Decomposition Analysis Using Differing Data Processing Methods to Identify Volatile Organic Compounds

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Background

When a body decomposes it emits volatile organic compounds (VOCs). VOCs from decomposing remains have been studied before,[1] however there is a gap that remains in the knowledge on VOCs that evolve from submerged decomposing remains.[2] In this study, submerged animal tissue was tested to see how the VOCs can be utilized in forensic settings.

This study used data obtained last summer using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS). Pigs (*Sus scrofa domesticus*) are often used as analogs in decomposition studies due to similarities with humans.[3] In this study, pork belly samples were submerged in mason jars filled with water and stored at different temperatures. The GC×GC-TOFMS instrument was used to collect odor from above water samples for a period of twelve days.

GC×GC is often used to analyze complex odor profiles. When analyzing samples with GC×GC, there is a lack of standardization to process the data acquired from time trials. There is a need to discover effective ways to track VOCs for chemical identification and longitudinal analysis.

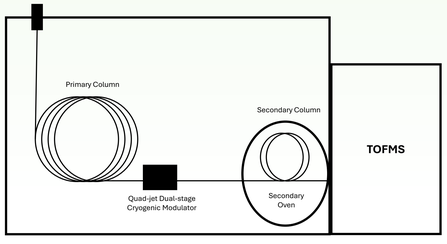

Figure 1. Schematic of GC×GC-TOFMS Quad-Jet Dual-stage Cryogenic Modulator used in experiment.

The goal of this research was to compare different software approaches to analyze GC×GC longitudinal data to achieve accurate analyte identification, representation of data over time, and effective class characterization.

Methods

From the previous study, nine pork samples were decomposed in Mason Jars filled with tap water at varying temperature (32˚C, 22˚C, 5˚C). All samples were prepared in triplicate, with a control group of three mason jars filled with only tap water.

Table 1. ChromaTOF Software used and the purpose of each software in analyzing samples for identifying peaks.

Software	Purpose
ChromaTOF	Uses quant mass to integrate peaks and identify compounds directly on single chromatograms
ChromaTOF Tile Fisher Ratio	Finds statistically significant difference between sample classes using tile areas
ChromaTOF Tile Coefficient of Variance	Unsupervised comparison of individual samples by taking standard deviation and dividing it by the mean of the summed tile areas
ChromaTOF Tile Fold Change	Focuses on the largest differences across sample sets to find differences between tile areas
ChromaTOF Sync 2D	Processes data by differentiating co-eluting peaks and aligning peaks via quant mass

For the first four days, 5 mL of water was taken and run on the GC×GC-TOFMS. Thereafter, the samples were sampled and run every three days until day twelve. Data were analyzed initially using ChromaTOF (LECO Corporation). The samples were then analyzed using ChromaTOF Tile and ChromaTOF Sync 2D to compare how they can be used in a forensic setting.

Peak tables were curated in Tile and Sync 2D. Then, ChromaTOF was used as a guide to confirm which peaks were identified correctly. If the compound was correctly identified, the peak would be accepted or kept on the table. If not correctly identified, the peak would either be rejected or deleted. Once the tables were complete from the different software approaches, they were exported to Excel for further comparison.

In excel, the relevant hits that were identified as decomposition VOCs were compared across the software approaches. The compounds that were most commonly found were compared to existing literature on decomposition VOCs. From there, two tables were built; where one table was compounds confirmed with literature and the other table was compounds not confirmed with literature.

Comparison between software approaches included looking at different criteria to see which method was the most effective and efficient. The criteria included how long the software approach took, the number of compounds found in each software approach, the accuracy of peak area in the software approach compared to ChromaTOF data, and principal component analysis (PCA) comparison to see which software approach was best used for forensic practice or research.

Table 2. Methods for preparing and collection decomposition VOCs in water

Samples	12 mason jars filled with tap water ▪ 9 with pork belly ▪ 3 only tap water
Conditions	▪ Hot (32˚C) ▪ Room (22˚C) ▪ Fridge (~5˚C) ▪ Water
Collection	5 mL taken from each sample each day of collection ▪ [0, 1, 2, 3, 6, 9, 12]

Results

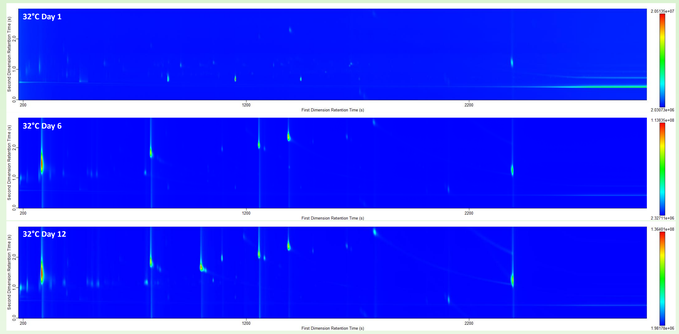

Figure 2. Total ion current contour plot from GC×GC-TOFMS at 32 ˚C over the duration of the trial. Chromatograms demonstrate how compounds appear over time.

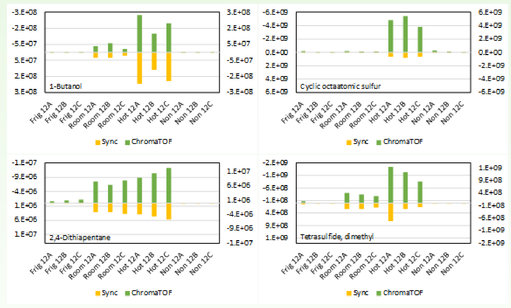

Figure 3 Bar graphs comparing abundances between Sync 2D and ChromaTOF. Sync 2D has similar abundances and trends for most compounds, but cyclic octaatomic sulfur's abundance was very high in ChromaTOF and not in Sync 2D.

