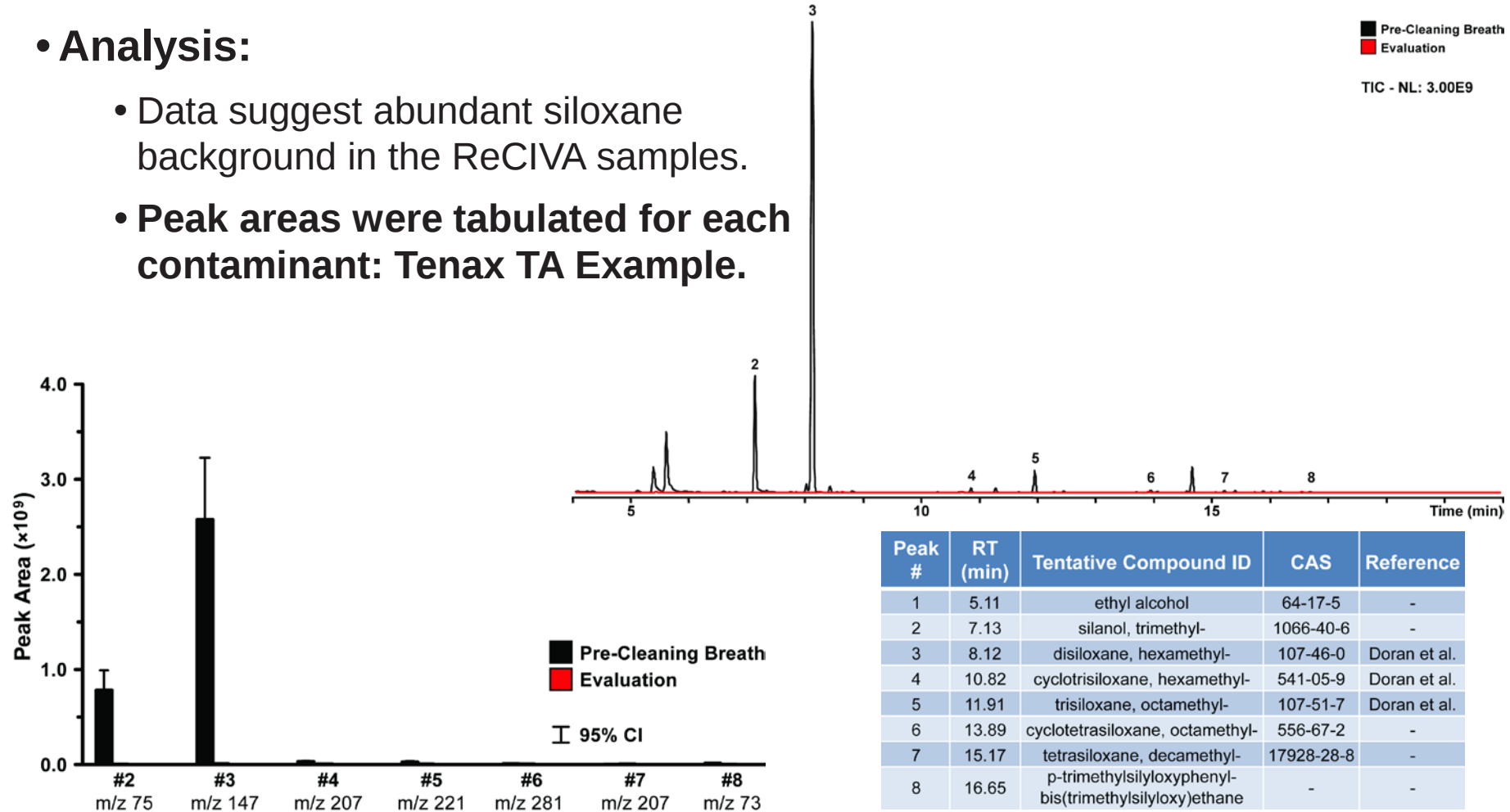
Peak #	RT (min)	Tentative Compound ID	CAS	Reference
1	5.11	ethyl alcohol	64-17-5	-
2	7.13	silanol, trimethyl-	1066-40-6	-
3	8.12	disiloxane, hexamethyl-	107-46-0	Doran et al.
4	10.82	cyclotrisiloxane, hexamethyl-	541-05-9	Doran et al.
5	11.91	trisiloxane, octamethyl-	107-51-7	Doran et al.
6	13.89	cyclotetrasiloxane, octamethyl-	556-67-2	-
7	15.17	tetrasiloxane, decamethyl-	17928-28-8	-
8	16.65	p-trimethylsilyloxyphenyl-bis(trimethylsilyloxy)ethane	-	-

Results

Is there background associated with the ReCIVA sampler? **Yes.**
Can the ReCIVA masks be cleaned?

