Delivering the Right Results

Analysis of Volatile Organic Components in Human Breath by GCxGC-TOFMS

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INTRODUCTION

Exhaled human breath is a complex mixture of compounds, containing hundreds or even thousands of components. Volatile compounds in the bloodstream are in equilibrium with the gaseous contents of the alveolar region of the lungs. By examining the contents of the lungs expelled during exhalation, we can gain an understanding of the presence and concentrations of compounds of interest relating to chronic conditions and/or metabolic conditions in a subject. Many of these compounds of interest occur at trace levels in the body. The increased detectability offered by GCxGC would allow for the detection of these trace peaks at lower concentrations. A significant concern when analyzing breath samples is their very high relative humidity. Sample collection and processing must include a means of removing water while maintaining maximum recovery of target analytes. In this work, breath samples were collected on polydimethylsiloxane (PDMS) foam-filled GERSTEL TDU tubes. The collected samples were injected using a GERSTEL TDU/CIS 4 thermal desorption inlet. Separation and identification were accomplished using a LECO Pegasus® 4D Comprehensive Two-Dimensional Gas Chromatograph–Time-of-Flight Mass Spectrometer (GCxGC-TOFMS).

BREATH ANALYSIS

Advantages

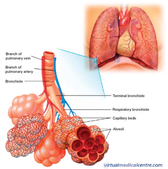
- Non-Invasive Sample Collection
- "Unlimited" Amount of Sample Available
- Complexity of Breath Composition can Provide a Wealth of Information

Challenges

- High Humidity of Breath Sample
- Variability of "Normal" Breath Component Concentrations
- Complexity of Breath Composition
- Stability of Reactive Components in Breath
- Sampling Variability

HUMAN BREATH ANALYSIS

- It is a noninvasive technique
- Interested in breath from the alveoli region of the lungs
  - Volatile and semi-volatile compounds in blood form an equilibrium between blood and breath across the Pulmonary Alveolar Membrane
- Signature "bio-markers" can be linked to metabolic states
  - Acetone for diabetes
  - Isoprene for hyperchlosterolemia
  - Straight chain hydrocarbons related to lipid peroxidation of polyunsaturated fatty acids found in cellular membranes
  - Increased hydrocarbons related to pulmonary, liver, bowel, and neurological diseases



EXPERIMENTAL OVERVIEW

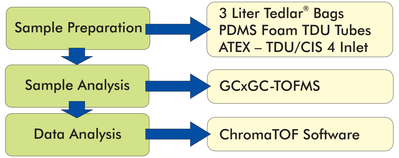


Figure 1. Experimental overview.

SAMPLE COLLECTION AND LOADING

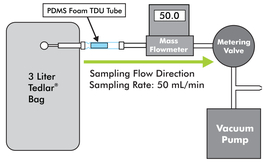


Figure 2. A schematic of the system used to load collected breath onto PDMS foam-filled GERSTEL TDU tubes. The metering valve is used to compensate the variable in the pneumatic restriction such that the flow through the TDU tube is consistent at 50 mL/min.

One of the major challenges in analyzing breath is consistency and methodology in sample collection. The breath that is of interest is the breath from the alveoli, deep in the lungs and is known as alveolar breath. This is where the equilibrium between breath and blood occurs. The breath contained in the bronchi, trachea, throat, and mouth, known as tidal breath, does not participate in this equilibrium. A challenge of sampling is to collect the alveolar breath while excluding the tidal breath. In order to accomplish this, the subject from whom the sample is being collected, exhales slowly for approximately 7 seconds, to account for tidal breath, and then immediately exhales maximally into the sample bag, without stopping the exhalation. The subject's nose is blocked to prevent contamination or loss of alveolar breath via the nose. The sampling bag containing the collected breath is attached to the sample loading device (see above figure). A conditioned TDU tube, containing the PDMS foam, is placed inline between the sample bag and the sample loading device. The volume of breath collected on the sampling tube is controlled by adjusting the amount of time that flow is collected (ex. 20 minutes at 50 mL/min = 1 Liter of breath collected). At the end of sample loading, the sample bag containing the breath sample is replaced with a sample bag containing UHP-grade dry nitrogen, and 100 mL of dry nitrogen are drawn through the loaded TDU tube. This "dry purge" is performed to remove as much water as possible from the loaded sample.

