Untargeted Exploration of Putative Emotional Chemo-Signals by Thermal Desorption Preconcentration GC-EI&CI-TOFMS

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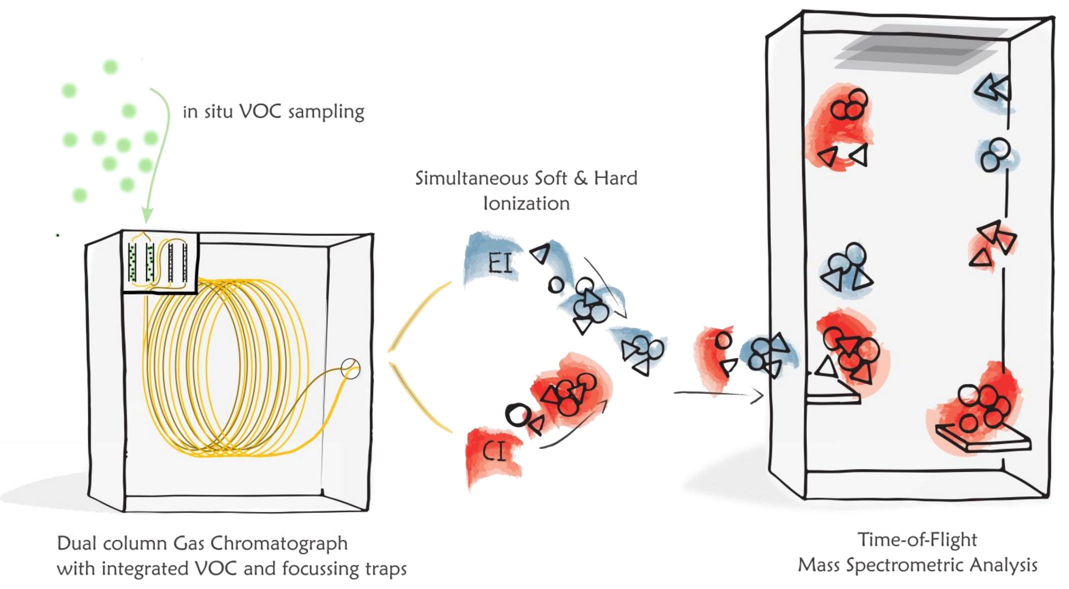
INTRODUCTION

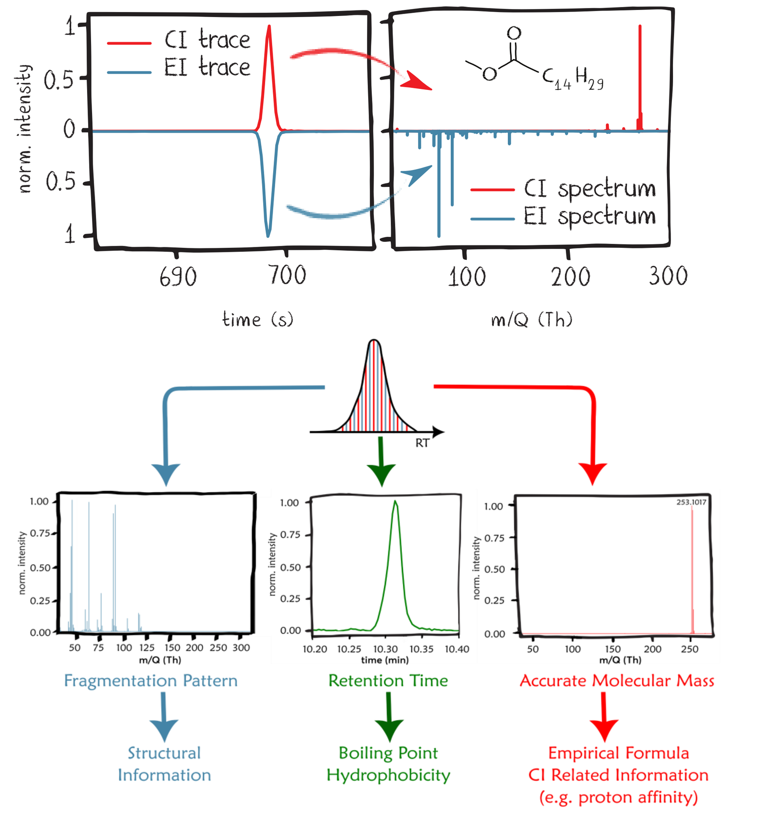
Untargeted analysis enables identification of unknown compounds in the complex samples. Human sweat contains a cocktail of volatile organic compounds (VOCs) that is changed by a variety of internal and external factors. Studies demonstrated that emotional states, such as fear or happiness, can trigger changes in the composition of the VOCs that are emitted. It is known that human chemo-signals elicit the same emotional responses in dogs. A goal of ongoing studies is to use dogs and their reaction to identify the exact compounds that are responsible for the chemo-signaling. However, the exact compounds that are released in fear, happy, and neutral conditions are still elusive.