- **Analysis:**

- Data suggest abundant siloxane background in the ReCIVA samples.
- **Peak areas were tabulated for each contaminant: Tenax TA Example.**

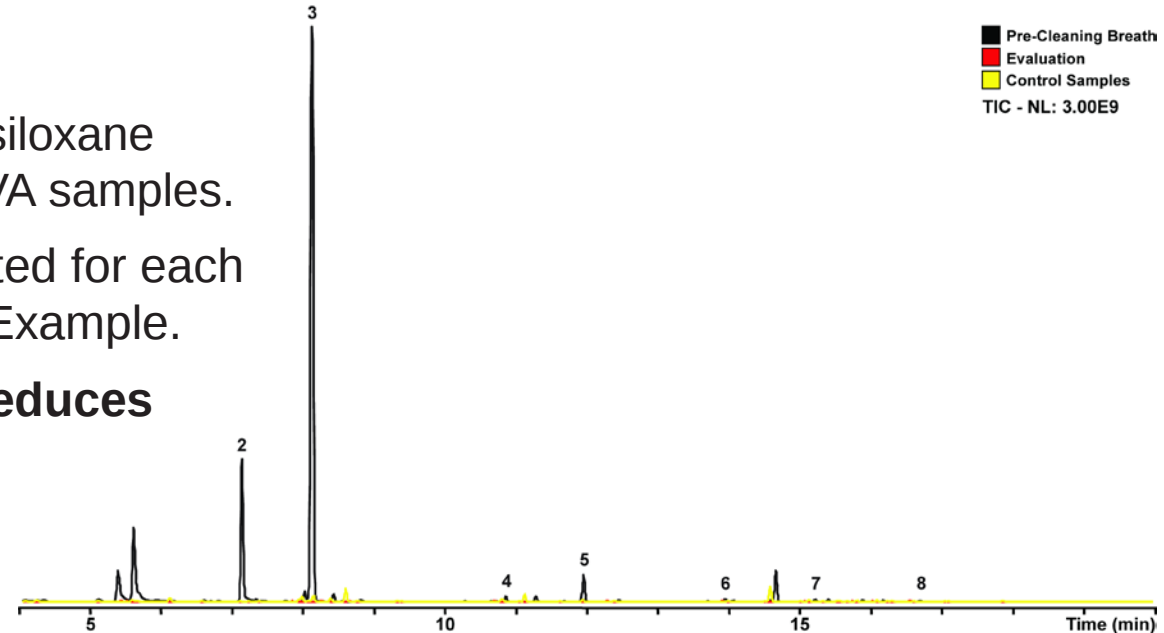
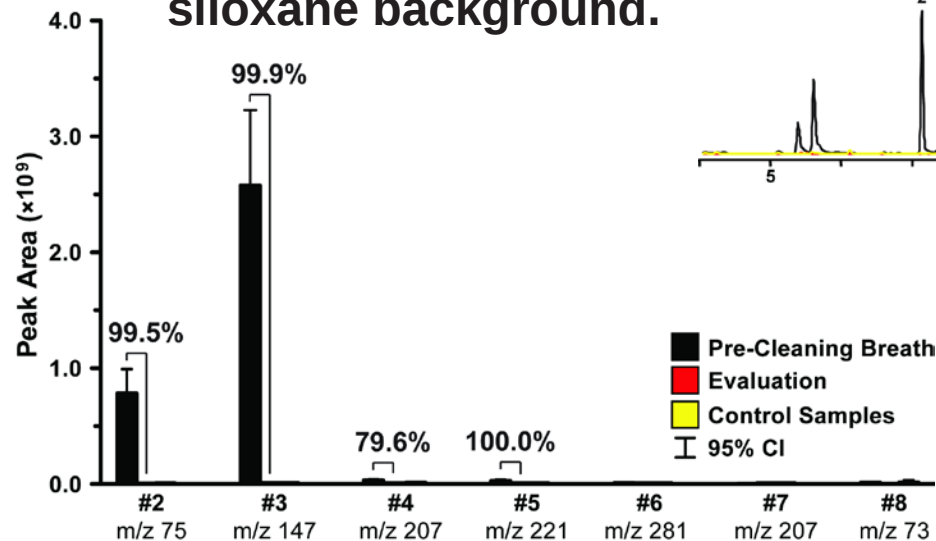