SAMPLE INTRODUCTION

The transfer of the collected breath from the PDMS foam tube to the GC is accomplished by means of a GERSTEL TDU/CIS 4 inlet system. The TDU thermally desorbs the analytes adsorbed on the PDMS foam. The desorbed analytes are then focused in the liner of the CIS 4 inlet. The CIS 4 is held at -120°C during the desorption of the TDU tube. The CIS 4 is then rapidly heated to 300°C to desorb the focused analytes onto the analytical column as a narrow plug. By using this dual stage inlet system, the collection tube can be thermally desorbed in a controlled manner while still being able to introduce the analytes to the analytical column as a sufficiently narrow injection plug.

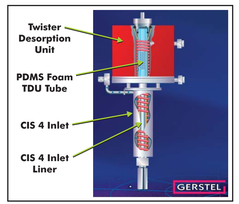
Experimental Conditions (Inlet)

Twister Desorption Unit (TDU)

- Operated in Splitless Mode
- Initial Temp: 30°C
- Ramp Rate: 700°C/min → 300°C, hold for 120 sec

Cooled Inlet System (CIS 4)

- Operated in Solvent Vent Mode
- Initial Temp: -120°C
- Ramp Rate: 12°C/sec → 300°C, hold for 120 sec



INSTRUMENTATION

Separation and identification in this work was accomplished using a LECO Pegasus 4D GCxGC-TOFMS. The complexity and sheer number of components in human breath, combined with the low concentrations of many components, make GCxGC combined with Time-of-Flight Mass Spectrometry an excellent technique for studying compounds in human breath.

GC Conditions

- Column 1: GERSTEL-MACH LTM: 10 m x 0.18 mm I.D. x 0.2 µm RTX-5
- Column 2: In GC Oven (No Secondary Oven): 1.0 m x 0.10 mm I.D. x 0.1 µm DB-17
- Temperature Program: 40°C → 5 min → 240°C → 2 min (+ 15°C Second Column Offset)
- Modulation Period: 5 seconds
- Flow: 1.50 mL/min
- Transfer Line Temperature: 280°C

MS Conditions

- Ionization Source: Electron Impact
- Ionization Energy: 70 eV
- Data Acquisition Rate: 200 spectra/sec
- Data Acquisition Range: 5 to 1000 m/z
- Stored Data Range: 40 to 350 m/z

EXPERIMENTAL

The goal of this work was to identify changes in the composition of human breath following consumption. A baseline or "blank" breath sample was collected from the subject, immediately prior to consumption and following a 12-hour overnight fast, where the subject consumed nothing but water. A post-consumption breath sample was collected 30 minutes after consumption. The subject's mouth was rinsed 3x with water, immediately following consumption, to remove as much of the consumed product as possible. Identical quantities of pre-consumption and post-consumption breath were analyzed for comparison.

PRE-ORANGE JUICE BREATH (1L)

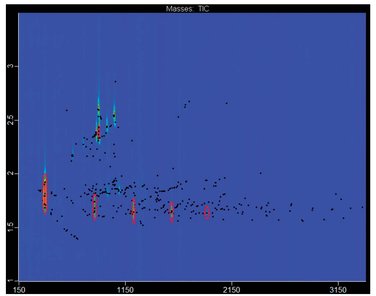


Figure 3. A Contour Plot from a 1 liter sample of human breath, collected prior to the consumption of orange juice. The peaks encircled by the dashed red circles are siloxane artifacts from the desorption of the PDMS Foam.