The newly introduced dual ionization GC-ecTOF simultaneously operates a chemical ionization (CI) and an electron ionization (EI) source on a single high-resolution time-of-fight mass analyzer (HR-TOFMS).

Compound identification confidence is significantly improved while complexity, resources, and time of the analysis are reduced by generating molecular and structural information on an analyte in parallel within a single GC run.

Here we introduce the GC-EI&CI-TOFMS for non-target analysis of human sweat. We identified unique compounds for different emotions that will be further examined on dogs. Dog training is very tedious; therefore, the experimental compounds must be selected with the highest confidence. By implementing the ecTOF, we can significantly improve compound identification through fast analysis, high performance, and high certainty.





CONCLUSION

- **The simultaneous EI&CI data using the ecTOF enables the acquisition of both molecular and NIST library searchable structural information in a single GC run.**
- **Unambiguous molecular identification is obtained with the combination of ARI GC and ecTOF in one GC run.**
- **Data treatment is less complex due to a perfect alignment and grouping of EI&CI peaks. Complex data alignment procedures are no longer necessary.**
- **We report high certain identification of significant VOCs emitted by humans under different emotions responsible for chemo-signaling.**

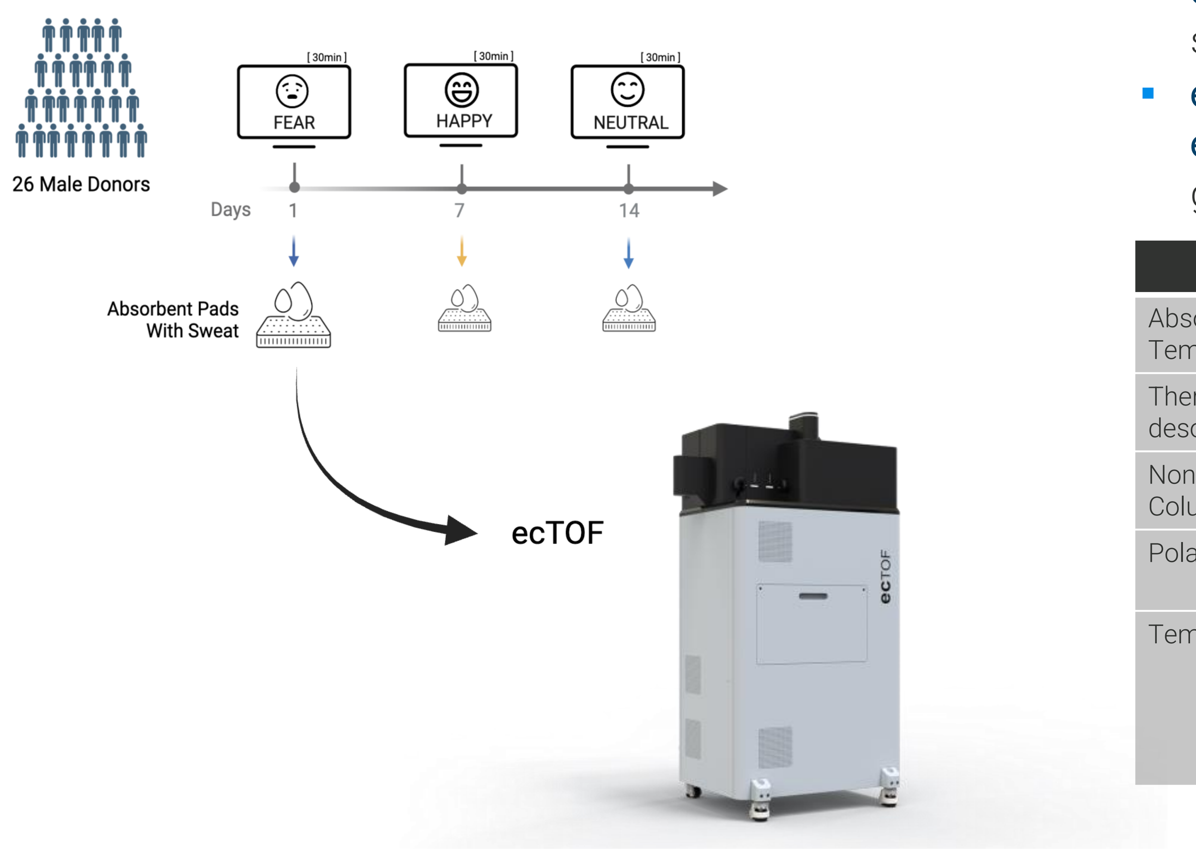
ecTOF

METHOD

Experimental method

Healthy male donors (N = 26, average age = 21) were exposed to 30 min videos inducing specific emotions: Fear, Happy, and Neutral (A). In total 37 samples of sweat in specific sterile absorbent pads were collected and stored at -80 °C until analysis.

A) Sample collection workflow



The adsorbent pads were added to 20 mL vials and heated to 37 °C for 30 min to establish a headspace. The samples were analyzed by *in situ* thermal desorption preconcentration gas chromatography (ARI GC) coupled to TOFMS (ecTOF).

- ARI GC (Aerodyne Research Inc., Billerica, CO, USA) enables a thermal desorption preconcentration and a dual column setup for polar and non-polar chromatographic separation in a single GC run.
- ecTOF (TOFWERK AG, Thun, Switzerland) includes parallel electron and chemical ionization for simultaneous generation of structural and molecular information.

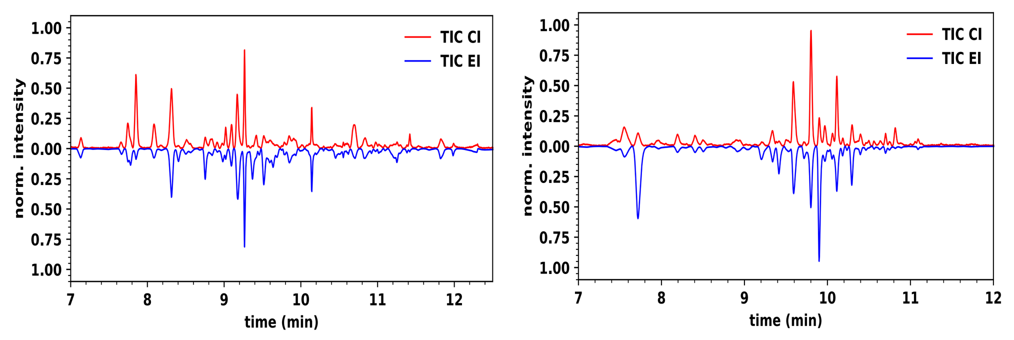
TDPC-GC Method		ecTOF Method	
Absorbant Trap Temp	20 ± 1 °C for 6 min at 30 cm^3/min	Flow Split	1:1 EI/CI
Thermal-desorption Temp	225 °C for 20 s at 10.5 °C/s	Heated Transfer Lines Temp	280 °C
Non-polar Column	DB-5 30m, 0.25mm ID, 0.25 µm	Ion Source Temp	EI 280 °C CI 280 °C
Polar Column	Wax GC 30m, 0.25mm ID, 0.25 µm	Ion Sources	EI StarBeam 70eV CI HRP using N_2H^+
Temp Program	35 °C for 40 s 35 °C/min to 100°C 15 °C/min to 150°C 30 °C/min to 235°C, held for 250s	Mass Range	15-500 m/z