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- **Data suggest baking reduces siloxane background.**



Peak #	RT (min)	Tentative Compound ID	CAS	Reference
1	5.11	ethyl alcohol	64-17-5	-
2	7.13	silanol, trimethyl-	1066-40-6	-
3	8.12	disiloxane, hexamethyl-	107-46-0	Doran et al.
4	10.82	cyclotrisiloxane, hexamethyl-	541-05-9	Doran et al.
5	11.91	trisiloxane, octamethyl-	107-51-7	Doran et al.
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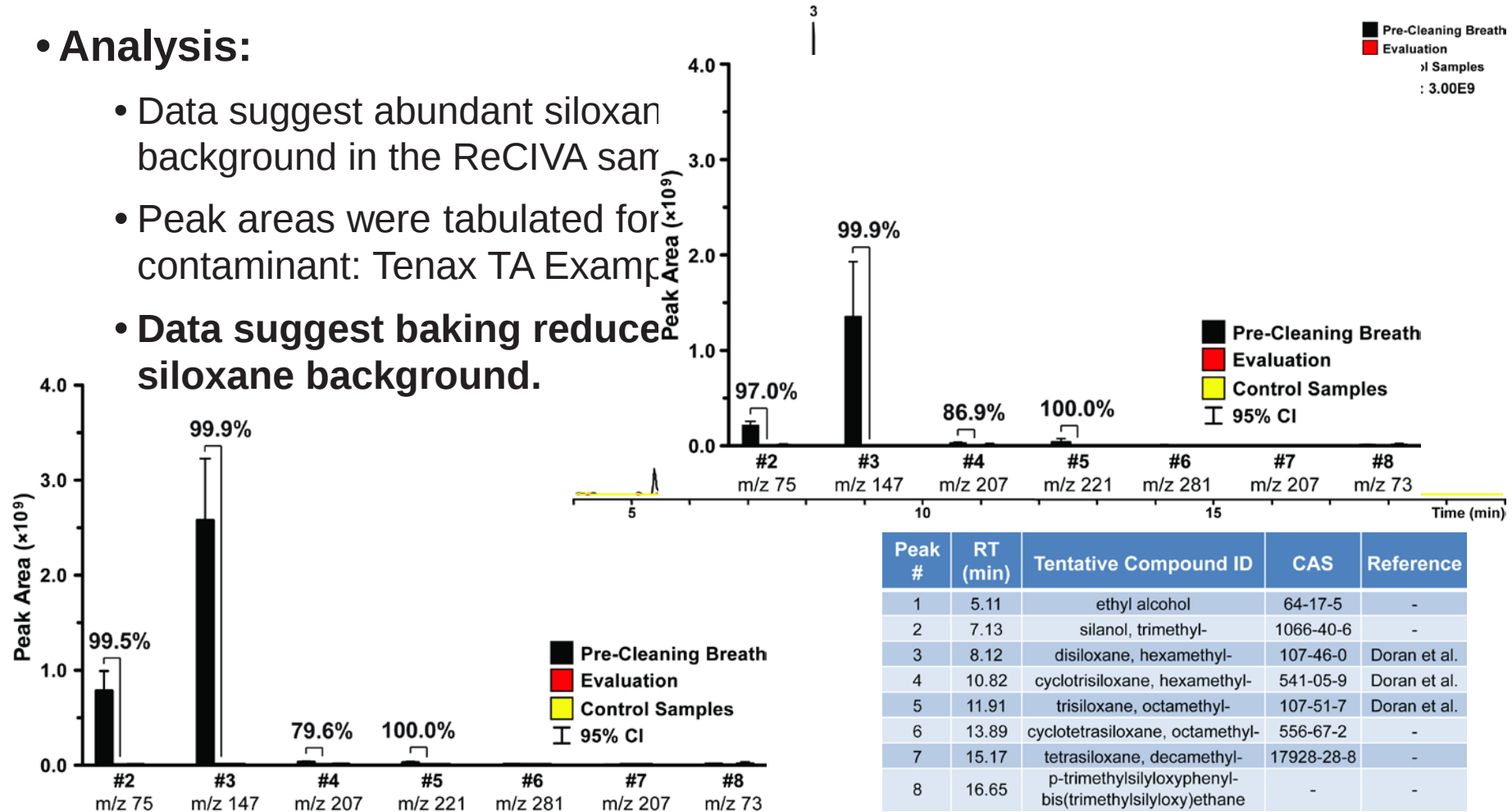
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- Peak areas were tabulated for contaminant: Tenax TA Examp
- Data suggest baking reduce siloxane background.