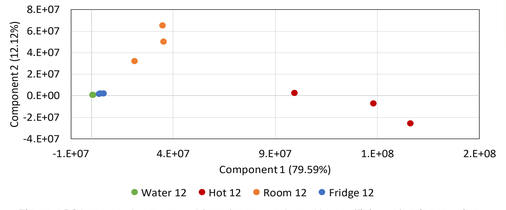

Figure 4 PCA scores plot generated from features selected by coefficient of variance using ChromaTOF Tile using Day 12 sample data.

Table 3. Compounds commonly found and confirmed with literature

dimethyl disulfide
dimethyl trisulfide
indole
p-cresol

Table 4. Compounds commonly found and not confirmed with literature

octyl propanoate
1-(2-methoxypropoxy)propan-2-ol
ethenyl decanoate
methylsulfanyl(methylsulfinyl)methane

The VOC compounds found that were confirmed with literature confirmed this study's GC×GC method's ability to find decomposition compounds. The VOC compounds found that were not confirmed with literature showed a capability of the GC×GC to identify compounds that appear to be unique to submerged remains.

Table 5. The timing and number of compounds found in the four methods.

Software	Timing	Compounds Found
ChromaTOF Tile Fisher Ratio	Day 1: 5 min 37 s Day 6: 5 min 8 s Day 12: 5 min 43 s	Day 1: 11 Day 6: 13 Day 12: 15
ChromaTOF Tile Coefficient of Variance	Day 1: 5 min 47 s Day 6: 4 min 10 s Day 12: 5 min 57 s	Day 1: 21 Day 6: 45 Day 12: 63
ChromaTOF Tile Fold Change	Day 1: 5 min 42 s Day 6: 6 min 41 s Day 12: 6 min 3 s	Day 1: 26 Day 6: 43 Day 12: 68
ChromaTOF Sync 2D	Day 1: 26 min 3 s Day 6: 40 min 14 s Day 12: 25 min 3 s	Day 1: 25 Day 6: 78 Day 12: 108

The timing for the software varied between Tile and Sync 2D but between the Tile approaches the time remained similar. Tile software had similar timing with some variation, while Sync 2D had much longer processing time overall **(Table 3)**.

Sync 2D found a drastically larger number of compounds on later days, while Tile had a variation of compounds found between approaches. However, Fold Change and Coefficient of Variance had similar numbers, while Fisher Ratio curated the least number of compounds. **(Table 3)**.

PCA plots showed that Tile had better class differentiation than compared to Sync 2D. In Sync 2D, the samples at 32˚C were more similar to the samples at 5 ˚C than the samples at 22 ˚C, which was in due part to grouping the compounds by similarities and differences. However, Fisher Ratio had PCA plots more accurately grouped samples based on differences of the compounds found. Fold Change and Coefficient of Variance also clearly differentiated the 32 ˚C samples most effectively and had similar distribution on the scores plot. Fisher Ratio had clear class differentiation based on differences of fewer compounds, thus the PCA was more bunched than in Coefficient of Variance and Fold Change. **(Figure 4, 5)**.

Sync 2D data were most comparable to ChromaTOF peak areas and longitudinal trends. While Tile software provides raw peak areas and thus lower peak area values, those areas did not represent longitudinal trends clearly, especially for sulfur compounds **(Figure 3)**.

Acknowledgements

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Conclusions

- Sync 2D showed the capability of showing trends in decomposition compounds
  - This software approach can be used in research environment where longitudinal data is needed to determine the trends of decomposition data
  - Best purpose: research applications requiring longitudinal tracking
- Coefficient of Variance's and Fold Change's PCA plots showed their ability for clear class differentiation
  - The two software approaches can be used to distinguish water containing decomposition in a crime scene
  - Best purpose: comparison at a single point and time [detailed analysis]
- Fisher Ratio showed a proficiency in identifying top differentiating compounds and showed aptitude for class differentiation
  - The software approach would be helpful for cursory analysis for crime scene evidence
  - Best purpose: quick comparison of biggest difference for swift identification

Future research into this topic includes:

- Create a profile database for longitudinal data regarding decomposition
- Test different conditions for decomposition
- Testing with whole cadaver systems for further validation

References

(1) Stadler, S., Stefanuto, P.-H., Brokl, M., Forbes, S. L., & Focant, J.-F. (2013). Characterization of volatile organic compounds from human analogue decomposition using thermal desorption coupled to comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. *Analytical Chemistry*, *85*(2), 998–1005. https://doi.org/10.1021/ac302614y

(2) Ho, J., Patel, D., Burr, W. S., Samson, C., & Forbes, S. L. (2024). Identifying VOCs from human remains detectable in water using comprehensive two-dimensional gas chromatography. *Forensic Chemistry*, *38*, 100561. https://doi.org/10.1016/j.forc.2024.100561

(3) Armstrong, P., Nizio, K. D., Perrault, K. A., & Forbes, S. L. (2016). Establishing the volatile profile of pig carcasses as analogues for human decomposition during the early postmortem period. *Heliyon*, *2*(2). https://doi.org/10.1016/j.heliyon.2016.e00070