RESULTS

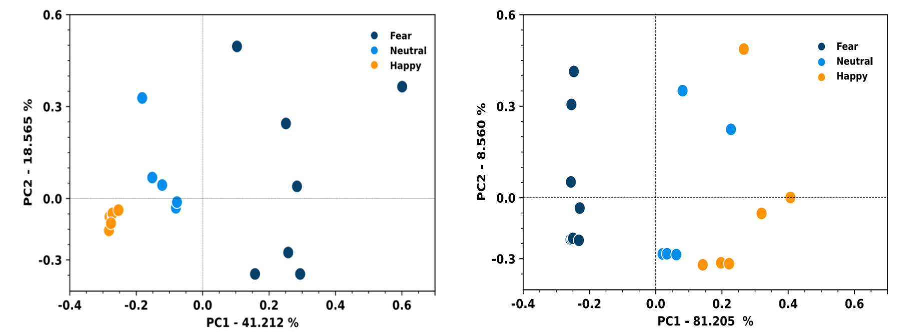
Data post-processing analysis

Data post-processing is simplified due to perfectly aligned EI&CI peak information (B). PCA analysis were used to investigate possible clustering behavior of different emotional samples (C). Further, volcano plots were used to identify significant differences in composition between the sweat samples (D) and significant compounds emitted only in a single emotional state were identified (E). An example utilizing EI and CI data acquired with the ecTOF is shown to highlight its increased confidence in compound identification (F).

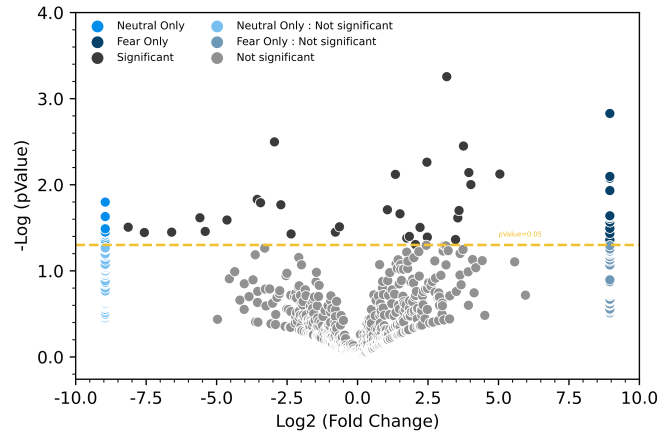
B) Total ion chromatogram (TIC) from CI (red) and EI (blue) of one of "Happy" sample measured on non-polar column (left) and polar column (right) showing perfect EI&CI peak alignment.



C) PCA analysis show variabilities between the different emotions measured by non-polar column (left) and polar column (right). With the non-polar column, Happy and Neutral emotional states are well separated.



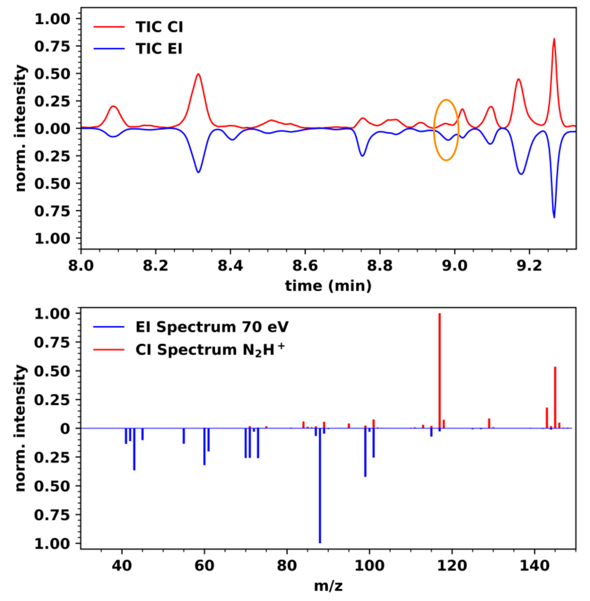
D) Identification of significant compounds between different emotional states. The chosen example shows the comparison between Fear and Neutral emotion.



E) Examples of unique compounds detected for each emotion and considered for further testing on dogs.

Happy	Fear	Neutral
2,3-Butanediol	3-Carene	Butanal
Propanoic acid ethyl ester	1,3-Hexadien-3-yne or 1,5-Hexadien-3-yne	Cyclohexane, methyl-
Butanoic acid ethyl ester		
Butanoic acid 3 methyl ethyl ester		
Octanoic acid, ethyl ester		
Hexanoic acid, ethyl ester		

F) The molecular information generated via CI greatly increases the compound identification confidence. An EI NIST library search for the peak at 9.0 min results in Hexanoic acid, ethyl ester (MW 144, match factor 903, reverse match factor 910, probability 89.5%). However, the EI mass spectrum did not show the molecular ion. Complementary CI spectrum includes mass peaks suggesting a sum formula $C_8H_{17}O_2^+$ with a mass accuracy for the $[M+H]^+$ - 1.3 ppm. The given NIST library information, including RI, as well as the CI information, a tentative identification with high confidence can be made.



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