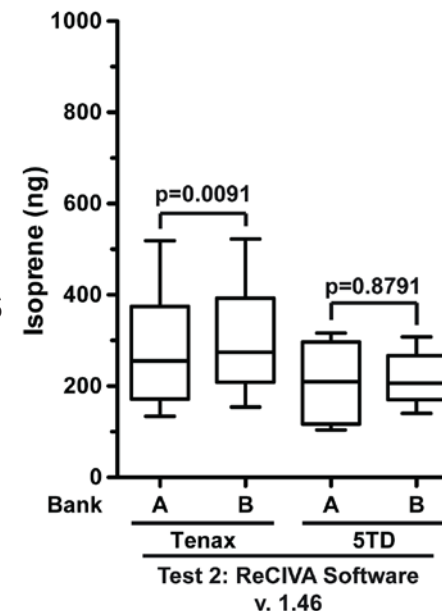
Results

Can duplicate samples be acquired across ReCIVA banks? **Yes, 5TD.**
Do different TD tubes perform similarly in the ReCIVA sampler? **No.**

- Analysis:

- All ReCIVA samples were quantified for isoprene (ng) and plotted as box-whisker plots.

- Data shows similar mean results are obtained between banks for 5TD tubes only.
- Results illustrate a difference in exhaled isoprene across adsorbent tube types.

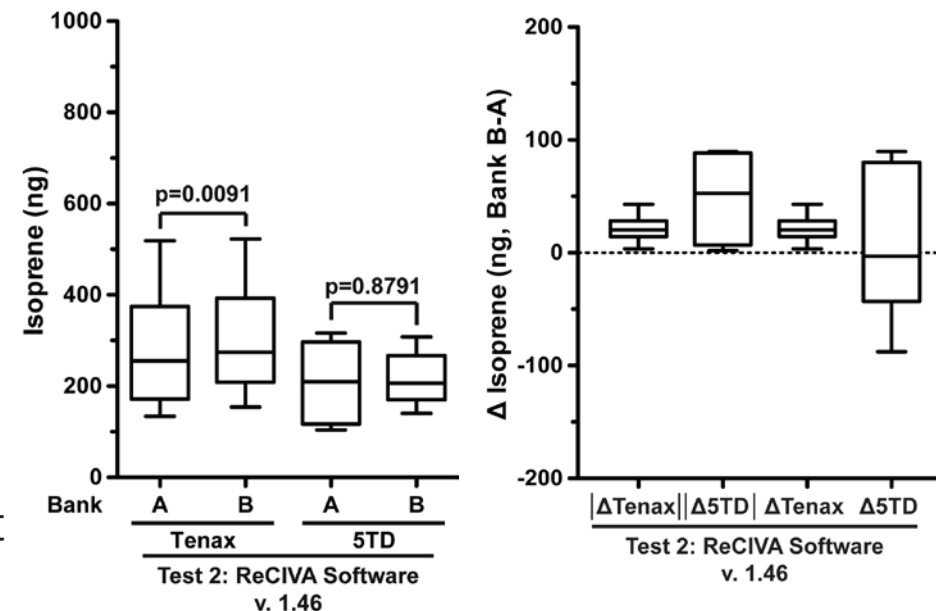


Results

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Do different TD tubes perform similarly in the ReCIVA sampler? **No.**

- **Analysis:**

- All ReCIVA samples were quantified for isoprene (ng) and plotted as box-whisker plots.
- **Delta bank results for each replicate were calculated and plotted as box-whisker plots.**
- Data shows similar mean results are obtained between banks for 5TD tubes only.
- Results illustrate a difference in exhaled isoprene across adsorbent tube types.
- **Data suggests banks may not give equal results with a bias in Bank B for Tenax TA tubes. Further analysis will be required.**