POST-ORANGE JUICE BREATH (1L)

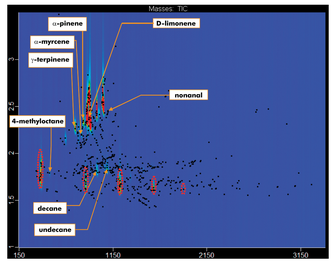


Figure 4. A Contour Plot from a 1 liter sample of human breath, collected following the consumption of orange juice. The peaks encircled by the dashed red circles are siloxane artifacts from the desorption of the PDMS Foam. The peaks indicated are examples of compounds that were either absent in the pre-consumption sample, or showed significant increases in peak area in the post-consumption sample.

Changes in the composition of human breath following consumption were determined by the method of sample subtraction. The sample of breath collected prior to consumption serves as a baseline. It is subtracted from the sample of breath taken following consumption. A 3D Surface Plot of the results of the subtraction is then generated. An area of the surface which shows no deflection indicates no change between the samples. Positive peaks in the plot are either peaks that were absent in the breath sample taken prior to consumption or peaks that showed an increase in peak area. Negative peaks in the plot are peaks that were either absent in the breath sample taken after consumption or peaks that show a decrease in peak area.

POST-SUGAR-FREE ENERGY DRINK BREATH (2L)

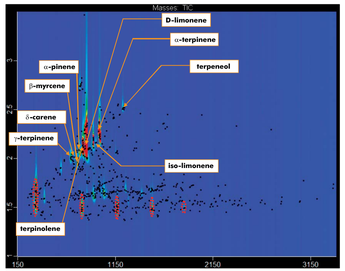


Figure 5. A Contour Plot from a 2 liter sample of human breath, collected following the consumption of sugar-free energy drink. The peaks encircled by the dashed red circles are siloxane artifacts from the desorption of the PDMS Foam. The peaks indicated are examples of compounds that were either absent in the pre-consumption sample, or showed significant increases in peak area in the post-consumption sample.

SAMPLE SUBTRACTION OF PRE- AND POST-SUGAR-FREE ENERGY DRINK BREATH (2L)

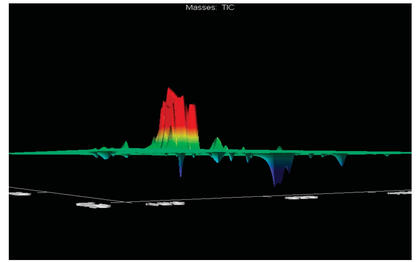


Figure 6. A 3D Surface Plot plot generated from a sample subtraction of 2 liter samples of human breath, collected prior to and following the consumption of a Sugar-free energy drink. The sample of breath taken prior to the consumption of a Sugar-free energy drink is subtracted from the sample taken after consumption of a Sugar-free energy drink. The surface of the plot indicates change between the two samples. Positive peaks in the plot are either peaks that were absent in the breath sample taken prior to consumption or peaks that showed an increase in peak area. Negative peaks in the plot are peaks that were either absent in the breath sample taken after consumption or peaks that show a decrease in peak area.

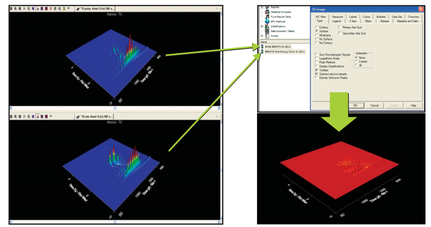


Figure 7. Two samples are selected and "Subtract Second Sample" is selected in "Properties". The resulting plot shows change between the two samples. The software used is LECO's ChromaTOF® version 4.20.

CONCLUSIONS

- ☑ The complexity of human breath and the low concentrations of many of its components make Comprehensive Two-Dimensional Gas Chromatography an excellent technique for its analysis.
- ☑ The LECO ChromaTOF software package's "Subtract Second Sample" feature allows for a quick and easy method of identifying areas of change between two samples.
- ☑ The sample collection methodology could be improved by eliminating the possibility of analytes partitioning into the condensation inside of the sample bag and through the use of a sample collection medium more favorable to the collection of polar analytes than PDMS.
- ☑ The analysis of human breath presents challenges in reproducibly collecting breath samples from subjects.