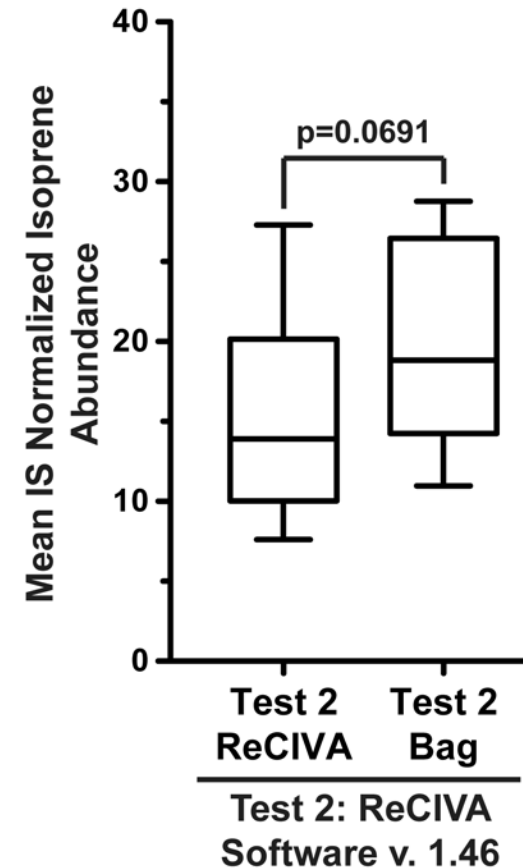


Results

Does the ReCIVA sampler perform similarly to breath bags? **Yes.**

- **Analysis:**

- All ReCIVA and bag isoprene areas were internal standard normalized and plotted as box-whisker plots.
- Data shows similar results are obtained from the ReCIVA compared to bags for Tenax TA tubes.

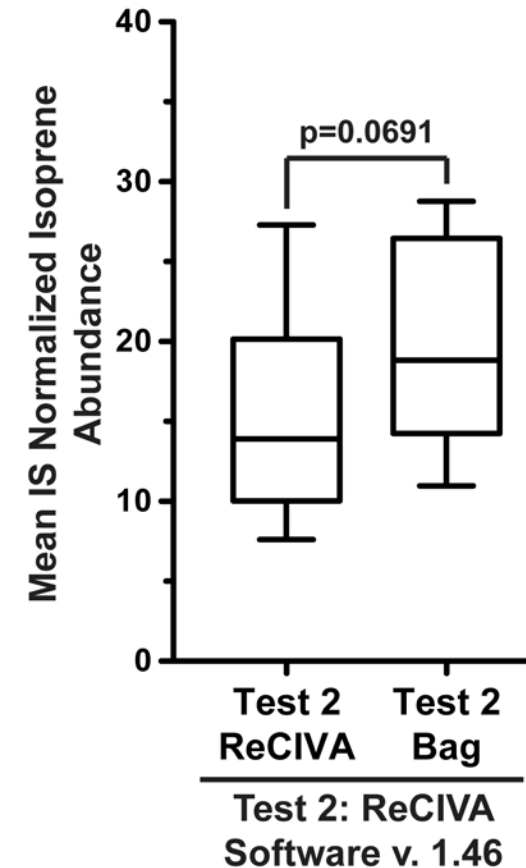


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- **Analysis:**

- All ReCIVA and bag isoprene areas were internal standard normalized and plotted as box-whisker plots.
- Data shows similar results are obtained from the ReCIVA compared to bags for Tenax TA tubes.



Conclusions:

- **Data suggests:**
 - **The ReCIVA control software version is CRITICAL to the proper function of the ReCIVA device.**
 - **Baking can significantly reduce the siloxane background observed from the ReCIVA masks.**
 - **Duplicate samples maybe obtained across banks of the ReCIVA sampler for the 5TD tubes.**
 - **Tenax TA TD tubes have a bias to Bank B within the ReCIVA sampler.**
 - **Similar isoprene abundances are observed between the ReCIVA sampler and breath bags for Tenax TA.**

Future Directions:

- **Evaluate multiple ReCIVA units to look for consistency among collectors.**
- **Evaluate the impact of breathing rate on ReCIVA collections.**
- **Work to further evaluate means for mask decontamination and reuse.**
- **Evaluate the impact of different breathing gases i.e. 100% O2 on results.**
- **Work with the manufacturer to provide feedback based on results observed. i.e. calibrating for Tenax tubes.**
- **Work toward developing a field applicable version for testing.**

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multiple samples on
multiple occasions.**

Questions?